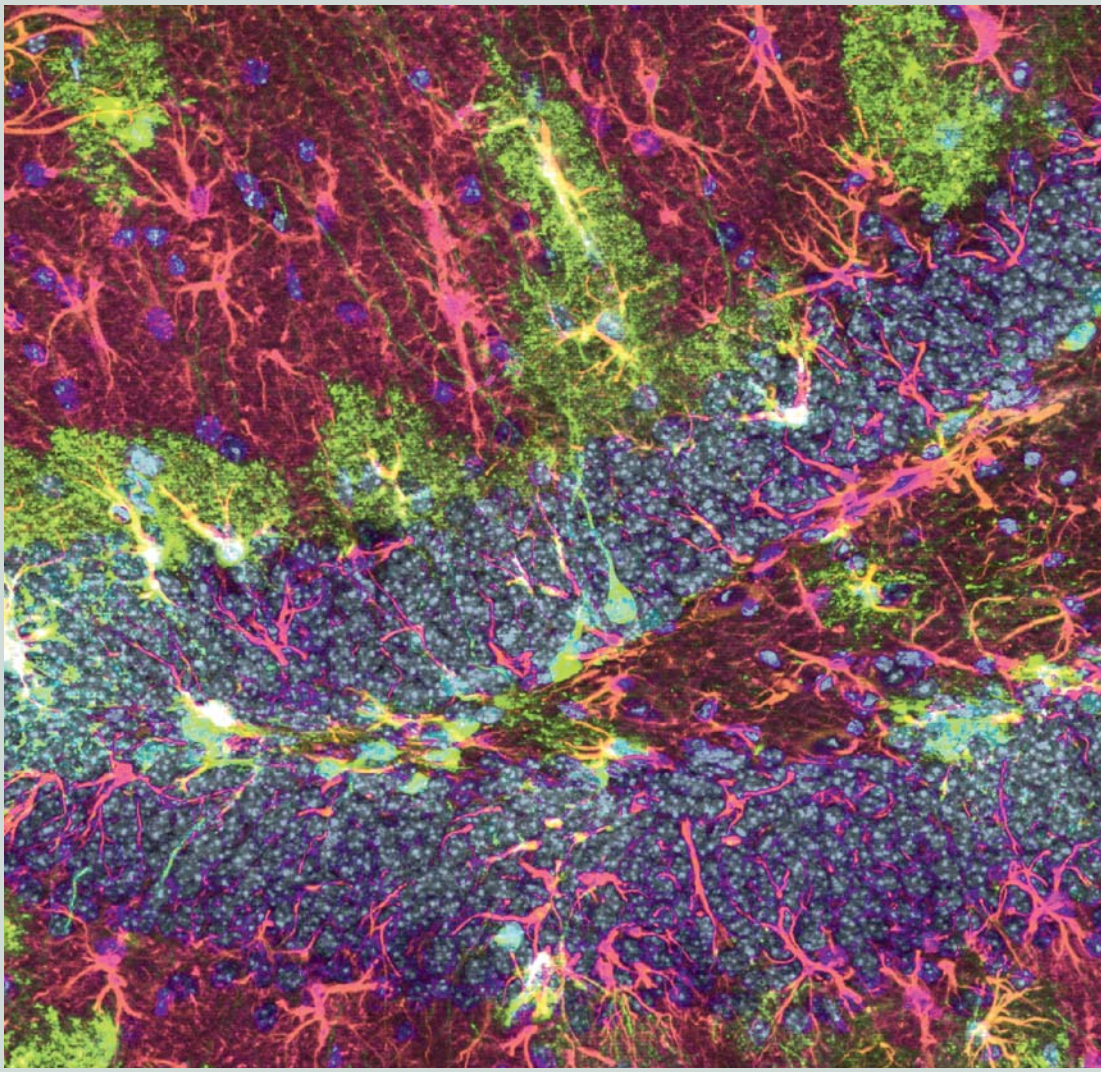
DBM 2011–2013

Department of Biomedicine



Departement
Biomedizin
Basel


UNI
BASEL


Universitätsspital
Basel


UKBB
Universitäts-Kinderspital
beider Basel

DBM 2011–2013

Department of Biomedicine

www.biomedizin.unibas.ch

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Cover Image
Dentate gyrus of the adult mouse hippocampus. Lineage-tracing of Hes5 expressing cells (green) including GFAP neural stem cells (red) and their production of immature neurons. Parenchymal astrocytes expressing S100beta (magenta).
Image courtesy: Dr. Chiara Rolando/Taylor lab

Contents

Preface

Departement Biomedizin Basel

The Department of Biomedicine (DBM) organizes the laboratory-based research of the faculty of medicine of the University of Basel. In the DBM, the laboratories of the former "pre-clinical institutes" as well as the clinical divisions of the University Hospitals are united under a common leadership with the goal to channel their efforts and to strive for excellence in biomedical research. In the 13 years since the department was founded, we enjoyed continuous growth and flourishing of our research. By providing a bridge between basic science and clinical medicine, the DBM is an important component in the University of Basel's strategic plan for the Life Sciences. The DBM concentrates on research in four focal areas: Oncology, Immunology, Neurobiology, and Stem Cells/Regenerative Medicine.

DBM's research groups obtain a large proportion of their research funds from competitive grants by national foundations, the EU and other countries. More than 60% of the positions are funded by third parties. The DBM has attracted individual grants as well as synergy grants from the European Research Council (ERC), the Swiss Initiative in Systems Biology (SystemsX.ch) and the Swiss National Science Foundation (SNSF). In 2010, Roche and the University of Basel with the University Hospital have initiated the "Basel Translational Medicine Hub". This Innovation Fund brings together expertise from academia and industry, and fosters tight bonds between basic and clinical research.

In 2012, Markus Heim, research group leader at the DBM received the prestigious Otto Naegeli Prize for the promotion of medical research. The prize is awarded every two years and is considered one of the highest national honors. Michael Sinnreich received the Robert-Bing-Prize 2012. The National Research Council of the SNSF recently elected Rolf Zeller as a new member, where he will join Markus Heim and Marc Donath, both also from the DBM. In 2013, Daniela Finke and Radek Skoda were elected members of Swiss Academy of Medical Sciences (SAMS).

This report summarizes the activities of meanwhile over 60 DBM research groups during the period of 2011-2013. The reports are grouped thematically according to the four focal areas. Each research group has selected their most relevant publications from this period. A complete list of all publications can be found in the annex of this report. The DBM and our research groups are regularly evaluated by the Scientific Advisory Board that consists of eight internationally recognized experts. During their yearly visits, the Advisory Board members evaluate and make recommendations on how to improve the organization of the department. They also provide an important basis for decisions, including promotions and changes in future directions. Laboratory

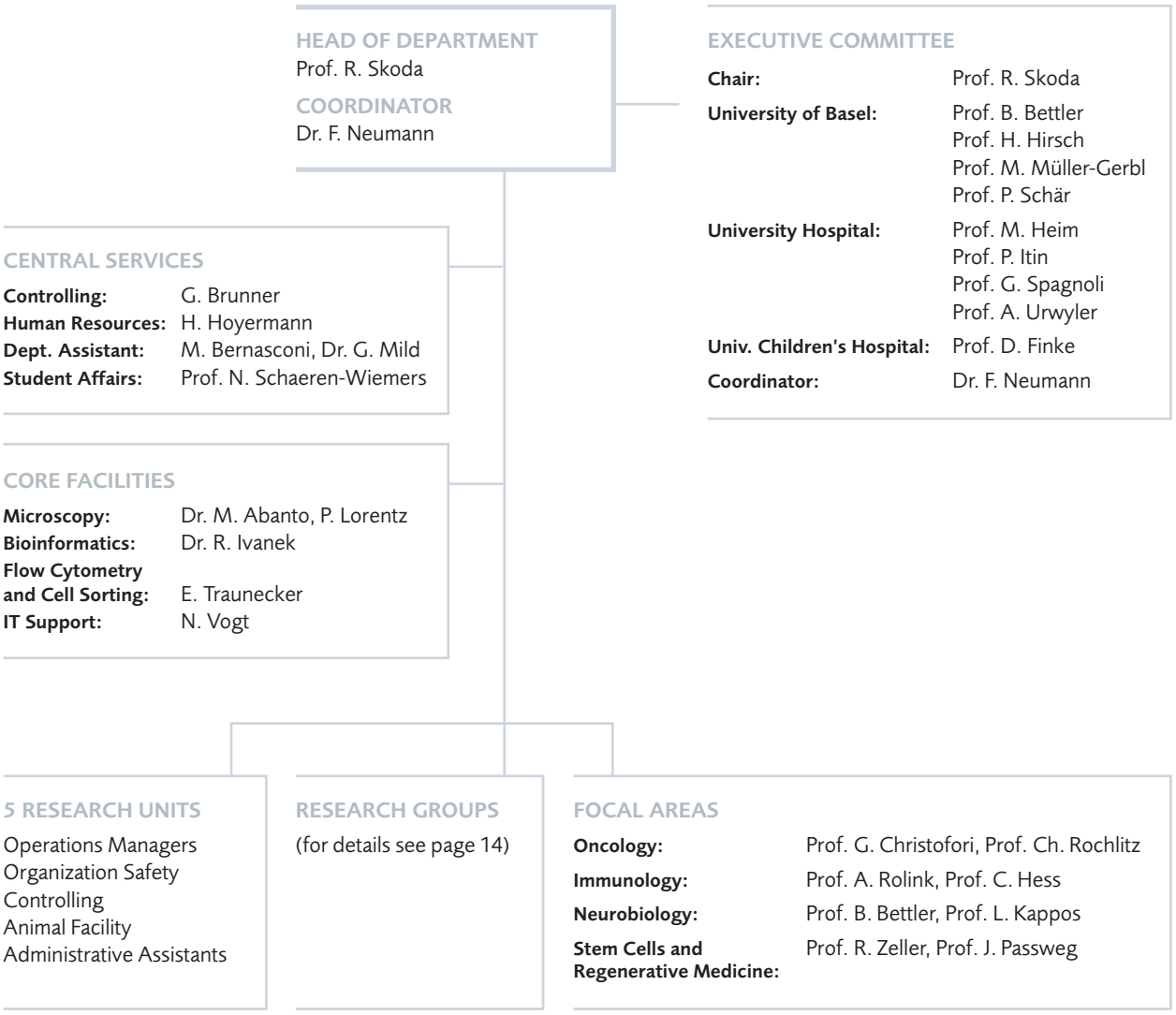
space remains a major problem, but in January 2014, the DBM will gain access to new laboratories on the 2nd floor of the DBM-Hebelstrasse, where the former medical library has been converted to lab space. We are looking forward to this much-needed expansion.

Key to the success of the DBM has been the enthusiasm of our scientists and clinicians from over 40 countries to communicate and to perform inter- and trans-disciplinary work resulting in benchmark biomedical research. The research is supported by a growing number of Core Facilities. While some Core Facilities are for the DBM only, others are joint ventures between our department with the Biozentrum (Faculty of Natural Sciences) and also the D-BSSE Institute of the ETH Zürich in Basel. The access to these key technologies is of immeasurable value to us. As a token of our esteem, we portray the core facilities in the current report.

The DBM is committed to the highest quality and innovation in research. I hope that this report will facilitate the exchange of information and interactions with our research groups and I wish you pleasant reading.

Prof. Dr. Radek Skoda
Head of the Department of Biomedicine

Organization Chart 2013



The Department of Biomedicine is led by the Head of the Department. The Coordinator and the staff of the Central Services assist the Head of the Department in all administrative and organizational issues. The overall strategy is defined by the DBM Executive Committee that is chaired by the Head of the Department and which consists of four representatives from the pre-clinical disciplines of the University of Basel, four representatives from the divisions of the University Hospital, and one representative form the University Children's Hospital.

Key Data 2012

Research groups	64
Diagnostics services and others	5
Tenured Professors	35
Not tenured Professors (Titular-, SNSF- and tenure track-assistant professors)	17
Employees total (of these 60% are paid by third-party funds)	711
Space	12'174 m^2

Budget 2012

Personnel	CHF	24'953'643
Supplies		9'019'393
Income (total)		- 9'381'759
Investments (equipment)		3'424'130
Total	**CHF**	**28'015'407**
Overhead costs (2011)		12'214'406
Total	**CHF**	**40'229'813**
Third-party funds (grants etc.)	**CHF**	**22'504'113**

Scientific Advisory Board

NEUROBIOLOGY

Prof. Greg Lemke
Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, USA

Prof. Christian Lüscher
Department of Basic Neurosciences and Service of Neurology, University of Geneva Medical School, Switzerland

IMMUNOBIOLOGY

Prof. Brigitta Stockinger
Head of Division of Molecular Immunology, MRC National Institute for Medical Research, London, UK

Prof. Kathryn Wood
Nuffield Department of Surgery, John Radcliffe Hospital, Headington, Oxford, UK

ONCOLOGY

Prof. Margaret C Frame
Edinburgh Cancer Research Centre, University of Edinburgh, Western General Hospital, Edinburgh, UK

Prof. Bob Löwenberg
Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands

STEM CELLS AND REGENERATIVE MEDICINE

Prof. Paolo Bianco
Stem Cell Laboratory, Biomedical Science Park San Raffaele and Department of Experimental Medicine and Pathology, La Sapienza University, Rome, Italy

Prof. Karl-Heinz Krause
Department of Pathology and Immunology, University of Geneva Medical School, Switzerland

Executive Committee

Prof. Radek Skoda
Head of the Department

Prof. Bernhard Bettler

Prof. Magdalena Müller-Gerbl

Prof. Daniela Finke

Prof. Primo Schär

Prof. Markus Heim

Prof. Giulio Spagnoli

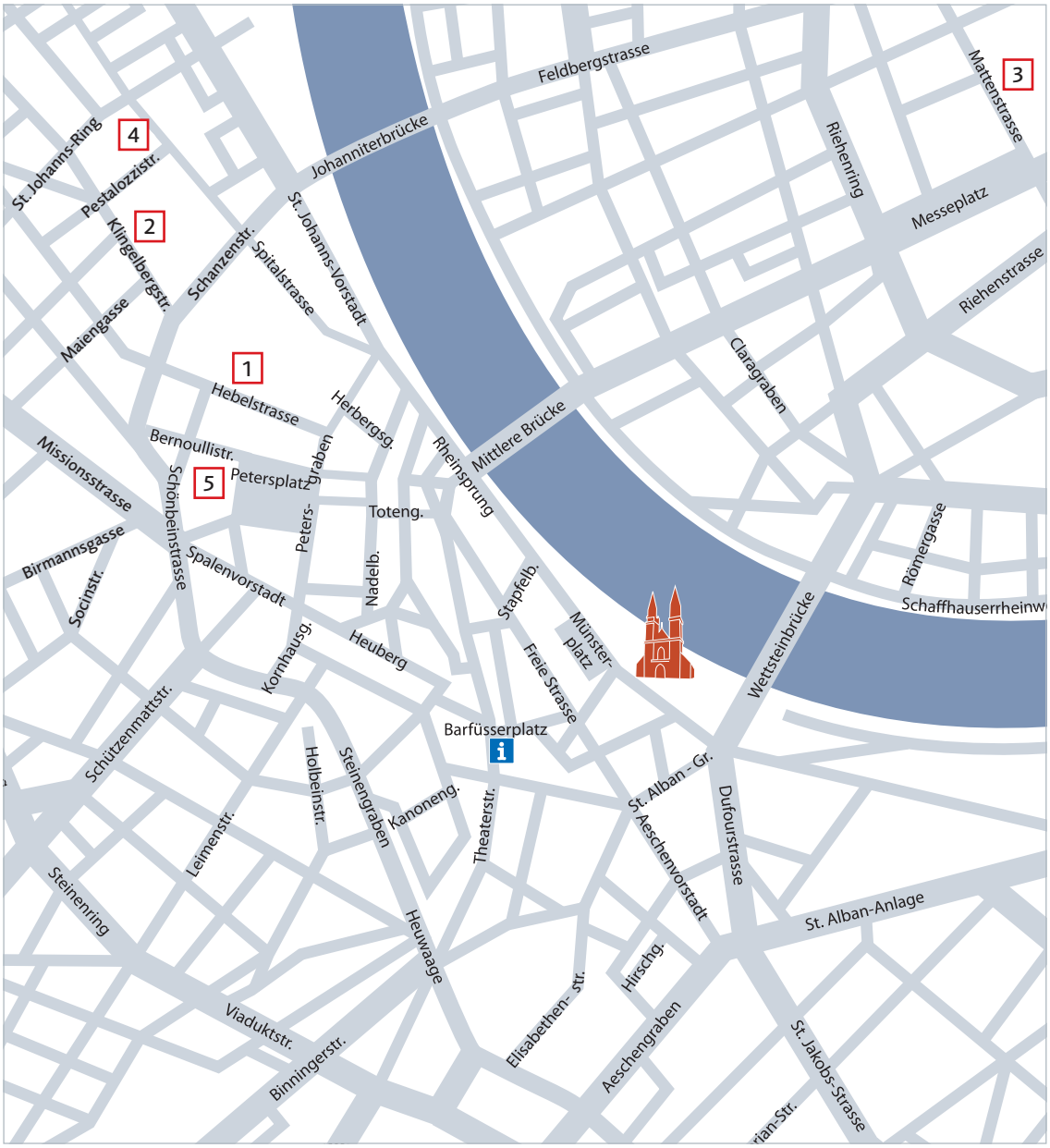
Prof. Hans H. Hirsch

Prof. Albert Urwyler

Prof. Peter Itin

Dr. Frank Neumann

Locations


Feldbergstrasse
Johanniterbrücke
St. Johanns-Ring
Pestalozzistr.
Klingelbergstr.
Schanzenstr.
Spitalstrasse
St. Johanns-Vorstadt
Maiengasse
Hebelstrasse
Herbergsg.
Bernoullistr.
Petersplatz
Petersgraben
Missionsstrasse
Schönbeinstrasse
Birmannsgasse
Socinstr.
Spalenvorstadt
Toteng.
Nadelb.
Rheinsprung
Mittlere Brücke
Claragraben
Riehenring
Messeplatz
Mattenstrasse
Riehenstrasse
Römergasse
Schaffhauserrheinw
Stapfelb.
Münsterplatz
Wettsteinbrücke
Freie Strasse
Barfüsserplatz
Kornhausg.
Heuberg
Schützenmattstr.
Holbeinstr.
Steinengraben
Kanonengr.
Theaterstr.
St. Alban - Gr.
St. Aeschenvorstadt
Dufourstrasse
St. Alban-Anlage
Leimenstr.
Steinenring
Heuwaage
Viaduktstr.
Binningerstr.
Elisabethen- str.
Hirschg.
Aeschengraben
St. Jakobs-Strasse
1
2
3
4
5

1 Hebelstrasse 20, 4031 Basel

2 Klingelbergstrasse 50/70
Pharmazentrum (7th floor), 4056 Basel

3 Mattenstrasse 28, 4058 Basel

4 Pestalozzistrasse 20, 4056 Basel

5 Petersplatz 10, 4001 Basel

Research Groups of the Department of Biomedicine

grouped according to location and focal area

Department of Biomedicine

Hebelstrasse 20

Brain Ischemia and Regeneration
Prof. Raphael Guzman

Clinical Neuroimmunology
Prof. Tobias Derfuss
Prof. Raija Lindberg

Neurobiology
Prof. Nicole Schaeren-Wiemers

Psychopharmacology Research
PD Dr. Matthias Liechti

Cardiobiology
Prof. Marijke Brink
Prof. Peter Buser

Cardiovascular Molecular Imaging
SNSF SCORE
PD Dr. Beat Kaufmannn

Cell and Gene Therapy
PD Dr. Andrea Banfi
Prof. Michael Heberer

Clinical Pharmacology
Prof. Stephan Krähenbühl

Dermatology
Prof. Peter Itin

Experimental Hematology
Prof. Radek Skoda

Gastroenterology
Prof. Christoph Beglinger

Gynaecological Endocrinology
Prof. Christian DeGeyter

Hepatology
Prof. Markus H. Heim

Inner Ear Research
Prof. Daniel Bodmer

Myocardial Research
PD Dr. Gabriela Kuster Pfister
Prof. Stefan Osswald

Ocular Pharmacology and Physiology
PD Dr. Albert Neutzner
Prof. Peter Meyer

Pulmonary Cell Research
Prof. Michael Roth
Prof. Michael Tamm

Signal Transduction
Prof. Therese J. Resink
Prof. Paul Erne

Stem Cells and Hematopoiesis
Prof. Claudia Lengerke

Tissue Engineering
Prof. Ivan Martin
Prof. Michael Heberer

Cancer Immunology and Biology
Prof. Alfred Zippelius
Prof. Christoph Rochlitz

Cancer Immunotherapy
SNSF Professorship
Prof. Giandomenica Iezzi

Childhood Leukemia
Prof. Jürg Schwaller

Gynecological Research
Prof. Viola Heinzelmann

Oncology Surgery
Prof. Giulio C. Spagnoli
Prof. Michael Heberer

Prenatal Medicine
Prof. Sinuhe Hahn

Clinical Immunology
Prof. Marten Trendelenburg

Diabetes Research
Prof. Marc Donath

Experimental Immunology
Prof. Gennaro De Libero

Immunobiology
Prof. Christoph Hess

Immunodeficiency*
SNSF Professorship
Prof. Mike Recher

Immunonephrology
Prof. Jürg A. Schifferli

Immunoregulation
SNSF Professorship
Prof. Simona Rossi

Immunotherapy
SNSF Professorship
Prof. Martin Stern

Infection Biology
SNSF Ambizione-SCORE
PD Dr. Nina Khanna

Molecular Immune Regulation*
SNSF Professorship
Prof. Lukas Jeker

Molecular Nephrology
PD Dr. Andreas Jehle

Translational Immunology*
SNSF Ambizione-SCORE
Dr. Christoph Berger

Transplantation Immunology and Nephrology
Prof. Ed Palmer
Prof. Jürg Steiger

Perioperative Patient Safety
PD Dr. Susan Treves
Prof. Thierry Girard

Department of Biomedicine
Mattenstrasse 28

- Embryology and Stem Cell Biology
  Prof. Verdon Taylor
- Cancer- and Immunobiology
  Prof. Matthias Wymann
- Cell Migration and Neuritogenesis
  SNSF Professorship
  Prof. Olivier Pertz
- Developmental Genetics
  Prof. Rolf Zeller
  Dr. Aimée Zuniga
- Human Genomics
  Prof. Sven Cichon
- Tumor Biology
  Prof. Gerhard Christofori
- Molecular Genetics
  Prof. Primo Schär
- Developmental Immunology
  Prof. Daniela Finke
- Developmental and Molecular Immunology
  Prof. Antonius Rolink
- Pediatric Immunology
  Prof. Georg A. Holländer

Department of Biomedicine
Pestalozzistrasse 20

- Developmental Neurobiology and Regeneration
  Prof. Josef Kapfhammer
- Cellular Neurophysiology
  Prof. Josef Bischofberger
- Musculoskeletal Research
  Prof. Magdalena Müller-Gerbl
- Integrative Biology
  Prof. Daniel Haag

Department of Biomedicine
Klingelbergstrasse 50/70

- Molecular Neurobiology Synaptic Plasticity
  Prof. Bernhard Bettler
- Neuromuscular Research
  Prof. Michael Sinnreich
- Brain Tumor Biology
  Prof. Luigi Mariani

Department of Biomedicine
Petersplatz 10

- Experimental Virology
  Prof. Daniel Pinschewer
- Transplantation and Clinical Virology
  Prof. Hans H. Hirsch
- Molecular Diagnostics
  Prof. Thomas Klimkait
- Rheumatology*
  Prof. Diego Kyburz

Core Facilities

- Microscopy
  Dr. Mike Abanto
  Pascal Lorentz
- Flow Cytometry and Cell Sorting
  Emmanuel Traunecker
- Bioinformatics
  Dr. Robert Ivanek
- Histology
  Prof. Konstantin Beier

Anatomy Museum
Prof. Magdalena Müller-Gerbl

Diagnostic Services

- PCR/HIV Laboratory
  Prof. Hans H. Hirsch
- Serology/Virology Laboratory
  Prof. Thomas Klimkait
- HLA Testing Laboratory
  Prof. Jürg Steiger
- Medical Genetics Laboratory
  Prof. Sven Cichon
- Pharmacology and Toxicology
  Prof. Stephan Krähenbühl

Focal Area:

- Neurobiology
- Stem Cells and Regenerative Medicine
- Oncology
- Immunology

- Other Research Topics
- Services and Core Facilities

* starting 2014

Core Facilities of the Department of Biomedicine

Scientific core facilities are becoming increasingly an integral part of research institutions nowadays – not only as a means of efficiency and cost optimization but also as centers of competence and customized scientific services. As centralized shared resources, they provide all researchers easy access to instrumentation, technology, and expert know-how. The highly trained facility managers are keeping up with the latest technological advances in order to serve the evolving needs of the research community.

DBM members have in-house access to core facilities for Bioinformatics, Flow Cytometry & Cell Sorting, and Microscopy and access to core facilities jointly operated with other research institutions including Quantitative Genomics Facility (for next generation sequencing), the Life Sciences Training Facility (for gene expression profiling), the Center for (Electron) Microscopy, the Transgenic Mouse Core Facility, and the Small Animal Facility for Nuclear Molecular Imaging.

DBM core facilities are unique in that they provide their service at no or very little costs. They are continually implementing the latest developments to better serve the DBM scientific community. As such they have a central role in creating a competitive research environment and contribute significantly to the scientific success of the Department of Biomedicine.

Bioinformatics

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http://biomedizin.unibas.ch/services/bioinformatics-core-facility/

The Bioinformatics Core Facility provides a centralized resource of expertise in computational biology and statistics, available to all researchers at the DBM. It offers services for analysis and visualization of large-scale biological data produced by high-throughput genomics experiments. The platform also provides training in bioinformatics and facilitates access to high-performance computational resources.

Design and analysis of the high-throughput biological data sets
One of the goals is to implement solutions for analysis, visualization, management and interpretation of large-scale genomic data generated by high-throughput techniques (microarrays and next generation sequencing) both in human and in model organisms. Over the first 18 months, the Bioinformatics Core Facility analyzed data from more than 30 studies from 16 research groups: 22 studies of gene expression, 11 DNA-protein binding (ChIP-, CLIP-seq), 1 DNA methylation study, 2 studies on detailed mapping of physical contacts between genomic elements (4c-seq), and 2 projects focused on identification of sequence variants. Beside the standardized approaches, the platform also develops customized solutions tailored towards the needs of individual research projects.

Bioinformatics training
The facility also provides regular bioinformatics training. Together with bioinformaticians from the Friedrich Miescher Institute for Biomedical Research we organize "**R** Introductory Course". This five-day practical course provides beginners with basic knowledge of the **R** software environment and with training on how to explore and visualize the data and perform wide range of statistical tests. Already 20 PhD students and Post-docs from the DBM attended this course in fall 2012 and in spring 2013 where they learned how to analyze their own data with **R** software.

Infrastructure
The platform's mission is to build and maintain an infrastructure that enables application of strong bioinformatics analysis to enhance and empower the biomedical research at the DBM. However, the rapid technological advances as well as the growing number of bioinformatics approaches and tools make it difficult to keep track of with limited resources at the facility. Therefore, the Bioinformatics Core Facility closely interacts with other bioinformatics units in the Basel area, namely with the group of Dr. Michael Stadler (Friedrich Miescher Institute for Biomedical Research) and the group of Prof. Torsten Schwede (Basel Computational Biology Center - $[BC]^2$, Swiss Institute of Bioinformatics) in order to implement the latest bioinformatics approaches. By joining the $[BC]^2$ in 2012, all researchers of the DBM got access to its high-performance computing cluster. In 2013, Dr. Ivanek became a member of the Swiss Institute of Bioinformatics.

Flow Cytometry and Cell Sorting

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DBM Hebelstrasse

http://biomedizin.unibas.ch/services/

Staff
Toni Krebs

Flow cytometry is a laser-based technology with which cell mixtures can be analyzed, counted and separated. The technology is based on light scattering and fluorescence characteristics of individual cells or other microscopic particles as they flow through a laser beam. Analyses of fluorescence markers, cell size and shape are routinely used to identify specific cell types. Cell populations cannot only be analyzed, but cells of interest can also be retrieved for further investigations. Sorting cells by flow cytometry has become a standard method used by DBM researchers from all focal areas, including applications in immunology, oncology, neurobiology, and stem cell biology. In medicine, the applications cover transplantation, hematology, tumor immunology and chemotherapy or prenatal diagnosis.

Service
The DBM Flow Cytometry and Cell Sorting Core Facility provides instrumentation, technical support and methodological assistance for a wide range of applications using flow cytometry for analysis and sorting of cell populations. We offer free-of-charge access to the most advanced technology and to a well-maintained equipment park to all DBM researchers, helping them to rapidly advance in their projects. Our facility is divided into two sections: (i) the Cytometry Unit, to which researchers have full access after a mandatory introductory training; (ii) the Sorting Unit, which is equipped with modern cell sorters and where our expert staff provides a full service in cell and particle sorting. We train researchers in basic and advanced handling of the equipment, and provide expert support on data acquisition and analysis. We also organize seminars on technological advances in cytometry and related fields.

Equipment
Cytometry: BD LSRII Fortessa, BD Accuri, Beckman Coulter Cyan,
Sorting: BD Aria III, BD Cytopeia Influx,

Outlook
The Flow Cytometry and Cell Sorting Core Facility aims at continually improving quality and capacity of their equipment and services. Our goal is to develop novel methods and keep up with technological advances in the field, e.g. by increasing throughput and sensitivity or by combining flow cytometry with microscopy.

Microscopy

Dr. Mike Abanto
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DBM Hebelstrasse

http://biomedizin.unibas.ch/services/microscopy-facilities/facility-hebelstrasse/

Staff
Beat Erne

Pascal Lorentz
pascal.lorentz@unibas.ch
DBM Mattenstrasse

http://biomedizin.unibas.ch/services/microscopy-facilities/biooptics-facility-mattenstrasse/

Light microscopy has revolutionized modern biology by creating a window for researchers to "see" the cellular and molecular world. The DBM Microscopy Facility enables researchers with limited microscopy knowledge to become experts and it allows experts to do creative experiments.
With over 100 introductions last year, a common use of the facility is training new users. Basic training is one-to-one and it begins with sample preparation, probe choice, demonstration and discussion. This is followed by hands-on experience at the microscope, and learning how to acquire a good image. Users finally convert their images into meaningful data through computer assisted image analysis and quantification.
The facility also invests in technological development. Two major questions in modern microscopy are: How small can we image and how much can we image? Towards answering the first question, single molecule imaging with quantum dots was recently implemented. This nano technique allows the observation of movement of individual proteins in live cells. Towards answering the second question, we have installed an Operetta microscope that brings the power of high content screening to DBM research and discovery. Advanced techniques such as super resolution microscopy, single molecule tracking, calcium imaging, FRAP, FRET, live cell imaging, slide scanning, high content screening and many more have been developed, implemented, and regularly executed at the DBM.

Two facilities – unique service
The DBM currently holds two microscopy facilities located at Hebelstrasse and at Mattenstrasse. Both facilities are used free of charge and there are a total of 17 microscopes, including four confocals, two stereomicroscopes, many different wide-field systems for live and fixed-cell or time-lapse microscopy, a laser dissection microscope, and a high content screening microscope. For image analysis we have Imaris, Huygens, Nikon, Zeiss, Olympus, Leica, Metamorph, and more software licenses. We also support freeware, particularly ImageJ.
All the academic microscopy facilities in Basel form an interactive network called the Basel Microscopy Network (BMN, http://microscopynetwork.unibas.ch), composed of the DBM, Biozentrum, D-BSSE, and FMI facilities. This facilitates exchange, fosters collaborations, and provides a wide net of microscopy possibilities across the Basel academic landscape.
Our outlook is to continue raising the basic level of microscopy at the DBM while also extending the frontiers of what is possible in super-resolution microscopy. We are developing microscopy courses in house and in the Basel area in collaboration with the BMN, and also invite speakers and companies to showcase the latest technology to our DBM researchers.

Joint Core Facilities

Life Science Training Facility (LSTF)

Prof. Dr. Andreas Papassotriopoulos
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Life Science Training Facility
Division of Molecular Psychology
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Switzerland

The Life Sciences Training Facility (LSTF) provides access to microarray and deep-sequencing technologies and contributes to the identification of novel molecular pathways in health and disease. The facility is primarily open to research groups affiliated with the University (e.g. Department of Biomedicine, Biozentrum, University Hospital), but it also collaborates with external groups. The LSTF implements technologies dedicated to the analysis and/or quantification of DNA and RNA including DNA/RNA microarrays for gene expression and genome-wide SNP genotyping, real-time quantitative PCR, Pyrosequencing, and library preparation for next generation sequencing.

Service, teaching and research
The LSTF, which is embedded in the university's Division of Molecular Neuroscience, is part of the Affymetrix Core Lab Program and is organized as a user-lab. Researchers receiving LSTF's services are tightly involved in all steps of their experiment since they perform the technical work themselves as well as the data analysis under our guidance and daily assistance. The facility has developed standardized procedures, which fit to the specificity of each project. The facility is dedicated to train young researchers at the bench through the organization of software training and courses associating theory and practice. To efficiently analyze their data, researchers have free access to commercial software as well as free third-party software.

Affiliation and staff
The LSTF is located in the Pharmazentum/Biozentrum and is funded by the major stakeholders (Department of Biomedicine, Biozentrum, Faculty of Psychology). The head of the university's Division of Molecular Neuroscience acts as the academic supervisor of the LSTF team, which is composed of one leading technical assistant, two technical assistants, and one scientific assistant.

Outlook
While we will continue to provide access to microarray services, we will also increase our efforts related to next generation sequencing and, importantly, to the statistical interpretation of the data. In addition, we will launch a program for genome-wide epigenetic studies through collaboration with expert groups from the pharmaceutical industry.

Quantitative Genomics Facility

Dr. Christian Beisel
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Department of Biosystems Science and Engineering (D-BSSE)
ETH Zurich
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Switzerland

The Quantitative Genomics Facility (QGF) is a central research and service facility located in the Department of Biosystems Science and Engineering (D-BSSE) of the ETH Zurich in Basel. The unit is supported and run jointly with the University of Basel and the Friedrich Miescher Institute (FMI) for Biomedical Research. QGF was established at D-BSSE in 2008 to allow researchers of the life science community in Basel and at ETH Zurich direct access to next generation sequencing (NGS) technology, and thereby facilitating the systematic quantitative investigation of genome-wide experiments.

The QGF team provides technical support for NGS applications in genomics and epigenomics, including high-throughput data management and analysis. Furthermore, QGF has installed state-of-the-art PCR platforms to enable high-throughput validation of NGS data down to the single cell level and to allow the absolute quantification of nucleic acids. The facility currently comprises an Illumina MiSeq benchtop sequencer and a HiSeq2000 sequencing system, accessory equipment for sample preparation and QC as well as the Fluidigm Biomark high-throughput qPCR and the BioRad QX100 ddPCR systems. While the operation of the NGS equipment is reserved for our trained technical staff the other preparative and analytical systems for sample preparation and QC as well as for PCR measurements are accessible by the users directly.

QGF is involved in grant-funded projects with research groups at DBM, Biozentrum and D-BSSE investigating the heterogeneous nature of tumour tissues as well as the developmental potential of mouse brain stem cells. An important new avenue in the genomics field is the evolving possibility to perform measurements in single cells for which we see great potential and establish suitable workflows in our facility. On the other hand, increasing needs in clinical research with regard to disease diagnostics and identification of biomarkers will be met by setting up robotics workstations for automated sample preparation and increasing sequencing capacity.
The growing impact of the sequencing service provided by QGF is reflected in the numbers of sequenced samples. While in 2009 we sequenced 150 samples, in 2013 we have processed more than 3'000. We envisage that the requests for genomics applications in the fields of systems biology, biotechnology and genetic diagnostics will further grow substantially, underlining the impact of QGF and its further development for the research in the Basel scientific community.

Small Animal Facility for Preclinical Nuclear Molecular Imaging

Prof. Dr. Thomas L. Mindt
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University Hospital Basel
Radiopharmaceutical Chemistry
Petersgraben 4, 4031 Basel
Switzerland

In fall 2013, the Small Animal Facility of the Clinic of Radiology and Nuclear Medicine of the University Hospital Basel was put into operation. The facility is designed to host up to 400 rats and mice for short and long-term experiments. It also comprises a SPECT/CT camera (NanoSPECT/CT; Bioscan) dedicated to small animal imaging as well as other equipment for the study of pharmacokinetic and –dynamic properties of radiolabeled compounds.

The use of nuclear imaging modalities with its unique high sensitivity (detection in the femtomole range) enables the study of molecules in a natural setting without interfering interactions such as receptor saturation effects. The facility provides a unique platform for translational research at the University of Basel.

The Small Animal Facility is managed by the heads of the divisions of Radiopharmaceutical Chemistry (DBM-associated Prof. Dr. T. Mindt) and Nuclear Medicine (Prof. Dr. Damian Wild).

Transgenic Mouse Core Facility (TMCF)

Daniela Klewe-Nebenius
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University of Basel
Biozentrum/Pharmazentrum
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Switzerland

The Transgenic Mouse Core Facility (TMCF) of the University of Basel provides research groups within the university access to transgenic and transgenic related techniques.

Service and set up

The service offers the injection of conventional DNA and BAC constructs into the nuclei of fertilized oocytes of various mouse strains, and carries out the injection of mouse embryonic stem (ES) cells into blastocysts to generate chimeras. As mouse line rederivation by embryo transfer has become increasingly important, TMCF supports the research groups in the handling of frozen or fresh mouse pre-implantation embryos, and is offering embryo cryopreservation including storage.

The service also provides gene targeting of mouse ES cells and supports research groups with tested material and methods for ES cell work. In addition, it offers de novo rederivation of mouse ES cells and embryonic fibroblasts from transgenic mouse lines. The facility works closely with the researchers and provides technical support during their experiments. TMCF continuously evaluates the technical needs and requirements in order to extend their services.

The facility consists of an injection suite, a cryo-preservation and mouse embryo thawing work station, lab space for general mouse embryo work, animal rooms, and a lab to carry out surgical procedures, a molecular biology lab for quality control and sample preparation, a mouse stem cell lab, and a workstation for primary mouse cell culture. We aim to provide state-of-the art support for all applied techniques according to the need of the scientific community. We strive to provide this support as informally and quickly as possible and to keep it as affordable as possible.

Staff, affiliation, and funding

TMCF was founded on the initiative of scientists from the Biozentrum and the DBM, and consists of the head of facility and five technical staff members, three of whom are employed part-time. The head of TMCF is newly supported by the TMCF scientific head, Prof. Dr. Verdon Taylor. Staff members are funded by the Biozentrum, the University of Basel, and the DBM. Clients are charged part of the actual costs for the consumables for each experiment. The remaining expenses are covered by the university.

Outlook

TMCF continues to provide a tailored service for all research groups at the University of Basel including the development of new protocols and methods in transgenic mouse technology. As of August 2013, part of the TMCF has been moved to the BioPark in the Rosental Areal.

Center for Microscopy (ZMB)

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University of Basel
Biozentrum
Klingelbergstrasse 50–70, 4056 Basel
Switzerland

The Center for Microscopy (ZMB) of the University of Basel provides electron microscope equipment of every description for research group projects and also plays a key role in education. The service accepts microscopy commissions from all disciplines in Life Sciences, Medicine and Natural Sciences, supporting the research groups in their projects. The ZMB also carries out its own research projects to develop and refine methods of preparation, imaging techniques, and image processing software.

The ZMB facilities comprise a preparation laboratory, three Transmission Electron Microscopes and three Scanning Electron Microscopes. We offer collaboration in imaging on electron microscopes either as 'full service', where the staff of ZMB is doing the imaging work, or by teaching users in how to operate electron microscopes on their own.

Administration of the ZMB is integrated into the University of Basel's Biozentrum. Professor Henning Stahlberg, head of the C-CINA, is currently the scientific director of the ZMB and at the same time director of the ZMB Users' Board. The Board makes the strategic decisions in keeping up with state-of-the-art electron microscopy and making the necessary new methods available to research.

Newly Appointed Professors 2010–2013 Faculty

Sven Cichon, born 1966 in Frankfurt/Main, Germany, studied Biology at the University of Bonn and graduated in 1995 with a doctorate on the identification of genetic variability in CNS-expressed receptor/transporter genes and their influence on neuropsychiatric disorders. He worked as a postdoctoral fellow at the University of Bonn and at Millennium Pharmaceuticals Inc. (USA), and as a research group leader at the Universities of Antwerp and Bonn, and at Research Center Juelich. In 2012, he was appointed Professor of Medical Genetics at the University of Basel and joined the Department of Biomedicine. His research focuses on the identification of genes influencing brain disorders and structural and functional variability of the brain.

Raphael Guzman, born 1971 in Lausanne, Switzerland, graduated from the Medical School in Bern in 1998 and completed his Neurosurgical Residency at the University of Bern. From 2004–2012 he worked at Stanford University School of Medicine in California where he did his postdoctoral fellowship and was then appointed Assistant Professor of Neurosurgery. In 2012 he was appointed Professor of Neurosurgery and his research group joined the DBM. His research group works on Neuroregeneration in Stroke and Neonatal Hypoxia with a focus on white matter regeneration.

Viola Heinzelmann-Schwarz, born 1969 in Kirchheim unter Teck, Germany, is gynaecology and obstetrics specialist and gynaecological oncologist. She holds the Ordinariat for Gynaecology and Obstetrics of the University of Basel since July 2012 and is Head Women's Hospital since July 2013. A trained gynaecologist (University Hospital Zurich) and granted with the Venia Legendi (University of Zurich, 2007), she subspecialised in gynaecological oncology at the Royal Hospital for Women in Sydney (AUS) from 2008–2012 (Prof. Neville Hacker and Prof. Michael Friedlander). She is not only a highly committed clinician but also a dedicated researcher, with focal interest in gynaecological cancers, in particular ovarian cancer.

Diego Kyburz, born 1964 in Basel, Switzerland, studied medicine in Basel and graduated 1989. 1990–1992 he attended the Postgraduate Course in Experimental Medicine and Biology of the University of Zurich. After his clinical training in internal medicine he went to the University of California in San Diego for a fellowship in rheumatology 1997–1999. After his return he completed his clinical training in rheumatology and worked as an attending physician at the division of rheumatology of the University Hospital of Zurich. In September 2013 he was appointed professor and chairman of the division of rheumatology in Basel. His research focuses on the role of the innate immunity in the development of chronic arthritis.

Claudia Lengerke, born in 1974 in Timisoara, Romania, studied medicine at the University of Tübingen, Germany, where she graduated in 2001. She completed clinical training in Internal Medicine, Hematology and Medical Oncology at the University Hospital Tübingen. After her postdoctoral fellowship at the Children's Hospital & Harvard Medical School, Boston, USA, she was a group leader at the University of Tübingen where she habilitated in 2011. In 2013, she was appointed Professor for Hematology/Stem Cell Research at the Department for Biomedicine in Basel. Her work focuses on the role of embryonic signaling pathways in healthy and cancer stem cell development and biology.

Daniel D. Pinschewer, born 1974 in Zurich, Switzerland, studied medicine at the University of Zurich (1994–2000) and obtained his medical doctorate in 2001. He then moved to The Scripps Research Institute (La Jolla, CA, USA) where he trained in molecular virology ("reverse genetics" of arenaviruses). Returning to Zurich in 2002 he became an independent group leader in 2004 and habilitated in infection immunology two years later. With the award of an SNSF professorship he was recruited to the University of Geneva in 2007. In 2013 he joined the DBM at the University of Basel as a chair in virology. His research combines virology and immunology, with an emphasis on antiviral immunity, viral pathogenesis and vaccination.

Verdon Taylor, born 1969 in Chesterfield, UK, studied Pharmacology at King's College London. In 1995 he obtained his PhD from the University of Basel working on kinases in brain development. He was a postdoctoral fellow at the ETH, Zurich, where he then became a group leader (Oberassistent) in Cell Biology. In 2002 he was awarded a Max Planck junior group in developmental neurobiology at the MPI, Freiburg, Germany. In 2009 he was recruited as Associate Professor to the University of Sheffield and then in 2011 as Professor for Embryology and Stem Cell Biology in the Department of Biomedicine in Basel. His research focuses on the mechanism regulating mammalian neurogenesis and neural stem cell fate.

Newly Appointed Professors 2010–2013 Junior Faculty

Christoph T. Berger, born 1977 in Basel, Switzerland, graduated from Medical School in Basel in 2002 and trained in internal medicine and clinical immunology. He spent his postdoctoral fellowships at the Ragon Institute of MGH, MIT and Harvard, in Boston, focusing on cellular immunity to HIV (Brander Lab, 2008–2010), and HCV infection (Alter Lab 2010–2011). He was awarded an Ambizione-Score Grant in 2012 to study the pathogenesis of giant cell arteritis, an autoimmune disease of the arteries. The work will focus on T-cell function, the identification of their immunological targets, and factors that might predict clinical outcome.

Giandomenica Iezzi, born 1970 in Lodi, Italy, studied Medicine at the University of Milan, Italy, where she graduated in 1994 and specialized in Allergology and Clinical Immunology in 2001. She started her research activity in Milan, at San Raffaele Hospital. Between 1996 and 2000 she worked at the Institute for Immunology, in Basel, then, from 2002 until 2007 at ETH, in Zürich. In 2007 she returned to Basel and joined the Oncology Surgery group at the Department of Biomedicine. In 2011 she obtained a professorship from the Swiss National Science Foundation and started with her own group. Her research focuses on immune responses developing in human colorectal cancer.

Lukas Jeker, born 1975 in Roma, Lesotho, studied medicine at the University of Basel where he obtained the medical Diploma in 2000. His interest in other cultures took him to Paris, Calcutta (medical student) and Baltimore, USA (lab research). He received clinical training in internal medicine in Davos, Liestal and Basel and basic research training as an MD-PhD student in Basel where he received his PhD in 2005 and his MD in 2009. From 2007 to 2010 he was a postdoctoral fellow at the University of California San Francisco, USA before getting promoted to Assistant Professor in 2011. Funded by a Swiss National Science Foundation Professorship he will return to Basel in spring 2014. His research is focused on molecular mechanisms of immune regulation with a particular emphasis on "non-coding" RNAs.

Nina Khanna, born in 1975 in Basel, Switzerland, studied medicine at the University of Basel, where she graduated in 2001. She completed clinical training in Internal Medicine, Infectious Diseases and Hospital Epidemiology at the University Hospital of Basel. She spent her postdoctoral fellowships in Transplant Virology at the Institute of Medical Microbiology, Department Biomedicine, University and University Hospital of Basel from 2006 to 2008 and in Gene- and Immunotherapy at the University Hospital of Würzburg, Germany from 2008 to 2010. She was awarded with an Ambizione-Score Grant in 2012 to study the host immune response to fungi and to improve the treatment using adoptive T-cell therapy.

Mike Recher, born 1975 in Basel, Switzerland, graduated from Medical School in Basel in 2000. From 2001–2005 he was member of the swiss postgraduate course for experimental medicine and immunology research fellow in the lab of Rolf M. Zinkernagel in Zürich. He studied immune responses in murine chronic virus infection models and completed his MD thesis on this topic. From 2005–2009 he did his clinical training (internal medicine and clinical immunology) in Basel and Zürich. 2009–2011, he spent his postdoctoral fellowship in the lab of Luigi D. Notarangelo at the Children's Hospital in Boston where he studied primary immunodeficiencies (PID). Mike Recher leads the PID clinic of the University Hospital Basel. As a SNSF Professor at the DBM he will focus on pathogenesis of autoimmunity in PID.

DBM Focal Area Neurobiology

Focal Area Coordinators

Prof. Dr. B. Bettler
Department of Biomedicine
Institute of Physiology
University of Basel

Prof. Dr. L. Kappos
Department of Biomedicine
University of Basel

Understanding the molecular events underlying diseases of the nervous system and exploiting this knowledge for improving treatment are among the major challenges in the life sciences. In view of the increasing social and financial burden generated by these diseases, especially in the setting of an ageing population, the Department of Biomedicine (DBM) has defined the neurosciences as one of its focal areas.

The Focal Area Neurobiology of the DBM complements parallel efforts at the Biozentrum and at the Friedrich Miescher Institute (FMI) and is part of the Neuroscience Network Basel (NNB), which was (has been) acknowledged as a center of competence by the University of Basel in fall 2008. The NNB follows a translational strategy and comprises more than 400 neuroscientists from 40 different laboratories associated with the University, the University Hospitals, the FMI and the Basel Life Science Industry.

Research is conducted at all levels – from molecules to behavior – thus providing outstanding research opportunities and an excellent platform for a strong educational program. Furthermore, the NNB offers weekly research seminars and lecture series at the graduate and postgraduate levels, covering all aspects of basic and clinical neuroscience. Finally, the NNB is part of the trinational educational and collaborative NEUREX network along with the neuroscience programs at the Universities of Freiburg (Germany) and Strasbourg (France).

A major aim of the Neuroscience groups at the DBM is to take advantage of the unique expertise in the neurosciences present in the Basel area to pursue translational research projects. As a consequence of these efforts, basic and clinical neuroscientists have successfully raised grant support for translational research projects from the Swiss National Science Foundation, the European Union, the Swiss Cancer League, the Swiss MS Society and various private foundations. The focus of these projects is on neuroinflammatory, neurodegenerative, psychiatric, neurological and neuromuscular disorders. Several members of the DBM/NNB are actively involved in the new National Centre of Competence in Research (NCCR) "The Synaptic Bases of Mental Diseases".

To promote the rapid translation of research results into clinical practice the DBM Focal Area Neurobiology co-organizes the Annual Basel Neuroscience Symposium "From Bench to Bedside". The one-day event provides a platform for exchange of ideas and is regularly attended by more than 150 local neuroscientists, including basic and clinical researchers from Novartis, Roche, Actelion, Santhera Pharmaceuticals, the FMI and the University of Basel.

Neonatal Hypoxia Ischemia

Stroke

Neuronal Stem Cells

White Matter Regeneration

Microglia

Neurodevelopmental disorders

Brain Ischemia and Regeneration

Prof. Dr. Raphael Guzman
Department of Biomedicine
and Division of Neurosurgery
University Hospital Basel
University Children's Hospital Basel

Group Members
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Pia Bustos (technician)
Dr. Sally Caine (visiting postdoctoral fellow)
Dr. Laurie Chicha (senior scientist)
Dr. Urs Fisch (MD/PhD student)
Stefan Moser (Master student)
Susanne Viehmann (Master student)

Therapeutic relevance of stem cells for white matter regeneration in neurodevelopmental disorders

Neonatal hypoxic-ischemic (HI) insults represent an important cause of cerebral palsy (CP), leading to devastating sensory-motor, cognitive and learning deficits in the growing child. White matter injury is a hallmark of HI and CP, and defects in myelination are also commonly identified in other neurodevelopmental disorders, including Autism Spectrum Disorders (ASD). White matter myelination generally reflects the progression of functional brain maturation and connectivity in the first years of life, and dysfunction in this crucial process might contribute to the etiology of common symptoms found in CP and ASD. Currently no available therapy targets the long-term consequences of early brain injury, making regenerative medicine a promising area for treatment exploration. Several reports suggest that transplanted neural progenitor cells (NPC) promote CNS tissue repair not merely through cell replacement, but by providing trophic and immunomodulatory support for endogenous repair mechanisms. We have promising preliminary data showing that endovascular injection of human embryonic stem cell (ESC)-derived NPC improve both sensory-motor and cognitive functions in a rodent model of neonatal HI. We observed that NPC treatment specifically stimulates white matter repair mechanisms such as oligodendrocyte progenitor proliferation and maturation with significant increase in myelin basic protein (MBP) expression. Our results also point to a direct interaction between NPC and microglia through NPC-secreted factors in vitro and in vivo, in the healthy animals, as recently published (Mosher et al., Nature Neurosciences 2012).

The objective of our newly formed DBM research team, is to implement those previous studies initiated at Stanford University, and further investigate the cellular and molecular mechanisms underlying human NPC-mediated repair in the context of rodent neonatal HI. As immune cells are known to be home to the CNS at the same time brain development initiates, we are particularly interested in the intermediate role microglia plays in NPC-induced white matter regeneration. Taking the example of another neurodevelopmental disorder, Rett's Syndrome, we are also aiming to specifically decipher the role microglia might play in the ontogeny of this devastating disorder, using induced pluripotent stem cell (iPSC)-based disease modeling approaches. Our group also teamed up with the CNS Discovery Department of Hoffmann-La Roche to evaluate potential cerebrospinal fluid biomarkers with a predictive value for neurodevelopmental impairments.

Besides embryonic and induced pluripotent stem cell culture know-how, our methodologies include powerful imaging techniques such as diffusion tensor imaging (DTI), bioluminescence imaging and synchrotron X-ray fluorescence, as well as behavioral assays, histology and transcriptomics/proteomics. Our laboratory is involved in several collaborations with clinical and research groups in Basel including the Neonatology and Pediatric Neurology Units at UKBB, and F. Hoffmann-La Roche. Active international collaborations also exist with Stanford University, UC San Diego, Duke University Center for in vivo microscopy and the University of Saskatchewan, Canada.

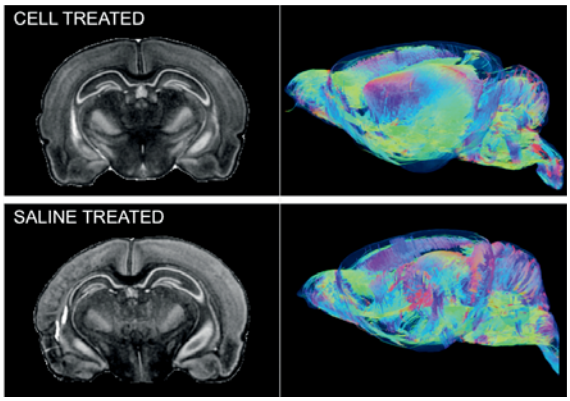

Fig. 1: High resolution magnetic resonance imaging was used to evaluate white matter tract integrity in saline- and NPC-treated rats following neonatal hypoxic-ischemic injury. Depicted are representative images of a cell treated animal (top row) and a saline treated animal (lower row). Diffusion tensor imaging (DTI) indicates that NPC-treated animals had improved white matter tract integrity as measured by higher mean fractional anisotropy (FA).

Connection to Clinical Practice

CSF biomarker assays in developmental brain disorders

Our group has ongoing clinical translational research projects to identify biomarkers related to endogenous neurogenesis and regenerative processes in the developing brain. We here concentrate on cerebrospinal fluid proteomics using different detection modalities.

Selected Publications

- Chicha L, Smith T, Guzman R. Stem cells for brain repair in neonatal hypoxia-ischemia. Childs Nerv Syst. (2014) 30:37-46.
- Lartey FM, Ahn GO, Shen B, Cord KT, Smith T, Chua JY, Rosenblum S, Liu H, James ML, Chernikova S, Lee SW, Pisani LJ, Tirouvanziam R, Chen JW, Palmer TD, Chin FT, Guzman R, Graves EE, Loo BW Jr. PET Imaging of Stroke-Induced Neuroinflammation in Mice Using [(18)F]PBR06. Mol Imaging Biol. (2014) 16:109-117.
- Mosher KI, Andres RH, Fukuhara T, Bieri G, Hasegawa-Moriyama M, He Y, Guzman R, Wyss-Coray T. Neural progenitor cells regulate microglia functions and activity. Nat Neurosci. 19, 1485-1487 (2012).
- Lee SW, Haditsch U, Cord BJ, Guzman R, Kim SJ, Boettcher C, Priller J, Ormerod BK, Palmer TD. Absence of CCL2 is sufficient to restore hippocampal neurogenesis following cranial irradiation. Brain Behav Immun. 30 (2013) 33-44.
- Rosenblum S, Wang N, Smith TN, Pendharkar AV, Chua JY, Birk H, Guzman R. Timing of intra-arterial neural stem cell transplantation after hypoxia-ischemia influences cell engraftment, survival, and differentiation. Stroke. 2012; 43:1624-1631.

Cortical Development
Projection Neurons
Cortical Injuries
Progenitor Cells
Transcriptional Regulator Ski
Cell Cycle Proteins

Cellular Neurobiology

Group left during report period

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Department of Biomedicine
Physiology
University of Basel

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Dr. Carine Bonnon Gaiser (postdoctoral fellow)
Manuela Dittrich (PhD student)
Dr. Alice Grison (postdoctoral fellow)
Lionel Nobs (PhD student)

Molecular mechanisms in neuro-development and neurodegeneration

The central nervous system (CNS) develops from self-renewing, multipotent neural stem cells present in different regions of the embryonic nervous system, where they are regionally and temporally restricted. Moreover, there is increasing evidence that mechanisms of regeneration are distinct from those of development. A central and challenging issue is to identify the extrinsic and intrinsic factors, which control the balance of self-renewal, proliferation, and cell fate decisions in a context-dependent manner. With our projects, we expect to obtain considerable insights into the expression and function of candidate genes controlling proliferation and differentiation of neural stem/progenitor cells during cortical development and following brain injuries.

Cortical development
In the developing dorsal telencephalon, neural stem and progenitor cells generate a large variety of neurons with specific functions in the mature cortex. The proto-oncogene Ski is a transcriptional regulator linked to the human 1p36 deletion syndrome, which involves a set of phenotypes including brain abnormalities (Bonnon and Atanasoski 2012). Ski shows a dynamic expression pattern during cortical development, and accordingly, the phenotype of Ski-deficient cortices is complex, involving altered cell cycle characteristics of neural progenitors, disturbed timing of neurogenesis, and misspecification of projection neurons (Baranek et al. 2012). In particular, Ski-deficient callosal neurons lose their identity and ectopically express the transcription factor Ctip2. The misspecified callosal neurons largely fail to form the corpus callosum and instead redirect their axons towards subcortical targets (Fig. 1). We identify the chromatin-remodeling factor Satb2 as a novel partner of Ski, and show that both proteins are required for transcriptional repression of Ctip2 in callosal neurons. We propose a model in which Satb2 recruits Ski to the Ctip2 locus, and Ski attracts histone deacetylases, thereby enabling the formation of a functional NURD repressor complex (Baranek and Atanasoski 2012). Our findings establish a central role for Ski-Satb2 interactions in regulating transcriptional mechanisms of callosal neuron specification.
Along this line, we tested the role of mTOR signaling during cortical development (Cloetta et al. 2013). We inactivated mTORC1 in mice by deleting the gene encoding raptor in the progenitors of the developing CNS. Brains deficient for raptor show a marked microcephaly. We find that changes in cell cycle length and increased cell death both contribute to the reduction in cell number. Moreover, differentiation of neural progenitors into glia but not into neurons was inhibited. The differentiation defect was paralleled by decreased Stat3 signaling, which is a target of mTORC1 and has been implicated in gliogenesis. Our results show that specific aspects of brain development critically depend on mTORC1 function.

Cortical injuries
Little is known about the molecular mechanisms driving proliferation of glial cells after an insult to the CNS. To test the hypothesis that the G1 regulator cyclin D1 is critical for injury-induced cell division of glial cells, we applied an injury model that causes brain damage within a well-defined region. For this, we injected the neurotoxin ibotenic acid (IBO) into the prefrontal cortex of adult mice, which leads to a local nerve cell loss but does not affect the survival of glial cells. We show that cyclin D1 immunoreativity increases drastically after neurotoxin injection. We find that the cyclin D1-immunopositive (cyclin D1+) cell population within the lesioned area consists to a large extent of Olig2 oligodendrocyte progenitor cells. Analysis of cyclin D1-deficient mice demonstrates that the proliferation rate of Olig2+ cells diminishes upon

loss of cyclin D1 (Fig. 2). Further, we show that cyclin-dependent kinase (cdk) 4, but not cdk6 or cdk2, is essential for driving cell division of Olig2-expressing cells in our injury model. These data suggest that distinct cell cycle proteins regulate proliferation of Olig2+ progenitor cells following a CNS insult (Nobs et al. 2013).

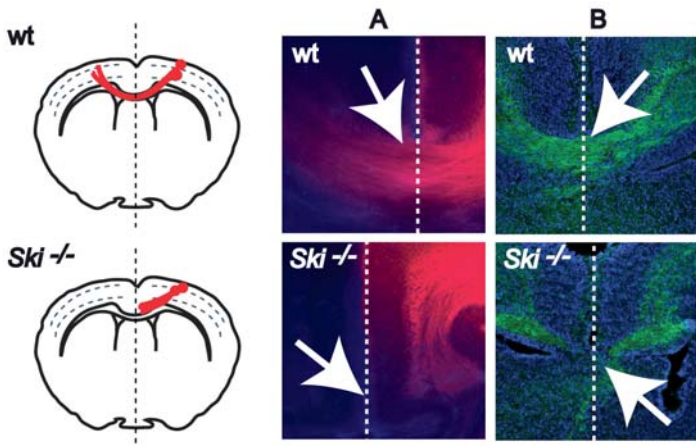

Fig. 1. Ski deletion leads to failure in the formation of the corpus callosum. (A) DiI labeling from the neocortex at E18.5 demonstrates that cortical efferent fibers form the corpus callosum in wt, but not in Ski−/− (arrows). (B) Immunohistochemistry for the axonal marker L1 on E18.5 coronal brain sections depicts axonal projections forming the corpus callosum. In comparison to wt (arrow in upper panel), the population of axons crossing the corpus callosum is completely missing in Ski−/− (arrow in lower panel).

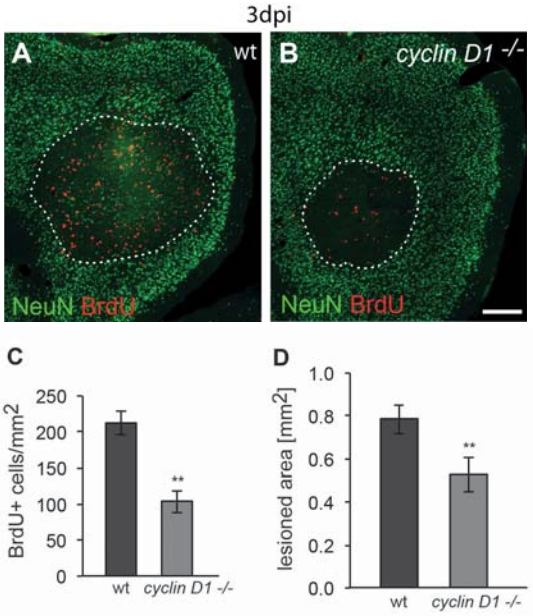

Fig. 2. Loss of cyclin D1 leads to a decrease in cell proliferation and to a reduction in the size of the IBO-induced lesion. (A, B) Double immunostainings for NeuN and BrdU on wt (A) and cyclin D1−/− (B) brain sections 3dpi demonstrate a marked decrease in proliferating cells per area within the lesion site in the mutant, as quantified in (C). Immunostainings for the neuronal marker NeuN on wt (A) and cyclin D1−/− (B) saggital brain sections reveal that the size of the lesioned area (delineated by dotted lines) 3d after injection of the neurotoxin IBO is significantly smaller in the cyclin D1 mutant, as quantified (D). Scale bar = 250 µm. Error bars indicate SEM. ** $P \leq 0.01$, versus wt (Student's t-test).

Selected Publications

- Bonnon C, Atanasoski S. 2012. c-Ski in health and disease. Cell Tissue Res 347:51-64.
- Baranek C, Dittrich M, Parthasarathy S, Bonnon CG, Britanova O, Lanshakov D, Boukhtouche F, Sommer JE, Colmenares C, Tarabykin V, Atanasoski S. 2012. Protooncogene Ski cooperates with the chromatin-remodeling factor Satb2 in specifying callosal neurons. Proc Natl Acad Sci U S A 109:3546-51.
- Baranek C, Atanasoski S. 2012. Modulating epigenetic mechanisms: the diverse functions of Ski during cortical development. Epigenetics 7:676-9.
- Cloetta D, Thomanetz V, Baranek C, Lustenberger RM, Lin S, Oliveri F, Atanasoski S[1], Ruegg MA[1]. 2013. Inactivation of mTORC1 in the developing brain causes microcephaly and affects gliogenesis. J Neurosci 33:7799-810. [1] equal contribution
- Nobs L, Nestel S, Kulik A, Nitsch C, Atanasoski S. 2013. Cyclin D1 is required for proliferation of olig2-expressing progenitor cells in the injured cerebral cortex. Glia 61:1443-55.

Adult Neurogenesis

Hippocampus

Synaptic Transmission

Neuronal Excitability

Dendritic Integration

Calcium Signalling

Cellular Neuro-physiology

Prof. Dr. Josef Bischofberger
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Stefanie Heigele (PhD student)
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Jörg Pohle* (PhD student)
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Martine Schwager (technician)
Dr. Mirko Vukcevic (postdoctoral fellow)

* left during report period

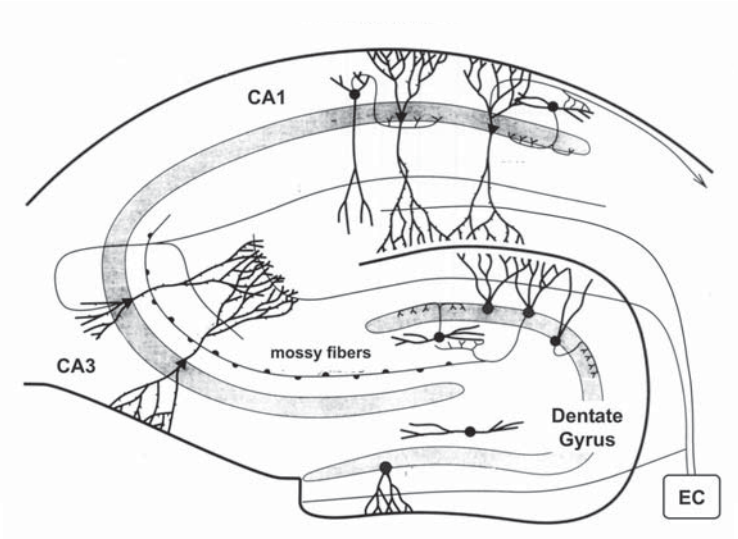
Adult neurogenesis in the the hippocampus

The hippocampal formation within the medial temporal lobe of the cerebral cortex is essential for our conscious memory for facts and events. Remarkably, the hippocampus is one of the very few regions in the CNS of adult mammals, including humans, where new neurons are continuously generated throughout life. This indicates that the new neurons are involved in learning and formation of new memories. In support of this hypothesis, we previously found that newly generated young neurons show enhanced excitability and synaptic plasticity as compared to the neighboring mature cells (Schmidt-Hieber et al. 2004, Nature 429:184-187).

Within the hippocampus neurogenesis is restricted to granule cells in the dentate gyrus (Figure 1). They receive excitatory inputs from the entorhinal cortex and project to the CA3 pyramidal cells. The dentate gyrus has some distinct structural features and is believed to serve distinct functions during memory processing. First of all, the granule cells form a so called competitive network as there is strong mutual inhibition via inhibitory GABAergic interneurons. By contrast, the CA3 pyramidal cells form an autoassociative network via mutually excitatory synaptic connections (Figure 1). Second, the number of granule cells appears to be ~5-times larger than the number of afferent entorhinal layer II principal cells and ~3-times larger than the number of CA3 pyramidal cells in the output region. This form of expansion recoding within a competitive network will generate a sparse and orthogonal (non-overlapping) representation, which will help to separate similar neuronal activity patterns – a function called 'pattern separation'. As a consequence, each memory item can be stored within the hippocampal network in a unique fashion. Finally, new granule cells can be generated throughout life from adult neural stem cells located in the subgranular zone of the dentate gyrus (Figure 2). Proliferation and differentiation of adult neural stem cells is tightly regulated in an activity dependent manner, indicating that the number of neurons might be adjusted to maintain sparse coding even with increasing memory load.

During the last three years we have focused on the process of synapse formation and synaptic integration of developing newly generated granule cells into the hippocampal circuitry. As extrasynaptic NMDA receptors are believed to support the generation of new spines, we have studied the functional properties of extrasynaptic ionotropic glutamate receptors in newly generated granule cells during and after synaptic integration (Schmidt-Salzmann et al. 2014). Using fast application of glutamate to outside-out membrane patches, we showed that all immature granule cells express already functional AMPA and NMDA receptors. The density of AMPA receptors was small in cells starting to receive excitatory synaptic input (~30 pS/μm^2) but substantially increased during synaptic integration to finally reach ~120 pS/μm^2 in fully mature cells. Interestingly, AMPA receptors showed a biphasic change in desensitisation time constant which was slowest during synaptic integration and substantially faster before and afterwards. This was paralleled by a biphasic change in the non-desensitising current component which was maximal during synaptic integration and about two times smaller in fully mature granule cells. Surprisingly, the NMDA-receptor density in young cells was already similar to mature cells (~10 pS/μm^2) and remained relatively constant throughout maturation. Also, functional properties of extrasynaptic NMDA-receptors were similar at different developmental stages. These data indicate that the non-desensitising AMPAR currents in newly generated young granule cells might support the effective activation of extrasynaptic NMDA receptors to induce Ca^{2+} influx and Ca^{2+}-dependent activation of Rho-GTPases important for new spine formation. Together with the previously described low Ca^{2+}-buffer capacity in young cells (Stocca, Schmidt-Hieber, Bischofberger, 2008 J Physiol 586:3795-3811), the large AMPA-currents might constitute a competitive advantage over mature cells for new synapse formation.

Fig. 1



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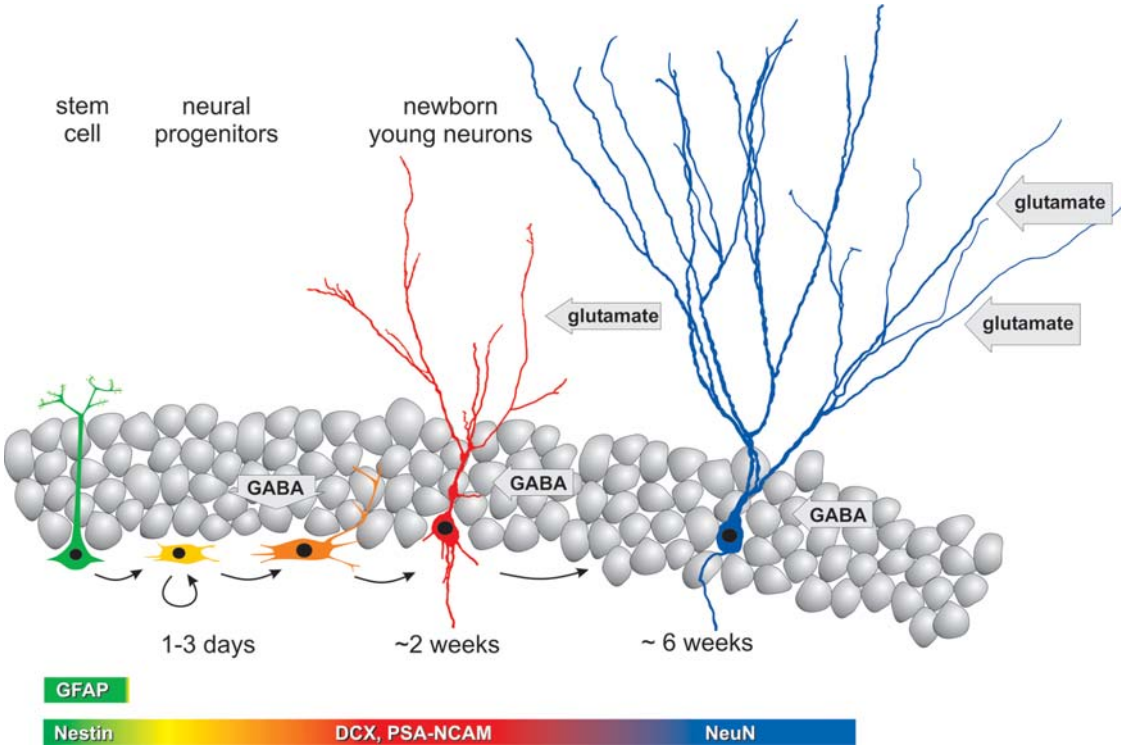
- Lepski G, Maciaczyk J, Jannes CE, Maciaczyk D, Bischofberger J, Nikkhah G (2011) Delayed functional maturation of human neuronal progenitor cells in vitro. Mol Cell Neurosci *47*:36-44.
- Herpfer I, Hezel H, Reichardt W, Clark K, Geiger J, Gross CM, Heyer A, Neagu V, Bhatia H, Atas HC, Fiebich BL, Bischofberger J, Haas CA, Lieb K, Normann C. (2012) Early life stress differentially modulates distinct forms of brain plasticity in young and adult mice. PLoS One *7*(10):e46004.
- Lepski G, Jannes CE, Nikkhah G and Bischofberger J (2013) cAMP promotes the differentiation of neural progenitor cells in vitro via modulation of voltage-gated calcium channels. Frontiers Cell Neurosci *7*:155.
- Schmidt-Salzmann C, Li L, Bischofberger J (2014) Functional properties of extrasynaptic AMPA and NMDA receptors during postnatal hippocampal neurogenesis. J Physiol 592.1 (2014) pp 125-140.
- Pohle J, Bischofberger J (2013) Young pyramidal cells generate large and supralinear Ca^{2+} signals via activity-dependent slow-down of extrusion. submitted

Fig. 2



Multiple Sclerosis
MicroRNA
Treatment Response
Immunomodulation
Prognostic Markers
Autoreactive B Cells

Clinical Neuro-immunology

Prof. Dr.
Raija LP Lindberg

Prof. Dr.
Tobias Derfuss

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and Division of Neurology
University Hospital Basel

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Dr. Anne-Katrin Pröbstel (postdoctoral fellow)
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Dr. Nicholas Sanderson (postdoctoral fellow)
Dr. Claudia Sievers (postdoctoral fellow)
Hedwig Wariwoda (technician)
Maria Zimmermann (PhD student)

* left during report period

Molecular and immunological analysis of multiple sclerosis

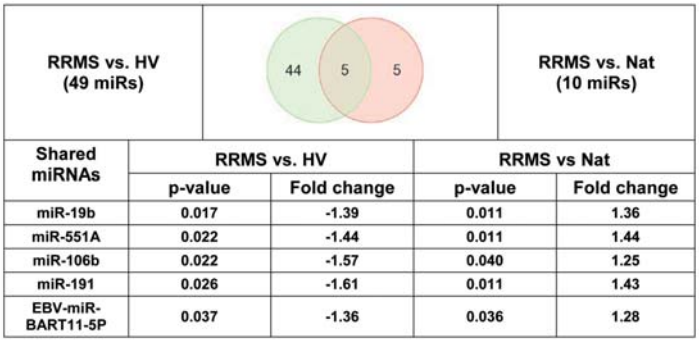
Our research focuses on the molecular and immunological analysis of multiple sclerosis (MS), an inflammatory, demyelinating central nervous system (CNS) disease. We have two main research lines: 1) genomic investigations (including genetic, transcriptional and protein expression analysis) and 2) studies on B cell involvement in MS pathogenesis. Both approaches provide tools for immunomonitoring of current and newly emerging treatments.

Immune regulation by microRNAs in MS
MicroRNAs (miRNAs) are small, endogenous noncoding RNAs, which are key regulators of many biological processes, e.g. cell proliferation, differentiation, apoptosis, signal transduction and organ development. Our miRNA expression profiling analysis of peripheral blood lymphocytes in relapsing-remitting (RR) MS patients revealed distinct miRNA patterns in CD4, CD8 and B cells. MiR-17, involved in autoimmunity, was up-regulated in CD4 cells from MS patients. This correlated with alterations in the expression of potential target genes of miR-17, i.e. PTEN and phosphatidyl-inositol-3-kinase regulatory unit 1 (PI3KR1), which were down-regulated upon stimulation of CD4 cells with antiCD3/CD28 in vitro. Functional experiments with a synthetic inhibitor of miR-17 supported the link between miRNA expression and the altered target gene expression. The more detailed analysis of B cells revealed a distinct set of 49 deregulated miRNAs in MS, including members of the miR-17-92 and the miR-106b-25 clusters. In addition, miR-181a, involved in B-cell development, was down regulated in untreated patients. Natalizumab, an approved treatment for RRMS, reverted selectively the expression of deregulated miRNAs both in T and B cells (Sievers et al., 2012) (Figure 1). We have expanded our studies to extracellular miRNAs in serum and CSF. We aim to define cellular and/or extracellular miRNAs as prognostic indicators for disease activity and treatment response.

B cells and their targets in MS
During recent years it has become clear that B-cells have a major role in the pathogenesis of MS. Depletion of B-cells leads to a remarkable amelioration of the disease whereas selective modulation of the B-cell response with a blocker of the TACI receptor induces worsening of MS. The mechanisms by which B-cells impact MS are incompletely understood. Our research focuses on the identification of novel B-cell autoantigens and the characterization of the interaction of autoaggressive B-cells with the CNS. We could show that antibodies against native myelin oligodendrocyte glycoprotein (MOG) identify a subset of pediatric patients with autoimmune CNS demyelination (Figure 2) (Pröbstel AK, 2011). Using an unbiased proteomic approach we identified potential novel autoantigens like neurofascin and contactin-2 that point to a critical role of the node of Ranvier for the demyelination process. Cells transfected with potential autoantigens and co-cultures of B-cells with antigen expressing cells or cerebellar slice cultures are currently used to identify patients with an autoaggressive B-cell response. This novel approach will shed light on the role of B-cells in the pathogenesis of MS and also provides means to identify autoantigens in their natural environment.

Immunomonitoring of new treatments and biomarker research
We aim to get a better understanding of altered immunological pathways in MS pathogenesis and to provide new insights into the mode of action of currently available and newly developed treatments. Genomic alterations e.g. deregulated miRNAs are evaluated as biomarkers for monitoring the efficacy of approved and experimental therapies. We are using a broad set of

immunological read-outs to monitor the immune status during novel immunomodulatory treatments (Ricklin ME, 2013). To develop biomarkers for neurodegeneration we established the sensitive detection of CSF neurofilament heavy chain (NfH), a degeneration product of CNS neurons (Kuhle J, 2011). Combination of these biomarkers with a standardized clinical and neuroradiologic assessment provides a comprehensive description of the disease in individual patients that can be used to predict future disease course and treatment.



Shared miRNAs	RRMS vs. HV		RRMS vs Nat	
	p-value	Fold change	p-value	Fold change
miR-19b	0.017	-1.39	0.011	1.36
miR-551A	0.022	-1.44	0.011	1.44
miR-106b	0.022	-1.57	0.040	1.25
miR-191	0.026	-1.61	0.011	1.43
EBV-miR-BART11-5P	0.037	-1.36	0.036	1.28

Fig. 1: Differentially expressed miRNAs in B lymphocytes of RRMS patients compared with those of healthy volunteers (HV) (light blue circle) and in untreated RRMS patients compared with natalizumab (Nat) treated patients (red circle), depicted with Venn diagram to illustrate group specific and overlapping, shared miRNAs (greenish area).

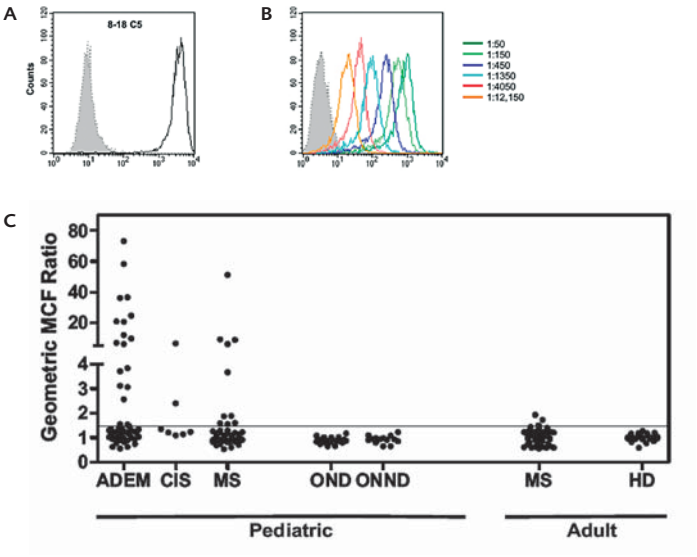


Fig. 2: Features of the autoantibody response against native conformational MOG. (A) Fluorescence intensity of the MOG transfected cell line (TE 671) is shown by staining the cells with the mAb anti-MOG 8-18C5 antibody. (B) Dilution of an ADEM serum shows anti-MOG antibodies even at the highest dilution. (C) Sera from childhood demyelination patients, pediatric controls, adult MS patients and adult healthy donors were analyzed by FACS with a MOG transfected cell line.

Connection to Clinical Practice

Our Clinical Neuroimmunology Laboratory is closely connected to the MS Outpatient Clinic of the Department of Neurology, University Hospital Basel that cares for more than 1000 MS patients per year. This allows access to a unique population of MS patients in different stages of the disease. There is also a close collaboration with the Division of Neuroradiology and the Medical Image Analysis Centre (MIAC) that enables characterization of patients with cutting edge neuroimaging techniques. This research is directed by Prof Till Spenger, who has a joint appointment at the Departments of Neurology and Radiology. Our Clinical MS Research Group plays a key role in organizing and conducting a series of international therapeutic studies in MS, e.g. with fingolimod, siponimod, MT-1303, fumaric acid, teriflunomide, the humanized monoclonal antibodies ocrelizumab (Kappos L, 2011), daclizumab, and GNbAC1. These trials provide unique possibilities to apply basic research approaches to understand disease mechanisms and therapeutic responses.
Development of biomarkers needs prospective, standardized, and high-quality clinical and neuroradiological data from large patient cohorts to allow for validation and implementation in clinical practice. The Swiss MS Cohort Study (SMSC), supported by the Swiss MS Society and coordinated by our MS Group was initiated in 2012. It aims at building and maintaining a long-term cohort of Swiss MS patients with follow-up clinical and MRI data as well as sampling of body fluids.

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- Pröbstel AK, Dornmair K., Bittner R, Sperl P, Jenne D, Magalhaes S, Villalobos A, Breithaupt C, Weissert R, Jacob U, Krumbholz M, Kuempfel T, Blaschek A, Stark W, Gärtner J, Pohl D, Rostasy K, Weber F, Forne I, Khademi M, Olsson T, Brilot F, Tantsis E, Dale RC, Wekerle H, Hohlfeld R, Banwell B, Bar-Or A, Meinl E, Derfuss T (2011). Antibodies to MOG are transient in childhood acute disseminated encephalomyelitis. Neurology *77*, 580-588.
- Ricklin ME, Lorscheider J, Waschbisch A, Paroz C, Mehta SK, Pierson DL, Kuhle J, Fischer-Barnicol B, Sprenger T, Lindberg RL, Kappos L, Derfuss T. (2013). T-cell response against varicella-zoster virus in fingolimod-treated MS patients. Neurology *81*, 174-181.
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- Kappos L, Li D, Calabresi PA, O'Connor P, Bar-Or A, Barkhof F, Yin M, Leppert D, Glanzman R, Tinbergen J, Hauser SL (2011). Ocrelizumab in relapsing-remitting multiple sclerosis: a phase 2, randomised, placebo-controlled, multicentre trial. Lancet *378*, 1779-1787.

Cerebellar Purkinje Cells
Dendritic Development
Glutamate Receptors
Voltage-gated Calcium Channels
Blood-Brain-Barrier
Organotypic Slice Cultures

Developmental Neurobiology and Regeneration

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The control of Purkinje cell dendritic development and plasticity of the vasculature in central nervous system (CNS) slice cultures

The shape of the dendritic tree of a neuron reflects its synaptic input because most synapses are located on the dendritic surface. Our group is interested in how functional activity does affect the growth and shape of the dendritic tree of cerebellar Purkinje cells, the principal cells of the cerebellar cortex. We take advantage of a special culture system which allows growing a thin cerebellar slice in a culture dish. In such cultured slices the dendritic development of Purkinje cells proceeds in a way very similar to the in vivo situation and results in Purkinje cells with a typical dendritic tree (Fig. 1) which has grown entirely in the culture dish. At the same time the culture setup allows for simple experimental manipulation of the system. The factors and molecules controlling growth and patterning of neuronal dendrites are not yet well understood. We have previously shown that the activity of metabotropic glutamate receptor 1 (mGluR1) or protein kinase C gamma (PKC) in organotypic cerebellar slice cultures of postnatal mice mediate the growth and development of the Purkinje cell dendritic tree. When we stimulated metabotropic glutamate receptors (but not other types of glutamate receptors) the dendritic development of Purkinje cells was severely inhibited and the resulting dendritic tree was much reduced in size and complexity. This inhibition of dendritic growth via activation of metabotropic glutamate receptors could be part of a negative feedback loop which limits the number of excitatory synaptic connections in Purkinje cells. We have now searched for potential signalling mechanisms limiting Purkinje cell dendritic growth and have concentrated on channels allowing the entry of Ca^{2+} ions. Using a combination of pharmacological blockade and genetically modified mice we have shown that two variants of voltage gated Ca^{2+} channels, the P/Q-type and T-type Ca^{2+} channels, are involved in the inhibition of dendritic growth seen after increased mGluR1 or PKC activity in cerebellar slice cultures. As shown in Fig. 2, pharmacological blockade of both P/Q-type and T-type Ca^{2+} channels results in a partial rescue of the Purkinje cell dendritic tree after mGluR1 or PKC stimulation. Our findings imply that Ca^{2+} entry through voltage-gated Ca^{2+} channels is crucially involved in the inhibitory effects on dendritic growth. In contrast, genetic absence or acute blockade of another type of Ca^{2+} channels, the TRPC3 channels, had no effect. At the moment our group is exploring whether further molecules involved in maintaining the Ca^{2+} equilibrium in Purkinje cells are also involved in the regulation of dendritic growth.

In a second line of research we are using the slice culture model for research on the blood brain barrier and the plasticity of the vasculature in the central nervous system. Transient ischemia causes delayed neurodegeneration in selective brain areas, particularly in the CA1 field of the hippocampus. This is accompanied by neurovascular impairment. It is unknown whether neurodegeneration is the cause or the consequence of vascular changes. In an entorhino-hippocampal organotypic slice culture system with well-preserved blood vessels we have studied the interplay between neurodegeneration and integrity of the neurovasculature. Short-term oxygen and glucose deprivation (OGD) resulted in up-regulation of hypoxic markers and with a delay of 24 to 48h in selective nerve cell death in CA1. In parallel, local vessel density decreased exclusively in the CA1 area affected by neuronal death (Fig. 3). The blood-brain barrier in this region was impaired as evidenced by reduction in the expression of the tight junction protein claudin-5. Pre-

venting neuronal death with tetrodotoxin or the AMPA receptor blocker CNQX rescued both neurons and blood vessels, suggesting that vessel loss is not due to OGD per se but a consequence of neuronal death. The mechanisms by which death of pyramidal neurons mediate vessel loss are not known. Our findings lay the groundwork for further study of the biological crosstalk between pyramidal neurons and the vasculature and for the role of AMPARs in ischemia-induced neuronal death.

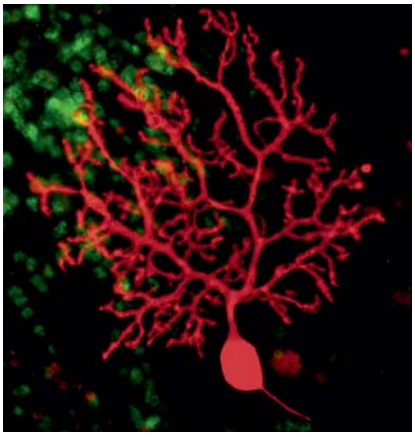

Fig. 1: View of a Purkinje cell in an organotypic slice culture after 12d in vitro. Anti- calbindin staining for Purkinje cells is shown in red. The elaborate dendritic tree of this cell has developed almost entirely during the culture period.

Selected Publications

- Chip, S., Nitsch, C., Wellmann, S., Kapfhammer J.P. (2013) Subfield-specific neurovascular remodeling in the entorhino-hippocampal-organotypic slice culture as a response to oxygen-glucose deprivation and excitotoxic cell death. J Cereb Blood Flow Metab. 33, 508-518.
- Gugger, O.S., Hartmann, J., Birnbaumer, L., Kapfhammer, J.P. (2012) P/Q-type and T-type calcium channels, but not type 3 transient receptor potential cation channels, are involved in inhibition of dendritic growth after chronic metabotropic glutamate receptor type 1 and protein kinase C activation in cerebellar Purkinje cells. Eur J Neurosci. 35, 20-33.
- Kapfhammer, J.P., and Gugger, O. S. (2012). The analysis of Purkinje cell dendritic morphology in organotypic slice cultures. J. Vis. Exp. (61), e3637, DOI 10.3791/3637.
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- Gugger, O.S., Kapfhammer, J.P. (2010) Reduced size of the dendritic tree does not protect Purkinje cells from excitotoxic death. J Neurosci Res 88, 774-783.

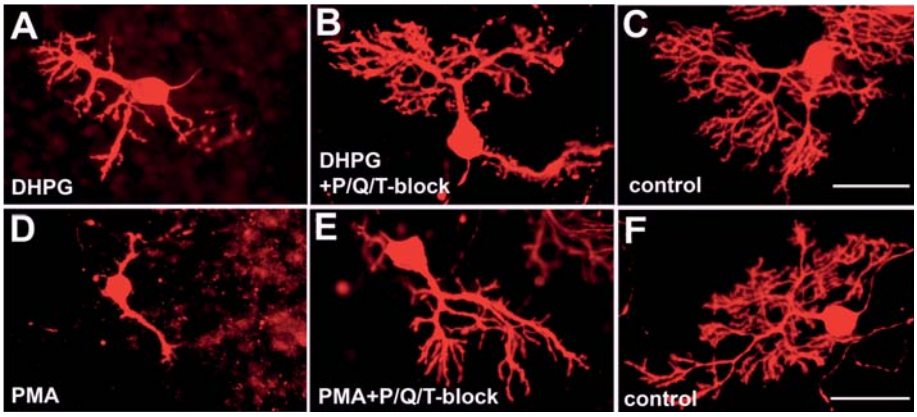

Fig. 2: Development of the Purkinje cell dendritic tree is severely impaired after stimulation of either mGluR1 or PKCγ activity (A and D, compare to control Purkinje cells in C and F). Pharmacological blockade of P/Q and T-type Ca^{2+} channels provides a partial rescue of the Purkinje cell dendritic tree (B and E) indicating that Ca^{2+} influx through these channels is required for the inhibition of dendritic growth. Modified from Gugger and Kapfhammer 2012.

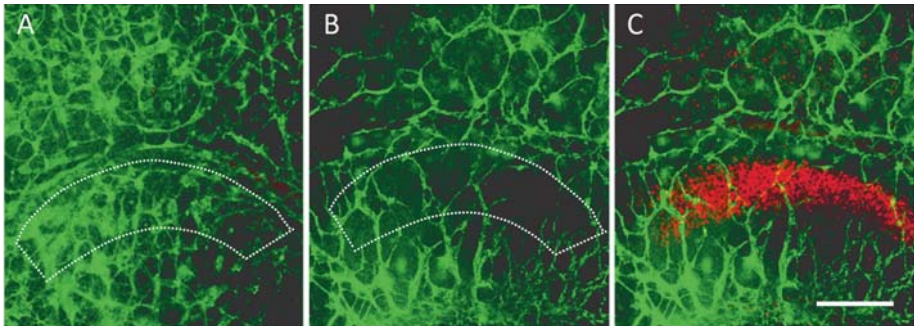

Fig. 3: Oxygen-glucose deprivation of entorhino-hippocampal slice cultures induces neuronal death specifically in the CA1 area of the hippocampus shown with propidium iodide staining (red cells in C). The blood vessels are shown by immunostaining for claudin 5, a blood-brain-barrier marker present on cerebral blood vessels. In untreated control cultures with intact neurons, blood vessels are present throughout the hippocampus (A). After oxygen-glucose deprivation, blood vessels are only lost in the area of neuronal cell death (B and C). Modified from Chip et al. 2013.

Synapse Formation

Neuromuscular Junction

Developmental Neurobiology

Muscle

Agrin

Molecular Neurobiology Synapse Formation

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Signaling mechanisms in synapse formation

Synapses are specialized points of contact for signal transmission between neurons and their follower cells. Their formation requires the coordinated formation of a nerve terminal and of a postsynaptic membrane rich in neurotransmitter receptors. At the neuromuscular junction (NMJ), the best known signals secreted from motor neurons and regulating the expression of acetylcholine receptors (AChRs) in the synaptic muscle membrane are agrin and neuregulin1 (NRG). However, little is known of the mechanisms involved. We have recently elucidated two relevant mechanisms.

Agrin acting through MuSK in the muscle is the major presynaptic organizer of postsynaptic differentiation; it is on its own sufficient to induce differentiation of functional synaptic membranes in the absence of motor nerves.
Neuregulin acts through its receptors, the RTKs ErbB2, -3 and -4. Unlike believed previously, it does not mediate the neural control of synapse-specific gene transcription such as the AChR genes. Nevertheless, the density of the AChRs at the synaptic membrane was reduced in mice lacking NRG/ErbB signaling.

In principle, synaptic AChR density can be regulated through AChR insertion into or increased removal from the synaptic membrane. Comparison of AChR removal from the synaptic membrane of NMJs in wild type mice and in mice lacking neuromuscular NRG/ErbB signaling showed that in the latter, anchoring of AChRs in the postsynaptic muscle membrane was destabilized, resulting in faster migration of AChRs from the synaptic to the perisynaptic membrane. Specifically, in mice in which NRG signaling to muscle was genetically or pharmacologically abolished, postsynaptic AChRs moved rapidly from the synaptic to the perisynaptic membrane, and the subsynaptic scaffold that anchors the AChRs was impaired. These defects combined compromised synaptic transmission at NMJs in vivo. Blockade of NRG/ErbB signaling in cultured myotubes abolished phosphorylation selectively of α-dystrobrevin1, but not of other components of the subsynaptic apparatus. Thus, NRG/ErbB signaling maintains high efficacy of synaptic transmission by stabilizing the postsynaptic apparatus via phosphorylation of α-dystrobrevin1.

So far, the only mechanism for agrin to regulate synaptic clustering of AChRs was thought to be by organizing a postsynaptic apparatus, a complex of proteins at the synapse trapping the AChRs and anchoring them to the actin cytoskeleton of the muscle fiber. We now found that agrin regulates AChR clustering also through organizing the focal delivery of AChRs into the synaptic membrane. This occurs via a network of microtubules focused with their plus ends to the postsynaptic membrane. At NMJs in vivo, this mechanism accounts for about 30% of the normal synaptic AChR density. Agrin leads to capturing of microtubules to the synaptic membrane, thus enabling AChR focal delivery to the synapse. MT capturing is regulated through local activation of PI3-K. This has two effects: 1) it phosphorylates (and thus inactivates) GSK3b at the synaptic membrane, which renders the plus end protein CLASP2 unphosphorylated locally at the synapse, thus increasing its affinity to MT plus ends and their ability to interact with the synaptic membrane; CLASP2-dependent capturing at the postsynaptic membrane is through interaction with LL5b, a phosphatidylinositol-3,4,5-triphosphate (PIP3) binding protein, and 2) LL5b itself is recruited to the synapse through its binding to PIP3.
In vivo, genetic deletion of CLASP2 or knock-down of LL5b by RNAi reduced the rate of AChR insertion and synaptic AChR density by about 30% each. Our experiments thus reveal a novel mechanism for agrin to cluster AChRs at the NMJ, i.e. through the organization of a subsynaptic MT network for focal AChR delivery to the synapse.

Selected Publications

- Schmidt, N., Akaaboune, M., Gajendran, N., Martinez-Pena y Valenzuela, I., Wakefield, S., Thurnheer, R., Brenner, H.R. (2011). Neuregulin/ErbB regulates acetylcholine receptors at the neuromuscular junction by phosphorylation of α-dystrobrevin. J. Cell Biol. 195, 1171-1184.
- Schmidt, N., Basu, S., Sladecek, S., Gatti, S., van Haren, J., Treves, S., Pielage, J., Galjart, N., Brenner, H.R. (2012). Agrin regulates CLASP2-mediated capture of microtubules at the neuromuscular junction synaptic membrane. J. Cell Biol. 198, 421-437.
- Basu, S., Sladecek, S., Martinez de la Peña y Valenzuela., I., Akaaboune, M., Smal, I., Pembley, H., Wittmann, T., Galjart N., Brenner, H.R. (2013). CLASP2/LL5β dependent microtubule capturing controls acetylcholine receptor delivery and density at the synaptic membrane of the adult neuromuscular junction, submitted.

G-protein Coupled Receptors

GABA-B

mGlu5

Trace Amine

Dopamine Receptors

Molecular Neurobiology Synaptic Plasticity

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Regulation of neuronal functions by auxiliary subunits of G-protein coupled receptors

We are interested in the mechanisms that control neuronal excitability, and to exploit these mechanisms for the treatment of neurological and psychiatric diseases. We are giving emphasis to the control of neuronal excitability by G-protein coupled receptors (GPCRs), in particular $GABA_B$ receptors, mGlu5 receptors, dopamine receptors and Trace Amine-Associated Receptor 1 (TAAR1).

$GABA_B$ receptors

$GABA_B$ receptors are the GPCRs for the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Their activity influences many neural systems and behavioral states (Gassmann and Bettler, 2012). $GABA_B$ receptors have been implicated in a variety of neurological and psychiatric conditions, including epilepsy, anxiety, depression, schizophrenia, obsessive compulsive disorder, addiction and pain. Despite the involvement of $GABA_B$ receptors in mental health disorders, the clinical use of $GABA_B$ receptor agonists is currently limited to the treatment of narcolepsy, neuropathic pain, spasticity and dystonia. One reason for this is that the main therapeutic effect of baclofen – the prototypical $GABA_B$ receptor agonist in clinical use – has unwanted side effects for mental health indications.

A large body of work suggests that native $GABA_B$ receptors vary in their kinetic and pharmacological properties. The origin of this variation is unclear. To some extent, it may be explained by the existence of associated proteins that alter receptor properties. In collaboration with B. Fakler (University Freiburg iBr) we affinity-purified native $GABA_B$ receptor complexes and identified their constituents using tandem mass-spectrometry. We found that $GABA_B$ receptors not only comprise principal $GABA_{B1a}$, $GABA_{B1b}$ and $GABA_{B2}$ subunits (Fig. 1A,C) but also auxiliary KCTD8, 12, 12b and 16 subunits (Fig. 1B,C). The KCTDs seem to be the missing components that confer fast activation kinetics, variation in the desensitization kinetics and distinct agonist potencies to native $GABA_B$ receptor responses. In the presence of KCTD12, $GABA_B$ receptor activation elicits a strongly desensitizing response (Fig. 1C). By contrast, in the presence of KCTD16, the activated receptors induce largely non-desensitizing responses (Fig. 1C). We found that distinct KCTD protein domains promote and inhibit receptor-mediated desensitization (Seddik et al., 2012). These differential effects, together with the distinct spatial and temporal KCTD distribution patterns (Metz et al., 2011), support the view that KCTDs contribute to the variation in native $GABA_B$ receptor responses (Ivankova et al., 2013). The discovery that KCTDs confer subtype-specificity on $GABA_B$ receptors presents opportunities for drug discovery. Indeed, drugs that target individual receptor subtypes would allow more-specific therapeutic interference with $GABA_B$ receptor signaling. The advantages of such drugs could include a reduction in side effects as well as entirely new therapeutic applications. To support drug discovery we are analyzing the mechanism of action of the KCTD proteins. Moreover, we are using a combination of knock-down and overexpression strategies to analyze whether $GABA_B$ receptor-associated proteins other than the KCTDs influence receptor distribution, neuronal processes and higher brain functions.

mGlu5 receptors

In collaboration with L. Lindemann (Roche, Basel) we have identified novel mGlu5 receptor-associated proteins. We are currently characterizing the newly identified proteins for their effects on mGlu5 receptor functions in vitro and in vivo.

Dopamine receptors

In collaboration with B. Fakler (University Freiburg iBr.) and C. Lüscher we have been awarded a Sinergia grant from the Swiss National Science Foundation to identify dopamine receptor-associated proteins. We are analyzing several receptor-associated proteins for their effects on dopamine receptor functions in vitro and in vivo.

Trace amine receptor 1

In collaboration with M. Höhner (Roche, Basel) we have found a cross-talk between TAAR1 and dopamine receptors (Revel et al., 2011). We are currently studying the underlying mechanism.

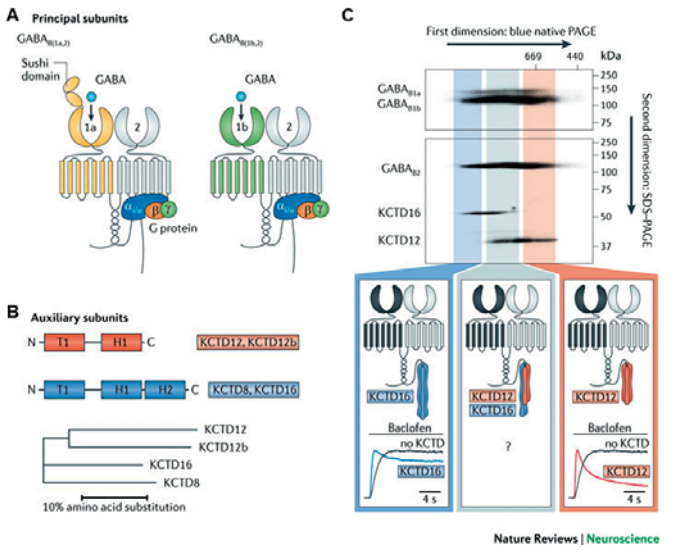

Fig. 1: $GABA_B$ receptor subunit composition. **A** | The principal subunits of $GABA_B$ receptors – $GABA_{B1a}$, $GABA_{B1b}$ and $GABA_{B2}$ – have the prototypical seven transmembrane domains of G protein-coupled receptors and form two distinct core units: $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$. $GABA_{B1a}$ and $GABA_{B1b}$ are subunit isoforms that differ by the presence of two amino-terminal sushi domains in $GABA_{B1a}$. Whereas these subunits contain the GABA binding site, $GABA_{B2}$ subunits couple to the G protein. **B** | The principal subunits associate with the sequence-related auxiliary subunits KCTD8, KCTD12, KCTD12b or KCTD16, which have a modular structure and feature a conserved tetramerization T1 domain as well as one or two carboxy-terminal 'homology' domains (H1 and H2). The T1 domains form homotetramers that bind to $GABA_{B2}$. The phylogenetic tree depicts the evolutionary relationships among the KCTDs. The branch length represents the percentage of amino acid substitutions between proteins. **C** | Biochemical experiments demonstrated the association of principal with auxiliary $GABA_B$ receptor subunits. The panel shows solubilized native $GABA_B$ receptor complexes from rat brain that were size-fractionated on non-denaturing blue native PAGE and SDS–PAGE. Receptor subunits were detected with specific antibodies. KCTD16 was associated with high molecular weight receptor complexes, whereas KCTD12 was associated with low molecular weight complexes (molecular weights are indicated in kDa). These findings demonstrate the existence of $GABA_B$ receptor subtypes that contain particular KCTD subunits. Medium molecular weight complexes possibly incorporate a mix of KCTD12 and KCTD16. The presence of KCTDs accelerates the rise-time of the $GABA_B$ response, which is shown here by baclofen-induced G protein-activated inwardly rectifying potassium channel (GIRK) currents in transfected Chinese hamster ovary cells. The presence of KCTD12 or KCTD12b (not shown) confers a marked desensitization on the $GABA_B$ response. It is unknown whether simultaneous incorporation of KCTD12 and KCTD16 into the same receptor complex produces receptor responses with intermediate desensitization kinetics (Gassmann & Bettler, Nature Reviews Neuroscience, 2012).

Connection to Clinical Practice

Prof. Dr. Markus Heim, Prof. Dr. Adrian Merlo
Gastroenterology and Hepatology, Neurosurgery

Constitutive Notch2 signaling in hepatic tumors and neural stem cells

Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCC) are the most common liver tumors and a leading cause for cancer-related death in men. Notch2 regulates cellular differentiation in the liver. Notch signaling is implicated in various cancers, but it is unclear whether Notch2 contributes to HCC and CCC formation. We generated mice that ectopically express activated Notch2 in the liver. In collaboration with M. Heim we found that liver-specific expression of Notch2 is sufficient to induce HCC formation and biliary hyperplasia. Using the diethylnitrosamine (DEN) HCC carcinogenesis model, we further showed that Notch2 signaling accelerates DEN-induced HCC formation (Dill et al., 2013). DEN-induced HCCs with constitutive Notch2 signaling exhibit a marked increase in size, and proliferation when compared with HCCs from DEN-induced control mice. Additionally, DEN treated mice constitutively expressing Notch2 eventually develop CCC. Our data establish an oncogenic role for constitutive Notch2 signaling in liver cancer development. In collaboration with A. Merlo (Neurosurgery) and B. Hemmings (FMI Basel) we found that constitutive Notch2 signaling in neural stem cells promotes tumorigenic features and astroglial lineage entry in mice (Tchorz et al., 2012).

Selected Publications

- Dill, M.T., Tornillo, L., Fritzius, T., Terracciano, L., Semela, D., Bettler, B.*, Heim M.H.*, and Tchorz, J.S*. (2013). Constitutive Notch2 signaling induces hepatic tumors in mice. Hepatology 57(4), 1607-1619. *Corresponding authors.
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Myelin Biology

Axon-Glia Interaction

Membrane Domains and Trafficking

Multiple Sclerosis

Peripheral Neuropathy

Neuroprotection

Neurobiology

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Molecular mechanisms of myelin formation and maintenance in health and disease

Generation of functional myelinated nerves requires a reciprocal communication between the myelinating cells and their associated axons. Myelination is established by highly specialized glial cells, oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system, that wrap axons with a multilayered myelin membrane for rapid impulse conduction. In addition, axonal signals regulate the survival, migration and differentiation of Schwann cells as well as the myelination process. We are using basic as well as clinical approaches for investigating the complex nature of the myelin membrane during myelination and in demyelinating diseases such as **multiple sclerosis (MS)** and **primary demyelinating peripheral neuropathies.** Our current projects in the lab involve the characterization of the functional role of the myelin proteins MAL, PMP22 and MAG in axon-glia interaction in health and disease, and the endogenous neuroprotective mechanisms in MS. The knowledge of the selective function of the different components of the complex myelin structure is a prerequisite to understand the different mechanisms, which may damage myelin in MS and in primary demyelinating neuropathies leading to axonal degeneration.

The **myelin and lymphocyte protein MAL** is a component of lipid rafts, and is important for targeting proteins and lipids to distinct myelin domains. MAL overexpression impedes peripheral myelinogenesis evident by a delayed onset of myelination and reduced expression of the myelin protein zero (MPZ/P0) and low affinity neurotrophin receptor p75NTR (Buser et al., 2009). We investigated the molecular mechanisms of MAL-overexpression on Schwann cell differentiation in more detail in primary mouse Schwann cell cultures that resemble the *in vivo* observation to a large extend. Since the reduced expression of MPZ and p75NTR was already determined before Schwann cell differentiation, the effect of MAL might be implicated during early developmental stages. Their transcription was robustly reduced, despite the fact that most transcription factors and receptors important for Schwann cell differentiation were not affected by MAL overexpression. In addition, the induction of the CREB and PI3-kinase signaling pathways was functional, highlighting that other rate limiting factors do exist. We identified a number of genes implicated in the cytoskeleton organization and plasma

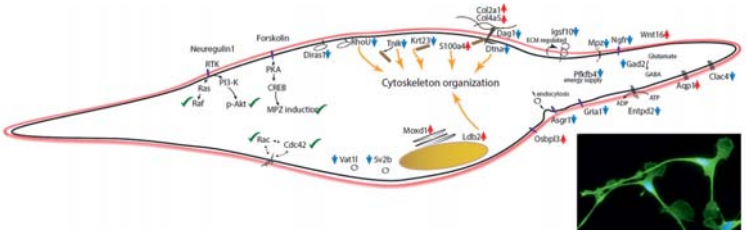

Fig. 1: Schematic illustration of putative functional roles of the identified differentially expressed transcripts in MAL-overexpressing Schwann cells.
Forskolin-dependent induction of myelin protein zero (MPZ) as well as phosphorylation of Akt were unaffected by MAL overexpression. Microarray analysis revealed that a number of differentially expressed transcripts in MAL-overexpressing Schwann cells were associated with the cytoskeleton organization and plasma membrane mobility (copied from Schmid et al., 2013). Inset shows immunfluorescent micrograph of cultured Schwann cells.

membrane dynamic (Figure 1) that are regulated in a MAL-dependent manner, underlining their possible role in influencing Schwann cell differentiation and myelination (Schmid et al., 2013).

Charcot-Marie-Tooth disease type 1A (CMT1A) is a hereditary demyelinating peripheral neuropathy caused by the duplication of the PMP22 gene. Demyelination precedes the occurrence of clinical symptoms that correlate with axonal degeneration. Apparent Schwann cell pathology led to the hypothesis that a disturbed axon-glia interface might contribute to altered myelination consequently leading to axonal degeneration. In a recent study, we examined the expression of MAG and Necl4, two critical adhesion molecules that are present at the axon-myelin interface, in sural nerve biopsies of CMT1A patients and in peripheral nerves of mice overexpressing human PMP22, an animal model for CMT1A. We show an increase in the expression of MAG and a strong decrease of Necl4 in biopsies of CMT1A patients as well as in CMT1A mice. Expression analysis revealed that MAG is strongly upregulated during peripheral nerve maturation, whereas Necl4 expression remains very low throughout development and in the adult. Ablating MAG in CMT1A mice results in separation of axons from their myelin sheath (Figure 2) demonstrating that MAG is important for axon-glia contact in CMT1A disease, and suggest that its increased expression has a compensatory role in the pathology of the disease. Thus, we demonstrate that MAG together with other adhesion molecules such as Necl4 is important in sustaining axonal integrity. Further, we identified that lack of Necl4 in CMT1A might contribute to the pathogenesis of this disease (Kinter et al., 2012).

Multiple sclerosis (MS) is a chronic inflammatory demyelinating CNS disease, predominantly affecting young adults and leading to substantial disability in a high proportion of patients. The pathology underlying this disease is the formation of multiple demyelinated lesions. Lesions are typically widely disseminated in the CNS with the prevalence of well myelinated areas. In recent years, damage to neurons and axons, as well as grey matter abnormalities gained increasing attention in MS research. Extended grey matter lesions detected throughout the cerebral cortex have been linked to clinical manifestations such as seizures, fatigue and cognitive dysfunction. By investigating otherwise pathologically normal appearing grey matter (NAGM) tissue from MS patients, we identified reduced transcriptional expression of genes predominantly expressed by astrocytes contributing to the functionality of astrocyte-neuron metabolic exchanges important for the maintenance of brain energy metabolism. Further, an increased expression of transcripts indicative of IL1β signaling pathway activation was detected in MS NAGM. Treatment of primary mouse cortical astrocyte cultures with IL1β or activation of inflammasomes by LPS plus ATP treatment elicited comparable changes as detected in MS NAGM. Reduced transcriptional regulation of MCT1 and CX43 was also observed in an animal model for peripheral immune response. Our data demonstrate that inflammation-mediated changes can directly influence the metabolic profile of astrocytes and by that, greatly influence CNS integrity. Our results highlight a possible detrimental role of chronic inflammation on the functional integrity of cortical grey matter and suggest that these alterations are a major pathogenic component in MS NAGM. Persistent reduction of astroglial metabolic components, essential for sustaining neuronal homeostasis and synapse activity, might actively contribute to the underlying molecular mechanisms of fatigue and cognitive dysfunctions encountered in MS patients (Zeis et al., manuscript submitted).

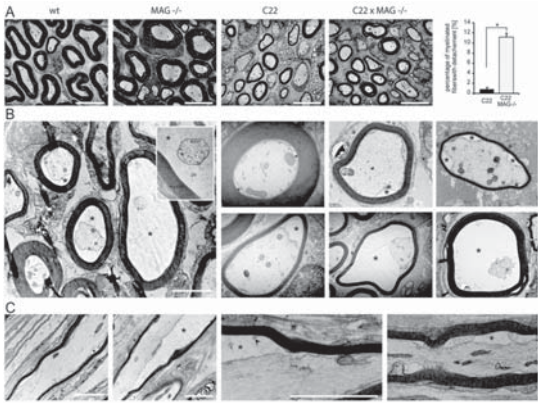

Fig. 2: Loss of axon-glia interaction in MAG-deficient CMT1A mice (C22 mouseline)
EM micrographs of sciatic nerves from wildtype, MAG-/-, C22, and C22xMAG-/- mice are shown (A). Ultrastructural analysis using electron microscopy revealed a significant number of myelinated fibers with increased periaxonal space in C22xMAG-/- (A, plot and B). Data are shown as mean and s.e.m. of three animals at P40 (*, p=0.00022). Different degrees of axonal detachments from the Schwann cell membrane could be detected (B, asterisk depicts periaxonal space). Longitudinal sections of fibers revealed that within one fiber different degrees of axon detachment can occur (C). First detachments can be observed at the age of P20 (data not shown). Scale bars: (A) 5 µm; (B, C) 2 µm. (copied from Kinter et al., 2012).

Selected Publications

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Muscular Dystrophy

Dysferlin Deficiency

Myotonic Dystrophy

FSHD

Autophagy

Gene Therapy

Neuromuscular Research

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Novel treatment strategies for muscular dystrophies

No treatment is currently available for patients with muscular dystrophies. Finding therapies is imperative as these disabling neuromuscular diseases have a high personal and socioeconomic impact. Our laboratory focuses on developing treatment strategies for muscular dystrophies due to dysferlin deficiency, myotonic dystrophy and facio-scapulo-humeral muscular dystrophy (FSHD). Additionally, our laboratory is interested in basic research questions regarding regulation of muscle homeostasis in health and disease. Dysferlin is a transmembrane protein implicated in surface membrane repair of muscle cells. Mutations in dysferlin lead to progressive muscle membrane damage and cause the muscular dystrophies Miyoshi Myopathy, Limb Girdle Muscular Dystrophy Type 2B and Distal Anterior Compartment Myopathy. Our laboratory has studied the dysferlin protein and the DYSF gene in great detail: we have identified dysferlin protein binding partners (Di Fulvio et al. 2011, Azakir et al. 2010), characterized lipid binding specificities of individual dysferlin domains (Therrien et al. 2009), elucidated the degradation pathway of mis-sense mutated dysferlin (Azakir et al. 2012a), identified the dysferlin domain requirement for membrane repair and generated functional mini-dysferlin proteins (Azakir et al. 2012b), inferred the impact of dysferlin gene mutations on protein structure (Therrien et al. 2006), and were the first to report a mild dysferlinopathy phenotype associated with an in-frame exon skipping mutation of the dysferlin gene (Sinnreich et al. 2006). Based on the insights gained, we are currently designing therapeutic strategies for dysferlinopathies.
We are investigating gene delivery of small recombinantly generated dysferlin molecules via adeno-associated virus (AAV) to mouse skeletal muscle. Dysferlin's large size precludes its encapsulation into AAV, the vector of choice for gene therapy to skeletal muscle. Therefore, we generated internally truncated dysferlin constructs, each lacking one of the seven dysferlin C2 domains, which mediate lipid and protein binding interactions. We demonstrated that certain C2 domains are dispensable for dysferlin's correct plasmalemmal localization and membrane repair function in patient derived muscle cells (Azakir et al., 2012b). Based on these results, we designed functional mini-dysferlin molecules, which are small enough to be incorporated into AAV, and which are capable of repairing membranes of patient derived myoblasts. We are currently testing these constructs in experiments with AAV mediated gene transfer to skeletal muscle of dysferlin deficient mice.

Myotonic Dystrophy type I (DM1) is a disabling, genetic disease affecting multiple organ systems. This disease is caused by expanded CTG triplet repeats in the 3'UTR of the Myotonic Dystrophy Protein Kinase (DMPK) gene. Disease severity is correlated to the repeat expansion size. On the RNA level such expanded CUG repeats (CUGexp) form hairpin structures, which lead to ribonuclear inclusions. More specifically, the RNA with expanded CUG repeats sequesters the splice-factor muscleblind-like 1 (MBNL1), which is necessary to regulate alternative splicing. Lack of available MBNL1 leads to mis-regulate alternative splicing of many different genes explaining the multisystem phenotype. We have screened libraries of small molecular weight compounds that are capable of liberating sequestered splice factors from toxic RNA hairpins. We are currently testing these compounds in mouse models of the disease.
In collaboration with the group of Professor Markus Rüegg at the Biozentrum (University of Basel), we study autophagy pathways in skeletal muscle. Autophagy is a catabolic process that ensures homeostatic cell clearance and is deregulated in a growing number of myopathological conditions.

Connection to Clinical Practice

Proteasomal inhibition for dysferlinopathies with mis-sense mutations

Dysferlinopathies are inherited in an autosomal recessive manner, and many patients with this disease harbour mis-sense mutations in at least one of their two pathogenic DYSF alleles. These patients have significantly reduced or absent dysferlin levels in skeletal muscle, suggesting that the protein encoded by dysferlin mis-sense alleles is rapidly degraded by the cell's quality control system. In a recent study performed on patient derived myoblasts (Azakir et al. 2012a), we showed that endogenous mis-sense mutated dysferlin is degraded by the proteasomal system. Inhibition of the proteasome substantially increased the level of mis-sense mutated dysferlin and the salvaged protein was functional as it restored membrane resealing and myotube formation in patient-derived muscle cells. We are currently conducting a proof-of-principle clinical study of Bortezomib (Velcade™) in dysferlinopathy patients with mis-sense mutations, in which we monitor dysferlin expression in skeletal muscle after Bortezomib administration.

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Psychopharmacology
Psychostimulants
MDMA
Cathinones
Addiction

Psycho-pharmacology Research

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Pharmacology of amphetamine-type stimulants: novel designer drugs

We are interested in the pharmacology of psychoactive substances, mostly psychostimulants. In particular, we study the pharmacology of amphetamine psychostimulants both in vitro and in humans. Amphetamine-type drugs include medications such as methylphenidate (Ritalin) used to treat attention-deficit hyperactivity disorder but also recreational drugs including methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) and many novel designer drugs known as cathinones ("research chemicals", "legal highs").
An important line of our research characterizes the pharmacological mechanism of action of the many novel designer drugs that continue to emerge including phenethylamines, cathinones, and piperazillines. We characterized the pharmacology of several of these new drugs of abuse (Fig. 1). Specifically, we determined norepinephrine, dopamine, and serotonin uptake inhibition in vitro using human cells that express the respective monoamine transporter, drug-induced efflux from monoamine-preloaded cells, and binding affinities to monoamine transporters and receptors. For example, we showed that mephedrone releases dopamine and serotonin similar to MDMA but it was a more potent dopamine uptake inhibitor. In contrast, 3,4-methlylenedioxypyrovalerone (MDPV) shows structural similarities to MDMA, but its pharmacology is very different. MDPV is an extremely potent uptake inhibitor of dopamine and norepinephrine but it does not release monoamines, more similar to cocaine but with a much higher potency and an expected higher abuse potential due to is predominant dopaminergic effects.

Pharmacology of amphetamine-type stimulants: mechanism of action of MDMA in vitro and in humans
In a larger series of translational and experimental clinical studies we characterized the mechanism of action of MDMA. We showed that MDMA mainly releases the neurotransmitters serotonin and norepinephrine in vitro and that both monoamines are also important mediators of the psychoactive and adverse effects in humans (Fig. 2). Ecstasy use can result in cardiovascular and hyperthermic complications, which are rare but potentially lethal. Ecstasy-induced hyperthermia may be triggered by additional factors such high ambient temperature, physical activity, and dehydration. However, we showed that MDMA increased body temperature even in the absence of such predisposing factors and that the thermogenic response to MDMA in humans is mediated through α_1- and ß-adrenergic receptors. Based on these experimental data in humans, α-ß-blockers such as carvedilol could be useful in the treatment of ecstasy-induced hyperthermia. More recently, we have also started to explore drug effects on social cognition. Ecstasy is reported by recreational drug users to enhance empathy and sociability. We therefore explored whether MDMA indeed alters social cognition or behavior in humans in a controlled experimental setting in addition to its direct subjective emotional effects. Indeed, in a test of recognition of basic facial emotions, MDMA impaired recognition of negative facial emotions such as sad or fear. Consistently, MDMA also impaired the correct identification of subtle negative affective states in the reading the mind in the eyes test. In contrast, MDMA enhanced mind reading for positive emotions (Fig. 3). Further, MDMA enhanced emotional empathy and prosociality in the laboratory setting. This means that MDMA interacts with the processing of emotions and aspects of social cognition that are of importance for human interaction behavior and likely contribute to the appeal of this drug to young people. Finally, we are similarly investigating the social-emotional effects of methylphenidate (Ritalin) in healthy subjects because methylphenidate is used as

neuroenhancer ("brain doping"). Our preclinical and clinical research is interdisciplinary and involves partners from pharmaceutical sciences, toxicology, psychology, emergency medicine, psychiatry, and pharmaceutical industry. Funding is provided by the SNSF, Neurex, the University of Basel, the Roche Translational Medicine Hub, and others.

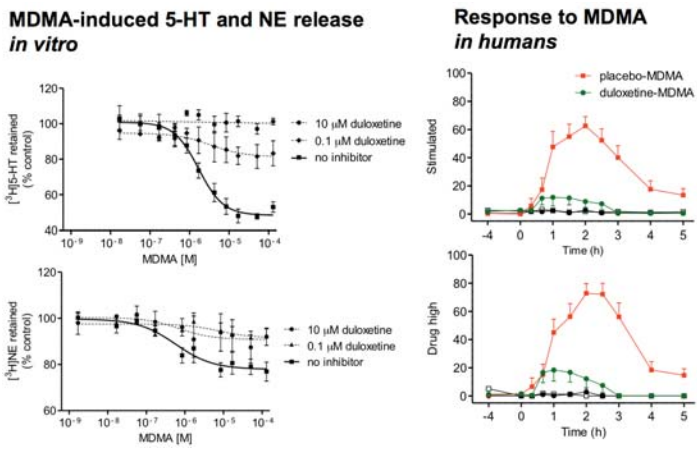

Fig. 1: The serotonin (5-HT)- and norepinephrine (NE) transporter blocker duloxetine reduced MDMA-induced 5-HT and NE release in vitro from transmitter-loaded and transporter-transfected human cells. Additionally, duloxetine prevented most psychotropic and cardiovascular effects of MDMA (125 mg) in human subjects in a randomized placebo-controlled cross-over trial in 16 subjects (P<0.001 compared with placebo). The findings indicate that the mechanism of action of MDMA involves 5-HT and NE transporter-mediated transmitter release and that this mechanism also mediated the psychological and physiological effects of MDMA in humans. Data represent mean and SEM.

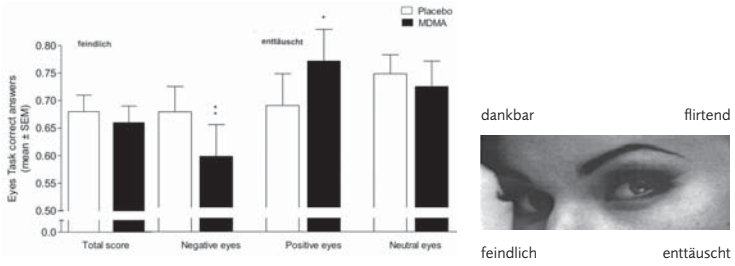

Fig. 2: The reading the mind in the eyes task was used to assess effects of MDMA on the ability to correctly infer mental states from looking at photographs of the eye region. Forty-eight subjects were tested in a placebo-controlled cross-over study. MDMA (125 mg) enhanced mind reading of positive emotions and impaired mind reading of negative emotions compared with placebo (*P<0.05, **P<0.01). The findings indicate a shift in the ability to correctly read socio-emotional information toward stimuli associated with positive emotions ("pink glasses") in addition to direct prosocial subjective drug effects. Data represent mean and SEM.

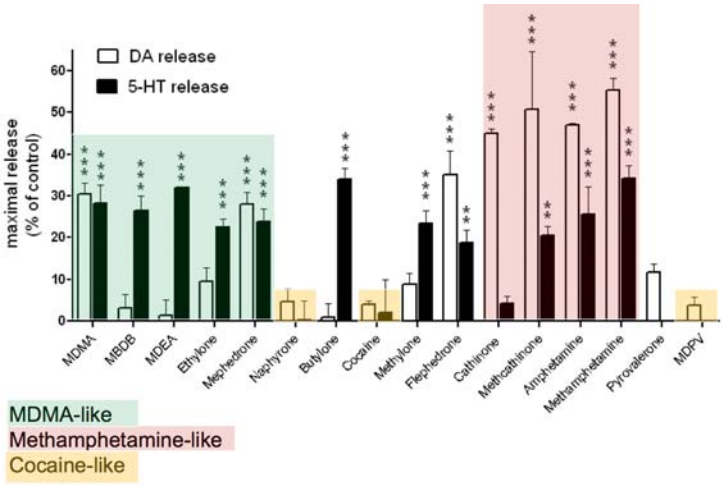

Fig. 3: Monoamine release profiles of novel designer cathinones and their non-b-keto-amphetamine analogs. Serotonin (5-HT) and dopamine (DA) release was studied in transmitter-loaded transporter transfected human cells. Maximal effects are shown induced maximal drug concentrations (100 mM) to test whether the new drugs are substrate releasers similar to amphetamines or only transporter inhibitors similar to cocaine. For example the novel cathinone designer mephedrone released 5-HT and DA similar to the non-b-keto amphetamine 3,4-methylenedioxymethamphetamine (MDMA, ecstasy). 3,4-methylenedioxypyrovalerone (MDPV) produced no 5-HT or DA release similar to cocaine. However, MDPV was an inhibitor of the 5-HT and DA transporter similar to cocaine but with significantly higher potency. These and other in vitro data help to understand the mechanism of action of novel designer drugs and are useful to infer their clinical effects. EC50 for release and uptake inhibition data are not shown. Data are mean and SEM of at least three independent experiments.

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DBM Focal Area
Stem Cells and Regenerative Medicine

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Stem cell research and regenerative medicine are major pillars within the Department of Biomedicine (DBM) and the life science strategy of the University of Basel. The last decade has seen substantial progress in identifying and isolating stem cells from different adult tissues and embryonic origin, which can be induced to differentiate into various specific cell-types relevant to regenerative medicine. The groups of this focal area are active in various aspects of this fascinating field with relevance to basic, mechanistic and clinically applied, translational research.

The basic research efforts aim to identify and isolate stem cells and understand how stem cells are maintained in their normal niches within the embryo and/or the body. As such, several groups are studying how stem cells of the blood are maintained in the bone marrow, differentiate into the various different cell-types of the hematopoietic system, and how their differentiation potential is altered in malignant states that are caused by aberrant stem cell-based cancers (e.g. leukemia or lymphomas). The close interactions of clinical with basic researchers allow bridging the gap between fundamental and translational research. For example, attempts to grow and differentiate mesenchymal stem cells from human and mouse bone marrow in vitro into different cell- and tissue-types, aim at developing cartilage and bone replacement therapies that can be translated to the clinic. The knowledge gained from these studies forms the basis for designing and developing clinically applicable tissue engineering strategies and in moving toward regenerative medicine.

One of the major aims of regenerative medicine is to reactivate and support the regenerative potential of the body in a controlled manner. To this aim, understanding the normal regulation of organogenesis and tissue homeostasis is crucial. While first attempts have given encouraging results, it is important to gain a much better knowledge of how stem cells interact with their niche to maintain their multi-potency and give rise to daughter cells that undergo transient amplification upon leaving the niche. These populations of transient amplifying cells will then initiate their specification and differentiation in a controlled manner. Our challenge is to establish culture conditions where stem cells can be maintained and their specification and differentiation into functional tissues can be induced in an efficient and precisely controlled manner. Any functional organ and tissue will consist of well-organized and functionally interacting cells with different identities. Therefore, it is important to e.g. understand the role of embryonic signaling centers in tissue patterning/organization and cell-type specification/differentiation.

The knowledge gained from analyzing cell-type, tissue specification and organogenesis during normal embryonic development is highly relevant to directed engineering of tissues from progenitor and/or stem cells. So-called induced pluripotent stem (iPS) cells – adult cells (e.g. skin cells) reprogrammed into stem-like cells – are increasingly used as they can be relatively easily obtained from patients for cell differentiation and tissue engineering studies. The generation and analysis of iPS cells fits the strategy of the DBM to promote collaborative efforts between basic research groups and clinicians with the aim to significantly reduce the gap between bench and bedside. In addition to interactions within the DBM, there are numerous collaborations with groups at the Biozentrum, FMI and the D-BSSE, which are funded by network grants such as Sinergia and SystemsX.ch. Many of our groups are actively participating in the Basel Stem Cell Network, which is one of the Competence Centers within the Life Sciences at the University of Basel. There, stem cell researchers have the opportunity to closely interact and collaborate with developmental biologists, geneticists and even mathematicians with the objective to foster interdisciplinary and innovative research.

Last but not least, with Verdon Taylor and Claudia Lengerke, we recently appointed two stem cell experts in the fields of brain development and hematopoietic stem cell signaling. Their groups help strengthening both basic and translational research efforts in this rapidly emerging and highly competitive research field.

Heart Failure
Hypertension
Obesity
Cardiac Metabolism
Signal Transduction

Cardiobiology

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Myocyte growth and metabolism in cardiac disease

The heart continuously needs to generate high amounts of ATP to perform its critical function as pump that circulates blood throughout the body. In the healthy heart, almost all ATP is derived from mitochondrial oxidative phosphorylation and the heart therefore relies on a continuous oxygen supply. When cardiac work has to increase, for example in pathological conditions such as hypertension, hypertrophy develops and metabolism is adapted to ensure that energy supplies meet the demands. Cardiovascular disease is often accompanied by cardiac cell loss via apoptosis or necrosis because metabolic substrates and oxygen supplies are inadequate. Reduced numbers of contractile cells along with insufficient performance of the remaining cardiomyocytes contribute to the progression to heart failure. Approaches taken to reduce cardiac disease may therefore aim to improve the performance of the differentiated cardiomyocytes, to prevent their death, or to generate new cardiomyocytes from precursor cells. The goal of our research is to provide a fundamental basis for such approaches by advancing the understanding of the molecular mechanisms that regulate growth and metabolism in cardiac cells. We are analyzing these mechanisms in primary cell cultures as well as *in vivo* models of obesity and cardiac pressure overload, as the latter mimic clinical conditions that represent a major health problem.

Our ongoing investigations are based on our earlier findings in an animal model of high blood pressure, which demonstrated that IGF-I modulates cardiac and skeletal muscle weight by regulating specific protein synthesis and degradation pathways. One of the key intracellular mediators of IGF-I-induced growth is the mammalian target of rapamycin (mTOR). mTOR integrates hormonal signals such as that of insulin and IGF with information on nutrient and energy availability as well as cellular stress. mTOR activates distinct substrates with tissue-specific functions depending on whether it is part of the multiprotein complex mTORC1 or mTORC2. In our studies, we are analyzing to what extent cardiac stress factors such as pressure overload, ischemia, nutrient deprivation, or a high fat diet change the activity of mTORC1 and mTORC2 and thereby modulate cardiac composition, geometry and function. Next to investigating how mTORC1 and mTORC2 play a role in cardiac protein synthesis, proteasomal degradation, autophagy (Fig. 1) and energy metabolism, we analyze stress-related changes in selected cardiac-specific proteins.

For these studies, we generated mouse models in which raptor or rictor, specific and essential components of mTORC1 and mTORC2, respectively, were ablated in a cardiomyocyte-specific manner. The deletions are induced during adulthood and followed by the experimental protocols mentioned above; functional and structural consequences are evaluated by ultrasound and immunohistochemical analysis (Fig. 2). In control mice, pressure overload causes an increase in cardiac weight sufficient to maintain normal cardiac ejection fractions. Raptor knockout mice in which cardiac mTORC1 is inactivated are not able to produce this adaptive hypertrophic growth and rapidly develop cardiac dysfunction. Even with a normal cardiac workload, sedentary raptor knockout mice develop cardiac dysfunction that culminates in death within 6 weeks (Shende, 2011). In contrast, rictor ablation, despite successfully reducing the phosphorylation of a range of mTORC2 targets, affects cardiac geometry or function under baseline conditions neither in young growing nor in adult mice during aging, up to 54 weeks. Pressure overload, however, causes eccentric hypertrophy and decreases ventricular function in rictor-deficient mice (Shende, 2013). While mTORC1 inactiva-

tion causes severe dysfunction mainly by reducing protein synthesis and mitochondrial content and by increasing apoptosis, mTORC2 inactivation leads to dysfunction via its effects on PKC signaling. Our ongoing studies are relating these effects to the activation of adrenergic and ErbB2 receptors (Pentassuglia, 2013).

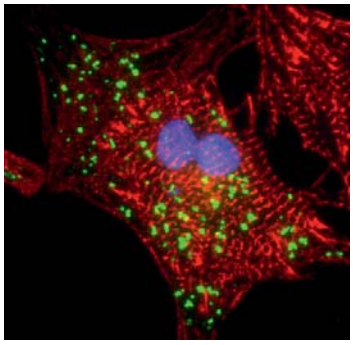

Fig. 1: Analysis of the regulation of autophagy is performed in isolated neonatal cardiomyocytes after transfection with pEGFP-LC3 to visualize the autophagosomes (green). The cells in this example were treated with pepstatin A and E-64d. Alpha-sarcomeric actinin labeling (red) reveals the cross-striation and DAPI staining the two nuclei of this cardiomyocyte.

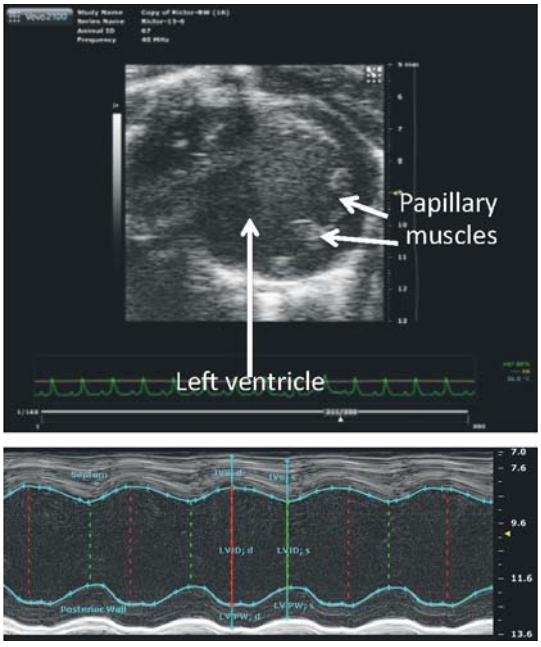

Fig. 2: Ultrasound analysis is used to follow over time *in vivo* the changes in cardiac geometry and function between control, raptor, and rictor knockout mice in experimental disease models such as aortic constriction (pressure overload) or high fat-induced obesity.

Connection to Clinical Practice

Cardiovascular disease in Europe

Cardiovascular disease causes over 4 million deaths in Europe each year, which represents 47% of all deaths in Europe (52% of deaths in women, 42% of deaths in men). Overall cardiovascular disease is estimated to cost the EU economy almost €196 billion a year. Levels of obesity are high in both adults and children, and the prevalence of diabetes has increased rapidly over the last ten years, increasing by more than 50% in many countries (European Cardiovascular Disease Statistics 2012 edition). Obesity is associated with some of the major risk factors for cardiovascular diseases such as hypertension. This is especially true for the elderly female population: there is a higher prevalence of obesity, diabetes and hypertension in older women associated with an increased prevalence of stroke, left ventricular hypertrophy and diastolic heart failure. Estrogen deficiency has been proposed as one of the reasons for this increase. Given the aforementioned epidemiological data, our basic research aims to acquire a deeper understanding of the mechanisms that underlie hypertension- and obesity-induced cardiac disease in male as well as in ovariectomized female mice without or with hormone replacement.

Selected Publications

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Molecular Imaging
Ultrasound
Microbubbles
Atherosclerosis
Vascular Inflammation
Myocarditis

Cardiovascular Molecular Imaging

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Ultrasound molecular imaging in cardiovascular diseases

Cardiovascular diseases are the leading cause of morbidity and mortality in the western world. Most important in that respect are complications of atherosclerosis (myocardial infarction, stroke, peripheral artery disease), but other disease entities such as myocarditis contribute to a considerable disease burden particularly in young individuals. Noninvasive imaging plays an increasing role in diagnosis, risk stratification and assessment of treatment responses in cardiology. Advances in image technology over the last years has allowed for depiction of the heart and blood vessels with ever increasing detail. Novel imaging technologies termed molecular imaging use detection of site-targeted contrast agents to depict the molecular footprint of a disease-relevant phenotype at the cellular level. It is thought that such techniques will in the future contribute to earlier detection of disease, to better risk stratification and to better assessment of treatment-responses. Molecular imaging with ultrasound contrast agents relies on the detection of microbubbles within diseased tissue. Microbubbles produce an acoustic signal owing to their resonant properties in an ultrasound field. Microbubble targeting is accomplished by either manipulating the microbubble shell for attachment of microbubbles to activated leukocytes, or by conjugation of disease specific ligands to the microbubble surface (Fig. 1).

Ultrasound molecular imaging of treatment responses in atherosclerosis

Up-regulation and surface expression of vascular endothelial cell adhesion molecules are early events in atherogenesis. P-selectin on the endothelial cell surface mediates rolling and activation of leukocytes. Firm adhesion is then mediated by Vascular Cell Adhesion Molecule-1 (VCAM-1) on the endothelial surface. Together, these molecules play a critical role in leukocyte arrest in blood vessels, and participate in the early stages of atherogenesis. We have previously shown that non-invasive imaging of P-Selectin and VCAM-1 can detect vascular inflammation during the very early stages of atherosclerotic disease. For pharmacologic interventions that are started early in the pathogenesis of atherosclerosis, or that include novel drug regimens with the goal of reducing vascular inflammation, the ability to non-invasively assess treatment effects on vascular inflammatory status will be important. We could show that ultrasound molecular imaging can detect the effects of statin therapy on early inflammatory processes in atherosclerosis at a timepoint when high-resolution imaging does not show differences in plaque thickness (Fig. 2). While statins are currently the drugs of choice for slowing atherosclerotic disease progression, other drugs, among them antioxidative compounds, continue to be developed. Apocynin is an antioxidative NADPH-oxidase-inhibitor with anti-inflammatory properties. We used contrast enhanced ultrasound molecular imaging to assess whether short-term apocynin therapy in atherosclerosis reduces vascular oxidative stress and endothelial activation. We showed that short-term treatment with apocynin in atherosclerosis reduces endothelial cell adhesion molecule expression. This change in endothelial inflammatory phenotype could be detected by molecular imaging before any measurable decrease in macrophage content, and was not associated with a detectable change in oxidative burden.

Ultrasound molecular imaging of myocarditis

Dilated cardiomyopathy as a consequence of viral myocarditis is a frequent cause for heart failure in young adults with a significant disease burden. In young patients presenting to the emergency department with either chest pain or signs of heart failure, myocarditis is a differential diagnosis. However,

the diagnosis of myocarditis in the emergency room is difficult, as clinical signs, the electrocardiogram and biomarkers (troponins) lack sensitivity or specificity. Therefore there is a need for a rapid, non-invasive imaging tool for the detection of inflammatory events occurring in myocarditis. We are currently evaluating in murine myocarditis models whether ultrasound molecular imaging can be used for this purpose.

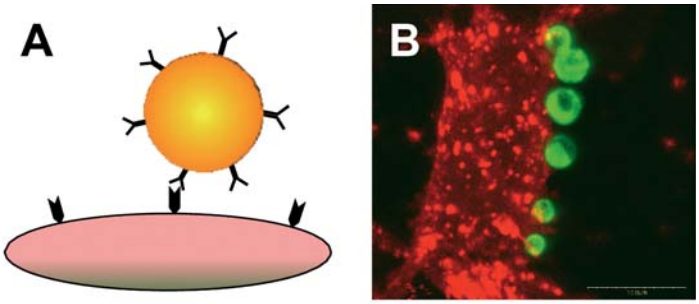

Fig. 1: Principle of site-targeting for ultrasound contrast agents. **(A)** Antibodies or other ligands for disease specific antigens are attached to the microbubble surface. **(B)** Attachment of microbubbles to VCAM-1 on an endothelial cell *in vitro*.

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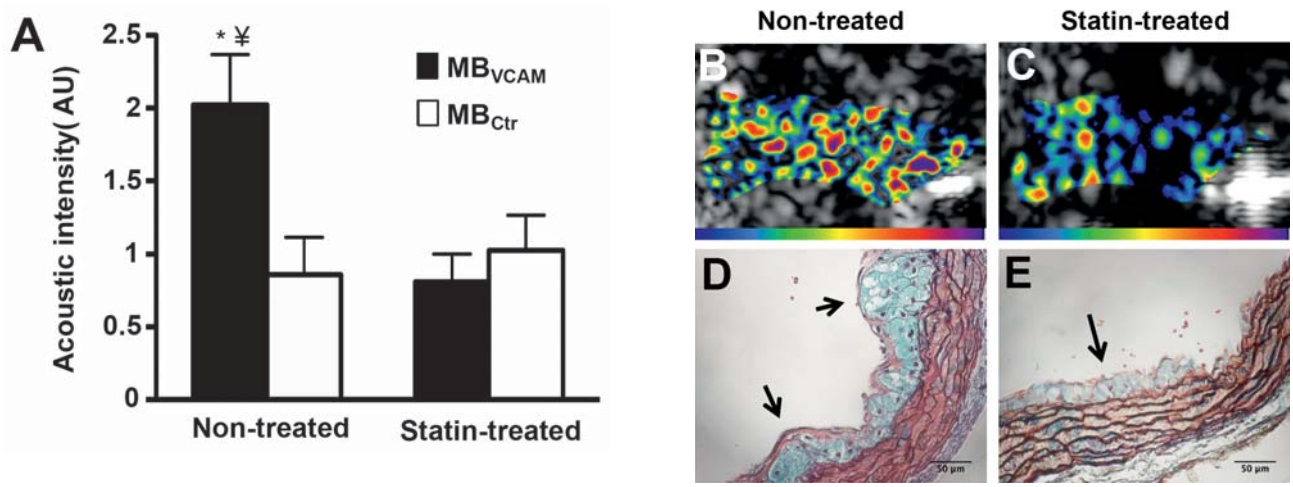

Fig. 2: **(A)** Mean ± SEM background-subtracted signal intensity for microbubbles targeted to VCAM-1 (MBVCAM) and control microbubbles (MBCtr) in non-treated and statin treated animals. * $p < 0.01$ vs MBctr in non-treated animals, ¥ $p < 0.01$ vs MBVCAM in statin treated animals. **(B)** Example of color coded image from a non-treated animal after injection of MBVCAM. **(C)** Images from a statin treated animal after injection of MBVCAM. **(D)** Histology from a non-treated animal, arrows denote large atherosclerotic plaque. **(E)** Histology from a statin treated animal, arrow denotes small plaque.

Angiogenesis
Myoblasts
Mesenchymal Stem Cells
Gene Therapy
Cell Therapy
Ischemia

Cell and Gene Therapy

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Therapeutic angiogenesis from vascular biology to regenerative medicine

Therapeutic angiogenesis aims at restoring blood flow to ischemic tissues by generating new vessels. Our research focuses on the basic principles governing vascular growth and their translation into rational therapeutic approaches to: 1) treat ischemic diseases, and 2) improve the vascularization of tissue-engineered grafts. We use precursor cells genetically modified to express controlled levels and combinations of factors, in order to provide both vascular growth and tissue regeneration, combining the specific advantages of cell and gene therapy.
Vascular endothelial growth factor (VEGF) is the master regulator of vascular growth. However, uncontrolled expression leads to the growth of vascular tumors (angiomas). By the close interaction of basic scientists and clinical surgeons, we are developing novel methods to deliver the VEGF gene alone or in combination with maturation factors to increase its safety and efficacy in vivo, through the use of transduced progenitors, gene therapy vectors and controlled release of recombinant proteins by smart biomaterials. Research is funded by Swiss agencies (SNSF and Swiss Heart Foundation), the European Union and industrial funds.

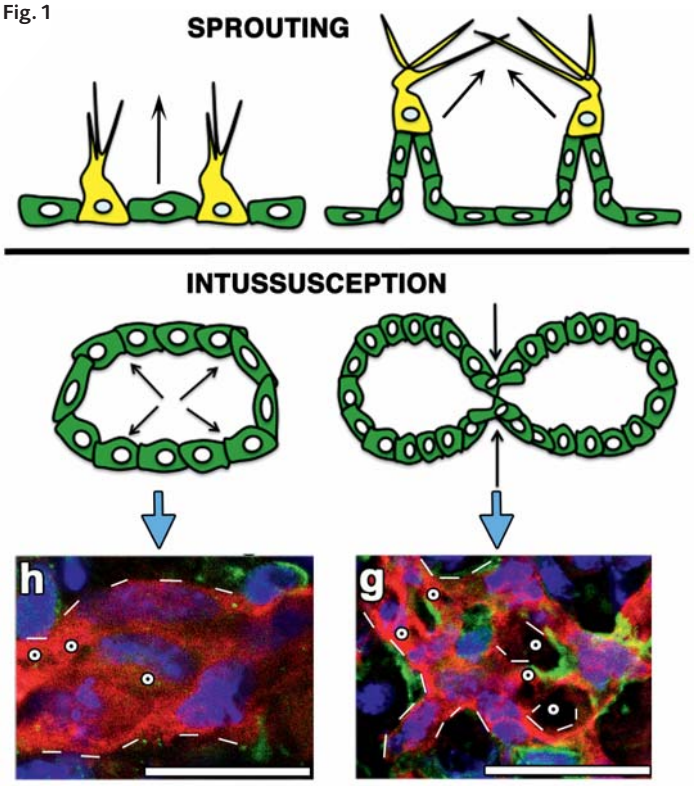
1) Controlled VEGF expression for therapeutic vascularization
We previously found that the transition between normal and aberrant angiogenesis depends on the VEGF amount in the microenvironment around each producing cell rather than on the total dose, since VEGF remains tightly localized in the extracellular matrix (Ozawa 2004; Banfi 2005). In order to translate this biological concept into a clinically applicable approach, we developed a high-throughput FACS-based technology to rapidly purify progenitors expressing specific VEGF levels after in vitro transduction (Misteli 2010; Wolff 2012). Controlled VEGF expression by FACS-purified populations of diverse progenitors could induce effective vascularization and cardiomyocyte differentiation in thick, engineered cardiac patches (Marsano 2013), therapeutic angiogenesis in ischemic myocardium (Melly 2012 and manuscript submitted) and increased in vivo vascularization of osteogenic grafts (Helmrich 2013).
To avoid the safety concerns raised by genetically modified progenitors, we took advantage of a state-of-the-art biomaterial platform for the controlled release of matrix-bound growth factors, developed by Jeffrey Hubbell (EPFL, Lausanne). We could achieve controlled release over 4 weeks and identify a 500-fold range of VEGF concentrations inducing only physiological capillary networks, which were long-term stable, functionally perfused and therapeutically effective in ischemic wound healing (Sacchi, manuscript submitted).

2) Cellular and molecular mechanisms of VEGF dose-dependent angiogenesis
Our understanding of angiogenic mechanisms is mostly based on developmental models, in which new vessels sprout to vascularize tissues. However, we found that VEGF delivery to skeletal muscle, at the doses needed for functional benefit, induces vascular growth without sprouting, but rather by circumferential enlargement of pre-existing vessels, followed by longitudinal splitting, or intussusception (Gianni-Barrera 2013; Fig. 1). Further, the molecular basis for the induction of angiogenesis by sprouting or intussusception by VEGF is provided by opposite patterns of activation of Notch1 signaling (Gianni-Barrera 2011 and manuscript submitted). We also found that the transition between normal and aberrant angiogenesis is not an intrinsic property of VEGF dose, but depends on

the balance between VEGF-induced endothelial stimulation and vascular maturation mediated by pericyte recruitment by PDGF-BB (Banfi 2012).

Taking advantage of the highly controlled cell-based gene delivery platform we developed, we are currently pursuing a systematic investigation of the mechanisms that regulate the switch between normal and aberrant angiogenesis in vivo, through the analysis of the stage-specific mRNA and miRNA transcriptomes of ex-vivo purified vascular cells, in collaboration with Hoffman-La Roche. The results are expected to help identify novel and more specific molecular targets for therapeutic angiogenesis approaches.

Fig. 1



Connection to Clinical Practice

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Prof. Dr. Friedrich Eckstein
Cardiac Surgery, University Hospital Basel
Prof. Dr. Dirk Schäfer, Plastic and Reconstructive Surgery, University Hospital Basel
Prof. Dr. Lorenz Gürke, Vascular and Transplantation Surgery, University Hospital Basel

Genetically modified progenitors for improved in vivo vascularization and tissue regeneration

The goal of the group is to translate the basic biological principles controlling the physiological generation of normal and functional vascular networks into the design of rational strategies to induce therapeutic growth of new blood vessels. We are currently pursuing this concept in three main areas of clinical interest:

1) To induce controlled angiogenesis in the myocardium and generate vascularized cardiac patches with transduced and FACS-purified VEGF-expressing adipose tissue-derived mesenchymal progenitors, in order to improve contractile function in the ischemic heart (Dr. med. L. Melly and Prof. F. Heckstein, Cardiac Surgery USB).
2) To achieve rapid vascularization of the inner core of clinical-size osteogenic grafts in order to favor progenitor survival and differentiation, leading to improved bone formation, by using VEGF-expressing transduced bone marrow-derived osteoprogenitors (Dr. med. R. Largo, Dr. med. M. Burger and Prof. D.J. Schäfer, Plastic and Reconstructive Surgery USB).
3) To achieve therapeutic angiogenesis in chronically ischemic muscle tissue for the treatment of peripheral artery disease patients, by sustained delivery of controlled levels of recombinant angiogenic factors by smart biomaterials (Dr. med. T. Wolff and Prof. L. Gürke, Vascular Surgery USB).

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Idiosyncratic Toxicity

Apoptosis

Cell Models

β-Oxidation

Respiratory Chain

IGF-1 Signalling

Clinical Pharmacology and Toxicology

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Franziska Päch (PhD student)

Exploring mechanisms of idiosyncratic toxicity of drugs

Drug toxicities can be related to drug exposure above the therapeutic range. This kind of toxicity is named intrinsic or type A toxicity, is often related to the pharmacological action of drugs and is almost certainly detected during drug development. Another type of toxicity is named type B or idiosyncratic toxicity. Idiosyncratic toxicity is rare, mainly not related to the pharmacological action of a drug and usually not detected during drug development. Target organs are often liver and/or skin, but may be any other organ. Mechanisms are immunological (antibody- or T cell-driven) or non-immunological (metabolic) toxicity.
Regarding the non-immunological type of idiosyncratic toxicity, many features can be reproduced in vitro cell cultures and/or isolated cell organelles exposed to high concentrations of a drug or drug metabolites. This observation has led to the concept that patients with idiosyncratic toxicity have risk factors rendering them more sensitive to such drug effects.

Our research in this field has two principle aims: 1) to explore mechanisms of idiosyncratic toxicity in vitro and in vivo in animals, and 2) to find out possible risk factors using cellular systems and/or animal models.

One example is the toxicity of benzbromarone and amiodarone, two benzofurane derivatives. Amiodarone, an antiarrhythmic drug, is toxic for many organs; one of them is the liver. The histological picture of affected livers shows fat accumulation, which is mainly a consequence impaired β-oxidation of fatty acids. In an early study, we could demonstrate that the N-desethylated metabolites of amiodarone are more toxic for β-oxidation than the parent compound. Since N-desethylation of amiodarone is mainly performed by CYP3A4, we assumed that a high activity of CYP3A4 may be a risk factor for amiodarone-associated cytotoxicity. To test this assumption, we overexpressed CYP3A4 in HepG2 cells and studied cytotoxicity. These investigations showed that amiodarone is indeed at lower concentrations cytotoxic in CYP3A4 overexpressing cells as compared to wild type cells (Biochem Pharmacol 2011;81:432-441). We could also show that cell death in this model was associated with impaired mitochondrial function, leading to cytochrome c release and apoptosis. Dronedarone, which is a follow-up development of amiodarone, also inhibits mitochondrial β-oxidation already at very low concentrations. In this case, however, the parent substance is more toxic than the N-desalkylated metabolites (Toxicol Sci 2013;131:480-90).

Statin-associated rhabdomyolysis is a second area of interest. In a first study, we could show that lipophilic statins are mitochondrial toxins and can induce apoptosis and/or necrosis in cultured skeletal muscle cells (Cell Mol Life Sci 2006;63:2415-2425). More recently, we demonstrated that statins inhibit cholesterol biosynthesis also in skeletal muscle cells and that this inhibition is associated with impaired O- and N-glycosylation of proteins (Biochem Pharmacol 2010;79:1200-1209) and with the inhibition of the activation of AKT, which is an important protein in the IGF-1 signalling pathway (Biochim Biophys Acta 2011;1813:2079-87). Subsequent studies showed that IGF-1 can prevent and up to a certain point also rescue cells from cytotoxicity associated with simvastatin. We are currently exploring the underlying mechanisms associated with this observation.

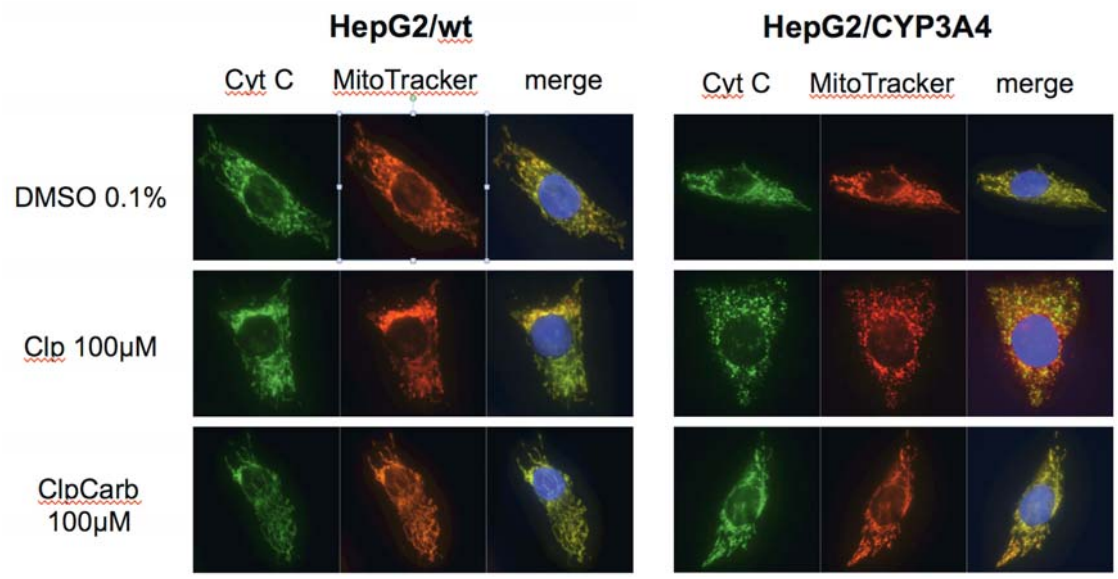

Fig. 1: Mitochondrial cytochrome c release after drug treatment in HepG2/CYP3A4 and HepG2/wt cells.
Representative images of cytochrome c (cyt c, green), mitochondria (MitoTracker, red), and their co-localization (merge, yellow). Cells were incubated with medium containing 0.1% DMSO, clopidogrel (Clp) 100µM or clopidogrel carboxylate (ClpCarb) 100µM for 12 h and cytochrome c was visualized by immunofluorescence staining with a monoclonal antibody. Cells containing CYP3A4 (HepG2/CYP3A4) incubated with clopidogrel contain much more mitochondria lacking cytochrome c (red color in the merged picture) than HepG2/CYP3A4 cells incubated with DMSO 0.1% (negative control) or clopidogrel carboxylate or HepG2 cells containing no CYP3A4 (HepG2/wt).

Future projects will go into two directions. First, we will continue to study the effect of statins on skeletal muscle cells and on cardiomyocytes with a focus on the IGF-1 signalling pathway. We are also interested in the effect of statins on the cellular proteome. A second direction is the effect of drugs and other xenobiotics on β-oxidation. We will try to find out biomarkers for impaired β-oxidation using cultured cells and also animal models. For that, we are going to use a proteomic and a metabolomics approach. Furthermore, we will refine the assays used so that we can localize exactly where β-oxidation is inhibited by specific drugs and other xenobiotics.

Connection to Clinical Practice

Avoiding idiosyncratic adverse drug reactions in patients

The start of our research is almost every time based on the observation of adverse reactions of drugs in patients. We then try to identify the mechanisms of the observed adverse reaction in suitable cellular systems. Once we have found plausible mechanisms, we confirm it in suitable animal models. The final goal is to go back to patients with the aim to avoid the adverse reaction by excluding patients with the suspected risk factor from treatment with the specific drug.

Selected Publications

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- Maseneni, S., Donzelli, M., Brecht, K., and Krahenbuhl, S. (2013). Toxicity of thienopyridines on human neutrophil granulocytes and lymphocytes. Toxicology *308*, 11-19.
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Genodermatosis

Skin Cancer

Epidermodysplasia Verruciformis

Ichthyosis with Confetti

Familial Adenomatous Polyposis Coli

Dermatology

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Genodermatoses as a clue to cancer development

Skin is the largest human organ and important in many different functions. As in other organs benign lesions as well as malignant neoplasms affect quality of life and life itself. Some benign skin tumours could occur as a sign of a severe disease.
We focus our research on genodermatoses correlated to neoplasms with the aim to identify basic mechanisms leading to tumour development.
Our first topic is the familial adenomatous polyposis (FAP). FAP patients suffer from increased risk for developing adenomatous colorectal cancer due to a mutation in the *adenomatous polyposis coli (APC)* gene. It is known that some of these patients also develop benign skin lesions (fibroma, lipoma, epidermal cysts), but detailed data were never collected. We hypothesized that these specific skin lesions could serve as a useful marker for presymptomatic diagnostic of FAP. We prospectively determined the prevalence of cutaneous lesions in genetically confirmed *APC* mutation carriers and assessed their potential usefulness in the identification of FAP patients. In close cooperation with Karl Heinimann (Research Group of Human Genetics) a skin examination in 56 adult *APC* mutation carriers was performed and compared to a control group of the general population. In nearly 50% of all FAP patients we could identify at least one FAP-associated benign skin lesion, but significantly more prevalent were only multiple lipomas and combined skin lesions in FAP patients. Additionally, these skin lesions occurred at younger age in *APC* mutation carriers (Fig. 1). On some lesional samples we could test our hypothesis of a second hit in the *APC* gene, comparable to other genodermatoses. Although in single cases an additional hit could be identified, the second hit model could not be confirmed as a basic mechanism in benign skin lesions of FAP.
Another research line is the cutaneous squamous cell carcinoma (cSCC), a type of malignant skin cancer, which is common in the general population and triggered by UV irradiation. Unique genodermatoses show an increased risk for developing these cSCC, for instance the epidermodysplasia verruciformis (EV). EV is a rare autosomal recessively inherited skin disease leading to an increased susceptibility to particular HPV types. The disease is associated with the increased risk for cSCC on the UV-exposed regions of the skin. It is assumed that EV functions as a model disease for the development of cSCCs also in the common population, but the pathomechanisms are not known. Our research in EV focuses on the identification of key mechanisms in the development of the EV-typical skin lesions as well as the cSCC by identification of homologies and differences to common cSCC. Actual aspect is the expression of microRNAs regulating the differentiation and growth of the tumor.
In contrast to EV, patients with a specific type of ichthyosis (ichthyosis with confetti (IWC)) are not reported to develop cSCC. This is interesting as these patients, who are heterozygous carrier of a *keratin 10 (KRT10)* mutation, accumulate a huge number of chromosomal rearrangements during their life. We could show that the patients are born with an erythematous and scaling skin (Fig. 2A). During childhood the patients develop thousands of white spots on their skin looking like normal skin (Fig. 2B). These spots are the result of a loss of heterozygosity (LOH), which leads to cells containing two alleles of the KRT10 wildtype allele. Although such chromosomal rearrangements are reported in cancer development, nothing is known about the cancer risk of patients with IWC. Future research of our group aims for identification of the mechanism underlying the disease and leading to a prognosis for the patients regarding their tumour risk, especially on the skin.

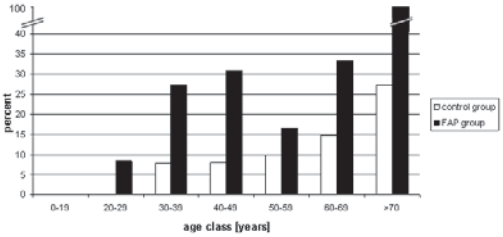

Fig. 1: FAP patients develop disease-associated benign skin lesion at younger age than the general population. Here we show the lipoma frequency of FAP-patients and the control group splitted in 10-year age classes (adapted from Burger et al. 2011. Oncologist 16, 1698-1705).

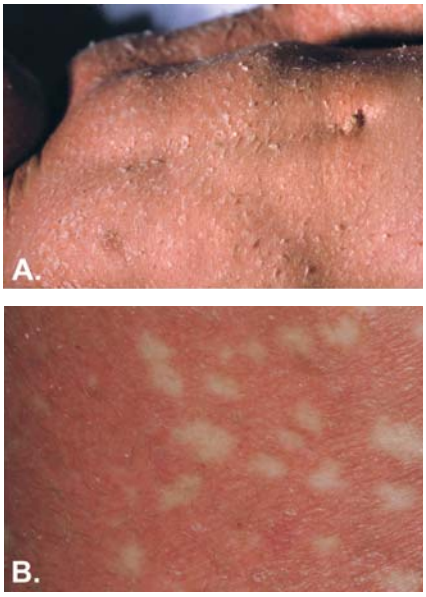

Fig. 2: Patients with IWC are born with erythematous and scaling skin (A). During childhood they develop a typical pattern of white spots on the erythematous skin (B).

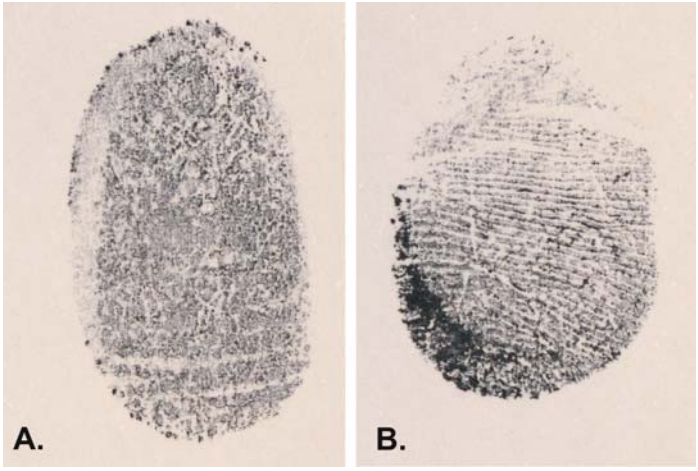

Fig. 3: Missing fingerprints are a rare phenomenon in otherwise healthy individuals (A). It is caused by mutations in a splice site variant of SMARCAD1. In a control you see a normal pattern of fingerprints (B).

Connection to Clinical Practice

Molecular investigation of genetically determined skin diseases

Our research focuses on rare genetic skin diseases, which could function as a model for general mechanisms. Most effort is applied to skin carcinoma development with the aim to understand basic mechanisms and identify new targets for tumour therapy. Patients who suffer from the related disease are under medical treatment in the Clinic of Dermatology. Since all of our research activities is close-by the needs of the patients we also examine single families outside of carcinoma topics. For instance, we could analyse the germline mutation in a family without fingerprints. In cooperation with an Israeli dermatological research group we identified a specific splice variant of *SMARCAD1* responsible for developing fingerprints on the palms and soles (Fig. 3).
The knowledge of the underlying cause of their skin disease is important for the patients, not only for estimation the cancer risk but also for the interpersonal relationships as skin is an important mediator between human individuals.

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Gene Regulation
Limb Bud Development
Mouse Genetics
Organogenesis
Signaling Networks
Systems Biology

Developmental Genetics

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Morphoregulatory signaling systems: functional analysis of their impact on gene regulation during normal and altered embryonic development

We use a systems biology approach that combines mouse molecular genetics, transcriptome analysis and biochemistry with mathematical simulations to gain insight into the signaling and transcriptional networks that orchestrate vertebrate limb bud organogenesis. We study how progenitor cells are selected to act as embryonic organizers and how these control growth and patterning of limb buds. One of our main topics is to investigate how limb bud mesenchymal progenitors integrate various signaling inputs into a transcriptional response that regulates their survival, fates, proliferation and differentiation potential. For example, we have shown that the transcriptional regulator GLI3 initially regulates establishment of Sonic Hedgehog (SHH) signaling by the organizer in the posterior limb bud mesenchyme. GLI3 interacts with HAND2 as part of the transcription regulatory network that initiates establishment of an anterior and a posterior compartment prior to the onset of SHH signaling.

As limb bud development progresses, GLI3 acts as a gatekeeper that regulates the exit of proliferating cells toward chondrogenic differentiation (Fig. 1). In particular, GLI3 directly regulates the cell cycle and expression of the BMP antagonist *Gremlin1* in limb buds. Our extensive genetic analysis has established that GREM1 is a key node within the self-regulatory signaling system that controls limb bud outgrowth and patterning. The highly dynamic *Grem1* expression is regulated by a large genomic landscape that integrates inputs from at least three different signaling pathways (BMP, SHH, FGF). Using 4C chromatin conformation capture in combination with ChIP-sequencing, we are analysing how this integration works and how its self-regulatory feedback features are controlled. While this feedback system keeps BMP activity low during limb bud outgrowth and patterning, we have shown that high BMP activity is required during initiation for setting up the ectodermal signaling centre and then again at late stages to initiate chondrogenic differentiation.

Other fascinating aspects of our research are briefly summarized below:

1. Evolutionary diversification of limbs in mammals: alterations in these signaling interactions not only cause congenital limb malformations, but also underlie evolutionary diversification of tetrapod limbs. We have recently been able to show that the loss of anteroposterior polarity and digits in bovine and artiodactyl limbs is due to degeneration of the cis-regulatory region that regulates the expression of the *Ptch1* receptor in response to SHH signaling. This loss of transcriptional sensitivity to SHH underlies the evolutionary adaptation of artiodactyl limbs, which diverged from other mammalians ~60 mio years ago.
2. Relevance to engineering of cartilage and bone from mesenchymal stem cells: the molecular networks controlling the dynamic modulation of TGFβ/BMP activity during limb bud development are highly relevant to the so-called developmental engineering of cartilage and bone from mesenchymal stem cells (MSCs). We are functionally analysing the molecular similarities and differences of normal chondrogenic differentiation and endochondral bone formation in embryonic limbs in comparison to induced differentiation of mouse MSCs.
3. The role of developmental modulators of SHH and BMP signaling in cancer: our genetic analysis shows that aberrant feedback signalling underlies malignant progression of medulloblastomas, which are the most common and deadly juvenile brain tumours in humans. In particular,

we have shown that genetic reduction of SerpinE2, an extra-cellular modulator of the Hedgehog pathway interferes with progression of pre-neoplastic lesions to medulloblastomas in the *Ptch1* heterozygous mouse model. Recently, we have obtained evidence that inactivation of *Grem1* also lowers the incidence of medulloblastomas in *Ptch1* mice.

In summary, we combine systematic genetic and cell-biochemical analysis with *in silico* simulations to gain insights into the complexity of the signaling networks that orchestrate normal organ and tissue development and aberrant malignant progression of medulloblastomas.

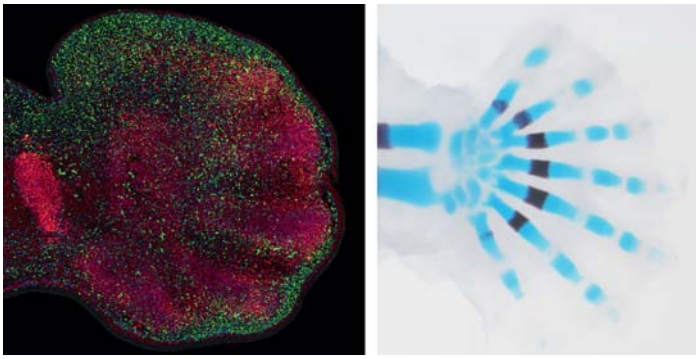

Fig. 1: The left panel shows the distribution of proliferating (green) and chondrogenic progenitors (red) in a mouse limb bud lacking the Gli3 transcriptional regulator. In the anterior part, mesenchymal cells continue to proliferate as their exit to chondrogenic differentiation is delayed (upper part; left panel). This delayed exit from the cell-cycle and overexpansion of progenitors results in formation of additional digits (7 in total – an anterior polydactyly; right panel). Gli3 restains the autopod to pentadactyly by acting as a gatekeeper at the exit of cell-cycle exit toward chondrogenesis (for details see Lopez-Rios et al., 2012)

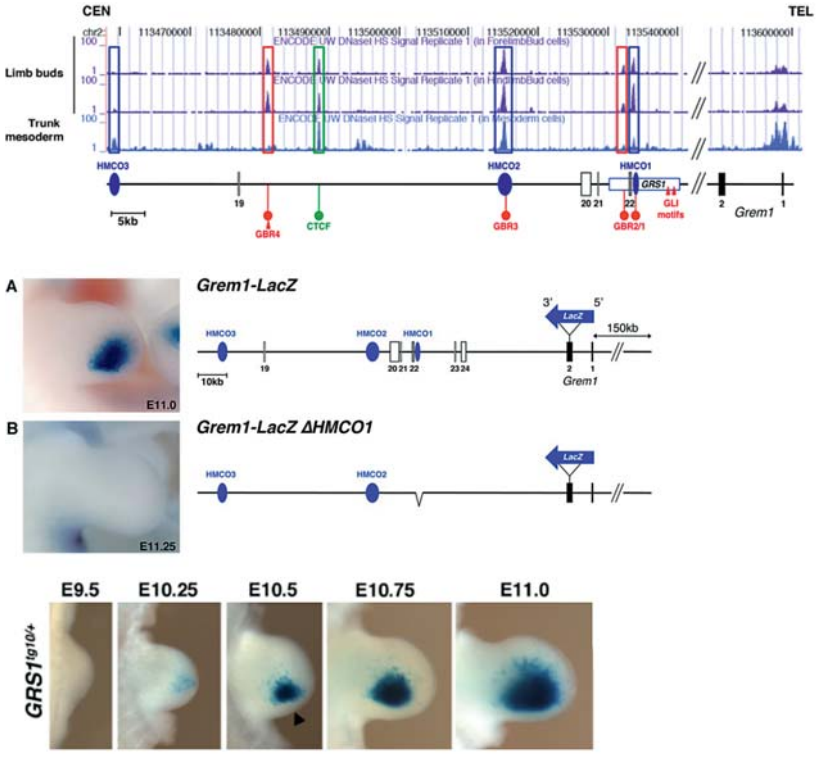

Fig. 2: The large, about 250 kb *Grem1 cis*-regulatory genomic landscape harbours multiple and distant *cis*-regulatory regions that regulate the dynamic *Grem1* expression during limb bud development (top panel). BAC transgenic approaches (middle panel) and detailed mapping resulting using smaller conventional transgenic constructs (lower panel) identify the core cis-regulatory regions that control *Grem1* transcription in mouse limb buds (for details see Zuniga et al., 2012).

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Neural Stem Cells

Neurogenesis

Notch Signaling

RNA Regulation

Neural Fate Determination

Embryology and Stem Cell Biology

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Genetic analysis of neurogenesis in the developing and adult mouse brain

The mammalian brain has a remarkable capacity for plasticity, critical for learning and memory and compensating for damage. However, the brains of mammals regenerate poorly, failing to generate appreciable numbers of new neurons. This was thought to be due to a lack of stem and progenitors cells in the postnatal brain, including in humans. It is accepted that the adult brain contains neural stem cells (NSCs) and in some species continue to generate neurons. Newborn adult neurons in the lateral forebrain and in the hippocampus contribute to olfaction and specific forms of memory, respectively. Using conditional mouse genetics and cell culture we are trying to understand the molecular mechanisms controlling NSC activity and fate during development and adulthood. We are also trying to elucidate why active NSCs are lost in infant humans and during aging.

Notch signaling and its control of neurogenesis

We and others have demonstrated the importance of Notch signaling in regulating NSC maintenance and cell fate during development. Notch controls the expression of a cascade of transcription factors critical for progenitor maintenance and differentiation. Although transcriptional regulation of target genes is pivotal, we have addressed other mechanisms controlled by Notch signaling and which contribute to neurogenesis. We performed genome-wide studies of NSC transcriptomes following ablation of Notch. We study a cluster of RNA-binding proteins and components of the microRNA pathway that are regulated downstream of Notch in NSCs. We showed that the RNAseIII Drosha and DGCR8/Pasha, key components of the microRNA microprocessor, play a central role in neurogenesis in the embryonic mouse forebrain. Drosha negatively regulates expression of the proneurogenic transcription factors Neurogenin2 and NeuroD1 through binding to and cleaving hairpin structures in their mRNAs to destabilize the transcripts. We continue to study the role on mRNA destabilization to expand our understanding of the targets of the Drosha/DGCR8 complex in NSCs of the mammalian brain.

Diversity in the adult forebrain NSC population

NSCs in the adult forebrain are confined to niches in the subventricular zone (SVZ) and hippocampus (Fig. 1). Most NSCs are quiescent, proliferate sporadically, and produce committed neurogenic progeny. The SVZ and hippocampus retain a remarkable capacity for repair indicating the importance of NSCs in the regeneration process. We uncovered a difference in Notch dependence between active neurogenic and dormant regenerative NSCs. Loss of Notch1, one member of the four-strong Notch family, results in a selective loss of activated neurogenic NSCs. In contrast, dormant NSCs are Notch1-insensitive until stimulated by a lesion to the SVZ. Hence, Notch1 is a key component of the adult SVZ niche promoting maintenance of neurogenic and activated NSC (Fig. 2). Using genetic markers and lineage tracing we addressed NSC heterogeneity in the adult brain. We identified subpopulations of adult SVZ NSCs (type 1-3) and found that activated NSCs express brain lipid binding protein (BLBP, FABP7) and epidermal growth factor (EGF) receptor (Fig. 1A). They proliferate in response to EGF and are a major clonogenic population in the SVZ. We found a similar population of BLBP-expressing mitotic progenitors in the postnatal human brain and these activated NSCs are diminished in aged rodents and humans leaving only dormant stem cells.

We also identified morphologically distinct NSCs in the hippocampus of adult mice that can shuttle between mitotic activity and quiescence (Fig. 2B). Radial and horizontal NSCs respond selectively to neurogenic and patho-

physiological stimuli including physical exercise and epileptic seizures. We found that the age-related reduction in neurogenesis in the hippocampus correlates with a loss of active horizontal NSCs and their transition to a quiescent state rather than a loss of all stem cells. These geriatric quiescent NSCs can be reactivated to rejuvenate hippocampal neurogenesis in aged mice. The selective response of NSC populations and reversible quiescence has important implications for adaptive learning and for regenerative therapy.

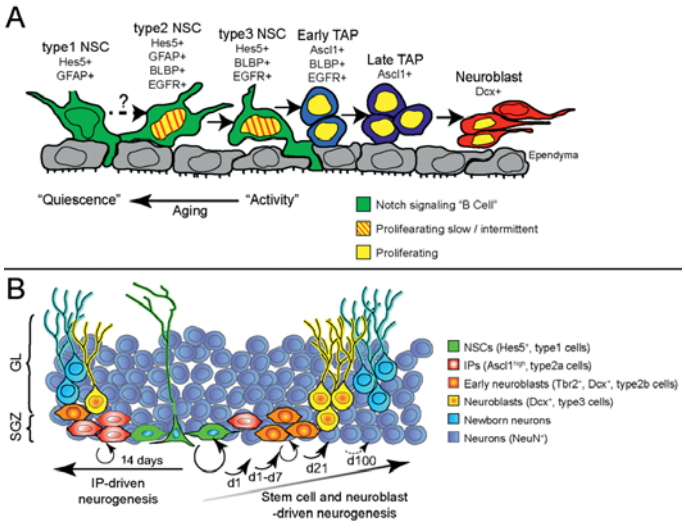

Fig. 1: NSC diversity in the adult SVZ and hippocampus.
A. Adult SVZ NSCs are Notch-dependent and express the canonical Notch target gene *Hes5* (green cells). The *Hes5*$^+$ NSC pool is subdivided into GFAP$^+$ type1, GFAP$^+$BLBP$^+$ type2 and BLBP$^+$ type3 populations which all show radial glia-like features and contact the lateral ventricles through ependymal pinwheel structures. BLBP$^+$ type2 and type3 NSCs do not express transient amplifying progenitor (TAP) markers (Ascl1) but express the EGF receptor and many are mitotically active whereas type1 NSCs are mitotically inactive. Type2 and type3 NSCs are diminished with age and this correlates with reduced neurogenesis, reduced mitotic activity and a shift towards type1 NSCs.
B. In the adult hippocampus type1 *Hes5*$^+$ NSCs can be quiescent or mitotically active. In the intermediate progenitor (IP) driven model, NSCs generate type2a Ascl1^{high} IPs through asymmetric cell division. Type2a IPs undergo symmetric self-replicating progenitor divisions before generating a pool of committed progenitors (type2b cells) and give rise to post-mitotic neuroblasts and newborn granule cells. In our stem cell and early neuroblast-driven neurogenesis model, active NSCs divide multiple times to generate type2a Ascl1^{high} IPs which produce mitotic Tbr2$^+$ early neuroblasts (type2b cells) without amplification. The type2b early neuroblasts divide to increase and expand the precursor pool before generating post-mitotic neuroblasts and newborn neurons. The average time taken for these process deduced from the lineage tracing experiments is shown in days. GL – granule cell layer, SGZ – subgranular zone.

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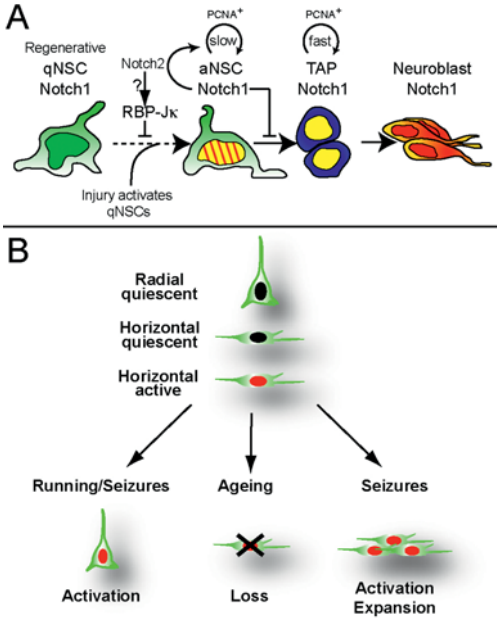

Fig. 2: Stem cell regulation of SVZ and hippocampal neurogenesis and regeneration.
A. Neurogenesis in the adult SVZ is maintained by active NSCs (aNSCs) which divide slowly (dashed yellow nucleus). aNSCs depend upon Notch1 for maintenance, self-renewal and neurogenesis, in the absence of Notch1, these aNSCs are compromised. Fast dividing transient amplifying progenitors (TAPs; yellow nucleus) express Notch1 and give rise to neuroblasts that migrate to the olfactory and generate neurons. Quiescent NSCs (qNSCs) depend on RBP-J but not Notch1 They enter the cell cycle in response to injury to regenerate the SVZ. In the absence of Notch1, activated NSCs fail to self-renew and effectively reinstate adult neurogenesis, resulting in a reduction of both qNSCs and a loss of aNSCs. RBP-J blocks cell cycle entry of qNSC and promotes aNSC maintenance. Slow/fast: Rate of cell division. PCNA: Proliferating Cell Nuclear Antigen.
B. We identified three distinct NSCs in the adult hippocampus. These stem cell populations respond differently to pathophysiological stimuli. Radial type1 NSCs are quiescent, remain in the hippocampus of aged animals and respond to physical exercise and epileptic seizures. Horizontal type1 NSCs are divided into active and quiescent pools and cells can transit between proliferation and quiescence. Active horizontal NSCs are lost during aging and expanded following epileptic seizure.

Hematopoiesis
Leukemia
Myeloproliferative Neoplasms
Kinase Inhibitors
Transgenic Mice
Familial Predisposition
Genomics

Experimental Hematology

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Molecular pathogenesis of myeloproliferative neoplasms

Myeloproliferative neoplasms (MPN) are a group of blood diseases characterized by aberrant proliferation of precursors of the myeloid, erythroid and megakaryocytic lineages. They represent clonal stem cell disorders with a tendency towards leukemic transformation. Currently, no curative therapy is available. MPNs comprise 3 entities: polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). The goal of our studies is to advance the understanding of the molecular events that cause MPN and influence its progression to leukemia. A recurrent mutation in exon 14 of the Janus kinase 2 (JAK2) gene that substitutes a valine to phenylalanine at position 617 (JAK2-V617F) is present in a majority of patients with MPN, in particular PV. This mutation leads to constitutive activation of the Jak2 kinase and represents a driver for the proliferation of hematopoietic cells. Activating mutations in exon 12 of JAK2 have been described in patients with PV that are negative for JAK2-V617F. Despite this progress, several questions remain unsolved including how a single JAK2 mutation causes three different MPN phenotypes, what other genes might be involved and what determines the progression to acute leukemia. We are examining these questions by combining three approaches: molecular studies in patients with sporadic MPN, genetic analysis of familial MPN and transgenic mouse models that mimic the human disease.

Analysis of clonal progression in MPN

In a subset of patients with sporadic MPN additional somatic mutations can either precede or occur after the acquisition of JAK2-V617F. The order of events can be established by examining individual colonies grown from patient's peripheral blood. Using this approach, we found that there is no fixed order of events between different gene mutations and a substantial proportion of patients even displayed two independent clones, i.e. bi-clonal disease. These results are compatible with the hypothesis that a clonal pre-JAK2 event is present in a subset of stem cells in these patients. This pre-JAK2 mutation predisposes these stem cells to acquire JAK2-V617F and in rare cases also to acquire other mutations and progress to acute leukemia (Fig. 1). We use next generation sequencing to efficiently screen patients for the presence of more than one gene mutation and then determine the clonal architecture of disease using the single colony approach to compare this data with clinical outcome and prognosis.

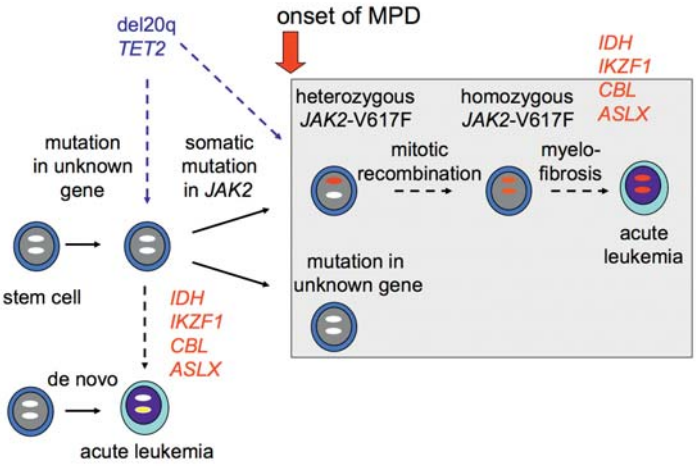
Familial predisposition for MPN

Familial syndromes resembling MPN can be grouped into two classes:

1. Inherited disorders with high penetrance and polyclonal hematopoiesis.
2. Hereditary predisposition to true MPN, with low penetrance, clonal hematopoiesis and occurrence of somatic mutations, e.g. in JAK2-V617F. We identified mutations in the thrombopoietin (THPO) gene as the cause for an inherited form of thrombocythemia in several families with a "class 1" phenotype. In another family we found a previously described mutation in the gene for the thrombopoietin receptor (MPL). However, in the majority of families neither THPO nor MPL is mutated. The search for these disease genes is ongoing. Families with "class 2" phenotype are more common than generally assumed. These germ line mutations increase the likelihood of acquiring a somatic JAK2-V617F mutation. We are using genetic methods to map the locus for these pre-disposing mutations.

Mouse models for MPN

We generated JAK2-V617F transgenic mice that express a human JAK2-V617F gene. This conditional construct can be activated by Cre-recombinase. Depending on the mode of Cre-mediated activation, these mice developed a phenotype resembling ET with strongly elevated platelet counts or a PV-like phenotype with increased hemoglobin, thrombocytosis and neutrophilia. We are using this mouse model for pre-clinical screening of Jak2 inhibitors and other potential therapeutic agents. Currently, a major focus of our research is to examine the nature of the MPN initiating stem cells and their interactions with the bone marrow microenvironment.



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Connection to Clinical Practice

Improved diagnostics of MPN and new therapeutic approaches: from bench to bedside

(C. Bucher and J. R. Passweg)

The first challenge in the diagnostic approach to MPN is to distinguish between reactive changes (i.e. elevated blood counts secondary to other diseases) and true MPN (i.e. primary disease of the bone marrow cells). In a second step, the definitive category of the MPN, i.e. polycythemia vera (PV), essential thrombocythemia (ET) or primary myelofibrosis (PMF), has to be established. The discovery of the JAK2-V617F mutation has completely changed the diagnostic approach to patients with a suspected MPN. Since JAK2-V617F is absent in reactive thrombocytosis, erythrocytosis or leukocytosis, the presence of a JAK2 mutation can be used to exclude such reactive changes. JAK2-V617F can be found in about 95% of patients with PV and in approximately 50–60% of PMF and ET and also in other chronic myeloid neoplasms, such as refractory anemia with ringed sideroblasts and thrombocytosis (RARS-T). Therefore, mutation screening for JAK2-V617F cannot distinguish between different forms of MPN and blood counts, erythropoietin levels and additional parameters (bone marrow trephine and cytology, cytogenetic analysis) and search for less frequent mutations (JAK2 exon 12, MPL, thrombopoietin, Epo-receptor and others) have to be taken into consideration. We developed a simultaneous multiplexed screening by next generation sequencing that allows a molecular classification of patients with myeloid neoplasm.

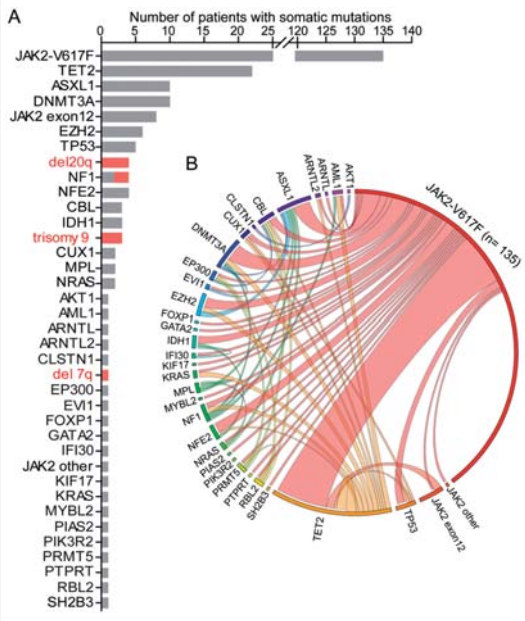

Fig. 2: Frequency and distribution of mutations in patients with MPN.

(A) Number of patients with mutations in the genes as indicated. Red bars and red text indicate chromosomal aberrations. (B) Circos plot illustrating co-occurrence of somatic mutations in the same individual. The length of the arc corresponds to the frequency of the mutation, while the width of the ribbon corresponds to the relative frequency of co-occurrence of two mutations in the same patient.

Nutrients

Taste Receptors

Gastrointestinal Tract

Signaling Molecules

Metabolic Control

Gastro-enterology

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The tasting gut

The global obesity problem supports the urgent need for research that aims to understand the basic mechanisms that regulate food intake, appetite and body weight. The variety of nutrients in developed countries presents modern humans with a problem: what are the best combinations of macro- and micronutrients that promote health and longevity in each individual? Why and how does an individual select among foods of different compositions and amounts when they are in surplus? Is food selection only due to particular taste and odor receptors or are there connections among taste and metabolic needs for energy or regulatory processes? That is, is there a genetic basis for food intake? These basic science questions have significant implications for individuals and society since an increasing percentage of the population is obese or overweight.
The gut "tastes" what we eat – sweet, bitter, fat, umami or amino acids – in much the same way as the tongue and through the use of similar signaling mechanisms. Indeed, the gut comprises a whole network of taste receptors and transporters in different cell types that cross-regulate each other's expression. The result is the release of gut hormones that help to control blood glucose levels but also communicate with the brain to control appetite and satiety when food reaches the gut. These taste receptors in the gut may therefore function as inhibitors of excess food intake, and their malfunction may play a role in the development of obesity, diabetes, and related metabolic conditions. This research has stimulated interest in these hormones as targets for the development of anti-obesity therapies. Our research focuses on the physiology, mechanism of action and interactions of the gut hormones GLP-1 and PYY as satiety hormones in relation to taste receptor activation in normal weight subjects and in patients with obesity before and after bariatric surgery to prepare the path for potential therapeutic application.

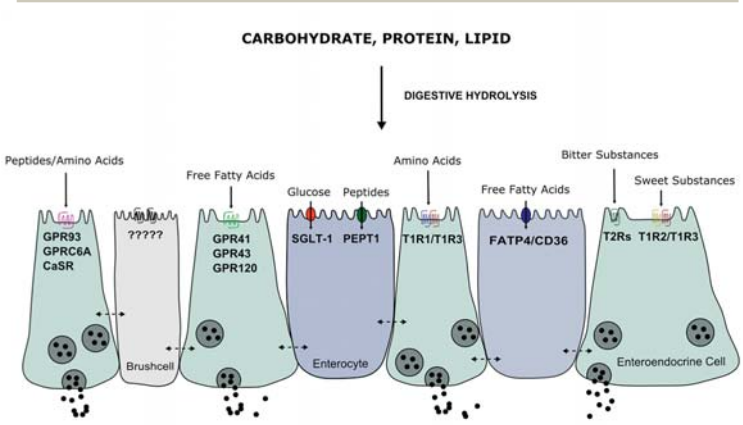

Fig. 1: Schematic of the GI mucosal cell layer indicating the principal nutrient-sensing molecules involved in ghrelin, CCK, GLP-1 and PYY secretion. Ingested macronutrients are subjected to digestive hydrolysis. The resultant free fatty acids, peptides, amino acids, oligosaccharides, and monosaccharides, as well as other sweet and bitter compounds, activate specific G-protein coupled receptors (GPCRs) and nutrient transporters expressed on the apical surface of enteroendocrine cells (green), brush cells (tan) and enterocytes (violet). This activation, via second and third messengers (not shown), leads to secretion of ghrelin, CCK, GLP-1 and PYY from the basolateral side of enteroendocrine cells into the GI-tract lamina propria. These peptides are then either absorbed into the hepatic portal vein and act on distal targets in an endocrine mode, or act locally on neurons or other cells in a paracrine mode, or drain into the lymph. Note that activated non-secretory cells communicate with adjacent cells via other mediators such as acetylcholine, vasoactive intestinal peptide, neuropeptide Y, adenosine 5'-triphosphate (ATP), serotonin (5-HT), etc.

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- Wölnerhanssen B, Peterli R, Steinert RE, Peters T, Borbely Y, and Beglinger C. 2011. *Effects of post-bariatric surgery weight loss on adipokines and metabolic parameters: Comparison of laparoscopic Roux-en-Y gastric bypass (LRYGB) and laparoscopic sleeve gastrectomy (LG) – a prospective randomized trial.* Surg Obes Related Dis 7(5):561-8.
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Disease Modelling

Stem Cells

Polycystic Ovary Disease

Cell Migration

Ubiquitination

Insulin Resistance

Gynecological Endocrinology

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Ovarian and reproductive disease modelling using stem cell technology

We combine human stem cell technology, including embryonic stem technology and induced pluripotent stem cells derived from cells of individuals with defined phenotypes, to unravel the origin of signaling pathways in early development and reproductive disease, with special focus on gonadal development. In addition, neurodevelopmental toxicity was selected as a model to understand variations in the differentiating potential of the five human embryonic stem cell lines derived and characterized in our laboratory. In addition, we are using induced pluripotent stem cells and long-term culture of differentiating embryoid bodies in closed bioreactors to unravel the mode of inheritance of insulin resistance in women diagnosed with polycystic ovarian syndrome, which is closely linked to the metabolic syndrome and diabetes mellitus. Using large data sets, either published by other research groups or created in our own laboratory, new genes or gene products involved in ovarian function are selected and their functions elucidated, most notably EULIR, an E3 ligase with affinity to the inhibin binding protein. As the EULIR-knockout mouse created in our unit displays a strong phenotype, including neural tube defects and subfertility, we are now in the process of elucidating its function. EULIR regulates cell migration through its interaction with a number of proteins involved in the formation and turnover of focal adhesions.

Selected Publications

- Kossowska-Tomaszczuk, K., Pelczar, P., Güven, S., Kowalski, J., Volpi, E., De Geyter, Ch., Scherberich, A. (2010) A novel three-dimensional culture system allows prolonged culture of functional human granulosa cells and mimics the ovarian environment. Tissue Engineering Part A 16: 2063-2073.
- Sieuwerts, A.M., De Napoli, G., van Galen, A., Kloosterboer, H.J., de Weerd, V., Zhang, H., Martens, J.W.M., Foekens, J.A., De Geyter, Ch. (2011) Hormone replacement therapy dependent changes in breast tissue of healthy postmenopausal women. Molecular Oncology 5: 504-516.
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Liver

Innate Immunity

Hepatocellular Cancer

Viral Hepatitis

Interferon

Jak-STAT Signaling

Hepatology

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Innate immune reactions to hepatitis C virus infection

Hepatitis C virus (HCV) infections become chronic in the majority of infected individuals, and chronic hepatitis C (CHC) can lead to cirrhosis and hepatocellular carcinoma. The innate immune system is central to host-virus interactions during the entire natural course of the disease (Heim MH, Journal of Hepatology, 2013). The HCV NS3/4A protease efficiently cleaves and inactivates two important signaling molecules in the sensory pathways that react to HCV pathogen associated molecular patterns (PAMPs) to induce interferons (IFNs), i.e. mitochondrial anti-viral signaling protein (MAVS) and Toll-IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF). Despite this viral escape mechanism, the innate immune system strongly reacts to HCV within the first days after infection. The sensory pathways, the type(s) of IFNs involved and the cellular source of IFNs are largely unknown. After 4 to 8 weeks, HCV specific T cells are recruited to the liver. IFN-γ stimulated genes get strongly expressed in the liver (Dill MT et al, Gastroenterology, 2012). In about 30% of patients the virus is eliminated during the acute phase of the infection by T cell mediated anti-viral mechanisms. In the remaining 70% of patients, HCV persists for decades. During this phase, T cell derived IFN-γ cannot be detected any more in liver biopsies. Instead, in about half of the patients, hundreds of type I or III IFN stimulated genes become again strongly expressed (Sarasin-Filipowicz M et al, PNAS, 2008). However, this innate immune reaction is ineffective against HCV. Moreover, patients with constitutive IFN stimulated gene (ISG) expression have a poor response to treatment with pegylated IFN-α (pegIFN-α) and ribavirin (Sarasin-Filipowicz M et al, MCB, 2009; Makowska Z et al, Hepatology, 2011; Dill MT et al, Gastroenterology, 2011). The viral escape mechanisms that protect HCV from IFN-mediated innate immune reactions are not entirely understood, but might involve blockade of ISG protein translation at the ribosome, localization of viral replication to cells with refractory IFN signal transduction pathways or to cell compartments that are not accessible to anti-viral IFN-stimulated effector systems. Recently, genetic variations near the IL28B (IFN-λ3) were found to be strongly associated to spontaneous clearance of HCV and to response to treatment with pegIFN-α and ribavirin (Rauch et al, Gastroenterology, 2010). The finding supports a central role of the innate immune in host-viral interactions (Heim MH, Nature Rev Immunology, 2013). The signaling pathways that link genetic variants of IL28B with immune answers to HCV remain to be elucidated.

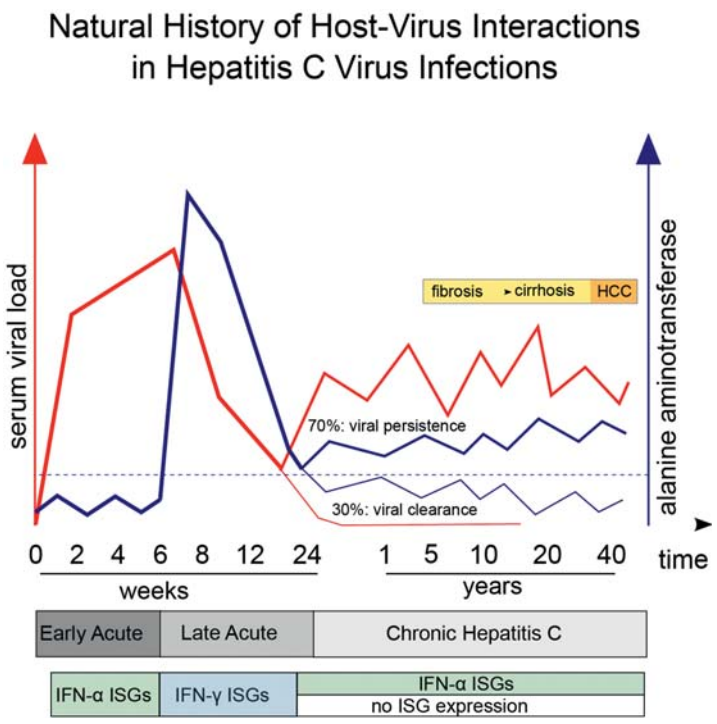

Fig. 1: In the early phase of acute infection (the first 4-8 weeks), HCV induces a type I or III IFN response that restricts viral replication (green box). With the recruitment of HCV specific T cells in the late phase of AHC, the gene expression profile in the liver switches to an IFN-γ pattern (blue box). In late AHC, viral replication is strongly inhibited, and in about 30% of patients, HCV is completely eliminated. In 70%, HCV persists, and can induce again a type I or III IFN response in about half the patients (upper green box). The other patients have little to no activation of ISGs in the liver (empty box). Changes in serum HCV load (red), alanine aminotransferase levels (ALT) (blue) and IFN stimulated gene expression (top bar) are shown. The dashed line shows the upper limit of normal for ALT.

Selected Publications

- Dill, M.T., Duong, F.H., Vogt, J.E., Bibert, S., Bochud, P.Y., Terracciano, L., Papassotiropoulos, A., Roth, V., and Heim, M.H. (2011). Interferon-induced gene expression is a stronger predictor of treatment response than IL28B genotype in patients with hepatitis C. Gastroenterology *140,* 1021-1031.
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- Heim, M.H. (2013). 25 years of interferon-based treatment of chronic hepatitis C: an epoch coming to an end. Nat Rev Immunol *13,* 535-542.
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- Makowska, Z., Duong, F.H., Trincucci, G., Tough, D.F., and Heim, M.H. (2011). Interferon-beta and interferon-lambda signaling is not affected by interferon-induced refractoriness to interferon-alpha in vivo. Hepatology *53,* 1154-1163.

Cochlea

Hair Cells

Inner Ear

Inner Ear Research

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Dr. Soledad Levano (postdoctoral fellow)
Dr. Vesna Radojevic (postdoctoral fellow)
Dr. Cristian Setz (postdoctoral fellow)

Understanding molecular events in the inner ear to restore hearing

Hearing loss has a huge impact on the affected individual as well as on society. Not only is one baby out of 1000 born with hearing loss, but more than 50% of over-65s suffer from hearing loss. Hearing loss of adult onset is one of the ten leading causes of disability-adjusted life years globally.

Inner ear
The complex architecture of the inner ear, named the labyrinth by early anatomists, houses the senses of hearing and balance. The main functions of the outer and the middle ear are transducing and amplification of sound, while the cochlea in the inner ear is the auditory sensory organ. The cochlea propagates mechanical signals as waves in fluid and membranes, and finally transduces them to nerve impulses. Its core component is the organ of Corti, which is distributed along the partition separating fluid chambers in the coiled tapered tube of the cochlea. The organ of Corti contains 16000 hair cells in each cochlea. The outer hair cells of the organ of Corti are mechanically active, while the inner hair cells of the same organ convert the stimulus into neuronal impulses via afferent synapses to the dendrites of primary auditory neurons (spiral ganglion neurons).

Hearing loss causes
Hearing loss can be caused by damage to either external, middle or inner ear. Today, hearing loss caused by diseases of the external and the middle ear can be treated satisfactorily, while disorders affecting the inner ear cannot. Often, only prosthetic devices offer some help. For mild to moderate hearing loss conventional hearing aids are used, while for profound hearing loss cochlear implantation is the standard of care today. Loss of or damage to hair cells and/or neuronal cells, which are the sensorineural elements of the inner ear, results in a so-called sensorineural hearing loss.However, the hair cells are the most vulnerable elements in the cochlea, and damage to them is the most common cause of sensorineural hearing loss. When the hair cells are lost from the adult organ of Corti, spiral ganglion dendrites retract and are possibly lost. Total loss of hair cells can result in degeneration of some cochlear neurons. Hair cell damage may result from a variety of causes, including genetic disorders, infectious diseases, overexposure to intense sound and certain drugs. As exposure to intense sound, drugs and diseases accumulates with aging, so the loss of sensorineural elements in the cochlea progresses with it, and many individuals experience noticeable hearing difficulty later in life.

Prevention of hearing loss?
Hearing loss due to sensorineural damage has been recognised for over a century and experiments to promote understanding of the phenomenon date from the early 1900s. Since cochlear hair cells of mammals, unlike those of fish and birds, do not regenerate, sensorineural hearing loss is often progressive and irreversible. Until recently, damage to cochlear hair cells and neurons has been regarded as an inevitable consequence of age, genetic conditions or exposure to certain environmental stimuli. This made avoidance of potentially harmful stimuli the primary means of protecting sensorineural structures. However, in the last few years, progress has been made in the understanding of hair cell damage. Our laboratory is studying different aspects of hair cell damage and death: we are investigating the somatostatinergic system in the inner ear, molecular aspects of age-related hearing loss, and we are employing the cre-lox system to generate inner-ear specific knock-out mice of genes involved in hair cell death. In addition, we are studying guidance cues for spiral ganglion neurites, in order to enhance the interaction of these neurites with cochlear implant electrodes.

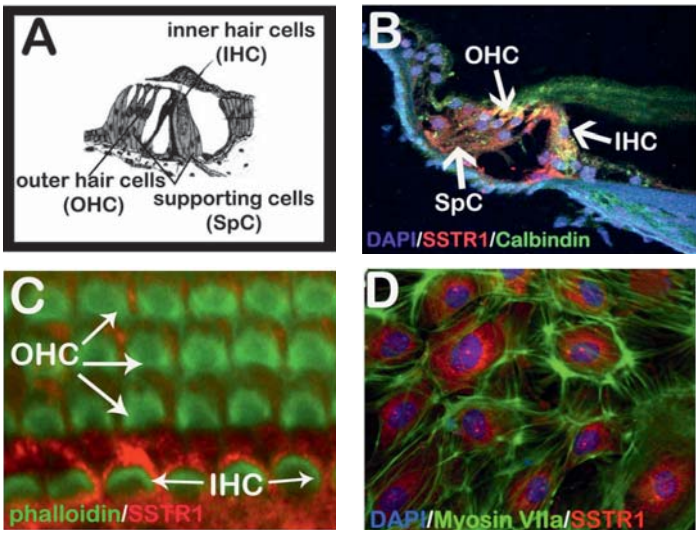

Fig. 1: Expression of SSTR1.
A) Diagram of the organ of Corti
B) Expression of SSTR1 in the adult mouse cochlea
C) SSTR1 expression in a organ of Corti explant
D) SSTR1 expression in neurosensory cell culture. (Radojevic et al. 2011)

Selected Publications

- Setz C, Brand Y, Radojevic V, Hanusek C, Mullen PJ, Levano S, Listyo A, Bodmer D. 2011 Matrix Metalloproteinases 2 and 9 in the Cochlea: Expression and Activity after Aminoglycoside Exposition. Neuroscience 181:28-39.
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- Brand Y, Setz C, Levano S, Listyo A, Chavez E, Pak K, Sung M, Radojevic V, Ryan AF, Bodmer D. 2011 Simvastatin protects auditroy hair cells from gentamicin-induced toxicity and activates Akt signaling in vitro. BMC Neurosciences 12:114.
- Radojevic V, Brand Y, Bodmer, D. 2012 Somatostatin Receptor Types 1 and 2 in the Developing Mammalian Cochlea. Develop. Neuroscience 34(4):342-53.
- Brand Y, Sung M, Chavez E, Wei E, Pak K, Housley G, Bodmer D, Ryan AF. 2013 Neural cell adhesion molecule L1 modulates type I but not type II inner ear spiral ganglion neurite outgrowth in an in vitro alternate choise assay. Journal of Mol Neuroscience (2013) 51:663-670.

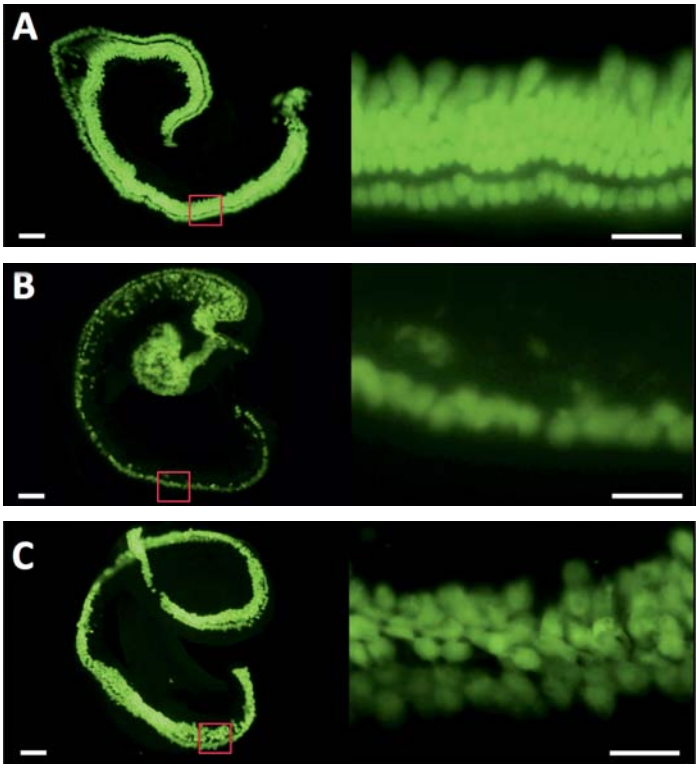

Fig. 2: Effect of simvastatin on gemtamicin-induced hair cell damage in vitro. Overview of the organ of Corti on the left (scale bar 100um) and high magnification on the right (scale bar 20um). A) Control, B) Gentamicin 50 µM, C) Gentamicin 50 µM + Simvastatin 100 µM. Simvastatin had a protectiv effect on gentamicin-induced hair cell damage. (Brand et al. 2011)

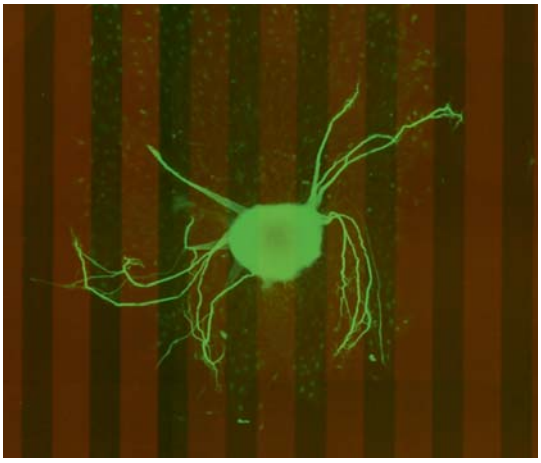

Fig. 3: Rat spiral ganglion explant cultured on L1 versus PLL alternating stripe pattern. There was a strong tendency for neurites to terminate upon/or grow along the L1 substrate. Stripe width 100 µm. (Brand et al. 2013)

Angiogenesis,
Notch Signaling
Liver Vasculature
Portal Hypertension
Angiosarcoma

Liver Biology

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Notch signaling in the hepatic microcirculation

Notch signaling in the liver
The Notch signaling pathway is an evolutionary highly conserved pathway regulating fundamental cellular processes including stem cell maintenance, cell fate specification, differentiation, proliferation, and apoptosis during development and renewal of adult tissues. Notch signaling plays an indispensable role in embryonic vascular development with global knockout of Notch1, Jagged1, Notch1/Notch4 double mutation, and Dll4 haploinsufficiency being embryonic lethal due to vascular defects. However, the role of Notch1 in the adult liver vasculature is not entirely elucidated. Our research focuses on the function of the Notch1 pathway in the ladult hepatic microcirculation in physiologic conditions as well is in chronic liver disease.
The hepatic sinusoidal microvasculature is composed of a highly specialized and differentiated endothelium characterized by a discontinuous, fenestrated endothelial lining without a basement membrane. The liver sinusoidal endothelial cells (LSECs) comprise half of the nonparenchymal cells of the liver and play a central role in many physiological functions, including liver organogenesis, liver regeneration, control of the vasomotor tone, scavenger functions, blood cell trafficking, prevention of hepatic stellate cell (HSC) activation, and production of paracrine factors such as hepatocyte growth factor and interleukin-6. Chronic liver disease can lead to endothelial dysfunction and dedifferentiation of LSECs with loss of fenestrations, deposition of a basement membrane, and surface expression of CD31, a process that has been termed sinusoidal capillarization and that precedes liver fibrosis. The determinants regulating the normal, differentiated LSEC phenotype are only incompletely understood. Paracrine secretion of vascular endothelial growth factor (VEGF) by hepatocytes and HSCs as well as autocrine production of nitric oxide by endothelial NO synthase have been shown to be essential to maintain the phenotype of LSECs. However, other signaling pathways, the absence of shear stress, and interendothelial and heterotopic contact with HSCs might be additional important determinants of LSEC differentiation. We have therefore developed a Notch1 KO animal model in order to study the function of Notch1 in the hepatic microcirculation composed of LSEC.

Notch1 signaling is required for vascular homeostatsis of the hepatic microvasculature
We have previously shown that MxCre-induced knockout of Notch1 led to nodular regenerative hyperplasia (NRH) of the liver, in the absence of fibrosis, with a persistent increase in proliferation of LSECs. Notch1 deletion caused LSEC dedifferentiation, vascular remodeling of the hepatic sinusoidal microvasculature, intussusceptive angiogenesis, and dysregulation of ephrinB2/EphB4 and endothelial tyrosine kinase (Tek). Time-course experiments revealed that vascular changes preceded node transformation. MxCre Notch1lox/lox mice had reduced endothelial fenestrae and developed portal hypertension and hepatic angiosarcoma over time. In contrast, mice with hepatocyte-specific disruption of Notch1 had a normal phenotype.

Notch1 functions as a tumor-suppresor in the liver vasculature

Notch1 has been shown to be an oncogene in many solid tumors and in leukemia. Depending on the tissue type, Notch1 also rarely can function as a tumor-suppressor gene (ie, in squamous cell carcinoma of skin and lung). Recently, blockade of Dll4 as well as loss of heterozygosity of Notch1 led to vascular tumors in animals. In our model, we observed persistent and cell autonomous LSEC proliferation, dedifferentiation, and eventually malignant transformation. Therefore, our findings of spontaneous development of hepatic angiosarcoma establish Notch1 also as a tumor-suppressor gene in LSECs.

Connection to Clinical Practice

Downregulation of Notch1 and EphrinB2 in patients with nodular regenerative hyperplasia

Nodular regenerative hyperplasia (NRH) is a rare liver disease characterized by small regenerative nodules without fibrosis and can cause portal hypertension. Aetiology and pathogenesis of NRH remain unclear. We have recently shown that Notch1 knockout induces NRH with portal hypertension through vascular remodelling in mice. The aim of this study was to analyse histological and clinical data of NRH patients and to explore if the endothelial pathways identified in our NRH mouse model are also regulated in human NRH. Patients were identified retrospectively from the pathology database. Clinical and laboratory patient data were retrieved. mRNA expression was measured in liver biopsies from a subset of NRH patients. Results: Diagnosis of NRH was confirmed in needle biopsies of 51 patients, including 31 patients with grade 1, 12 patients with grade 2 and 8 patients with grade 3 NRH. Grade 3 nodularity significantly correlated with the presence of portal hypertension: 50% of the patients with grade 3 NRH vs. 6.5% with grade 1 ($P = 0.0105$). mRNA expression analysis in liver biopsies from 14 NRH patients and in primary human sinusoidal endothelial cells revealed downregulation of identical genes as in the murine NRH model, which are implicated in vascular differentiation: Notch1, delta-like 4 (Dll4) and ephrinB2.

In this large NRH needle biopsy cohort, we demonstrated that advanced nodularity correlates with presence of portal hypertension. Downregulation of the endothelial signalling pathways Dll4/ Notch1 and ephrinB2/EphB4 supports the hypothesis that human NRH is caused by a sinusoidal injury providing first insights into the molecular pathogenesis of this liver condition.

Selected Publications

- Rothweiler, S., Terracciano, L., Tornillo, L., Dill, M.T., Heim, M.H., and Semela, D (2013). Downregulation of the Endothelial Genes Notch1 and EphrinB2 in Patients with Nodular Regenerative Hyperplasia. Liver International (Epub ahead of print).
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Functional Adaptation
Subchondral Bone Plate
Stress Distribution
Mechanical Properties
Osteoarthrosis
Osteochondral Unit

Musculoskeletal Research

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Subchondral plate mineralization patterns reflect physiological and pathological joint conditions

Meniscectomy leads to early changes in the mineralization distribution of the subchondral bone plate

It is generally recognized that the subchondral bone plate (SBP) is involved in the development of osteoarthritis (OA). However, the pathophysiological significance is not yet clear. The goal of this study was to investigate the extent of the changes that occur in SBP of the tibial plateau in the early stages of experimental OA.
Forty-three female rabbits were assigned to 5 experimental (n=8 each group) and one sham group (n=3). OA was induced by medial meniscectomy in the right knee, the left knee served as control. 2, 4, 8, 12, and 24 weeks after meniscectomy, cartilage damage was evaluated, and bone mineral density (BMD) and mineralization distribution of the SBP was measured by computed tomography osteoabsorptiometry (CT-OAM).
Cartilage damage started 2 weeks after meniscectomy with surface roughening. Cartilage defects increased over time. 24 weeks postoperatively, subchondral bone was exposed. As early as 2 weeks after meniscectomy, BMD in the medial tibial plateau decreased significantly. BMD increased again and reached the values of the non-operated knee 12 weeks postoperatively (fig.1). In addition, already 4 weeks after meniscectomy a significant shift of the densitiy maximum on the medial tibial plateau, which is normally centrally located toward the margin was observed (fig. 2).
In conclusion, the results of this study contribute to the concept of early involvement of the SBP in the development of OA. The hypothesis that changes in the SBP occur simultaneously to cartilage damage was confirmed.

A comparison of subchondral bone mineralization between the glenoid cavity and the humeral head

Mineralization distribution of the subchondral bone plate can be used as a marker for long-term stress distribution in diarthrodial joints. Severe injuries or pathological changes of the glenohumeral joint often end in osteoarthritis, where shoulder arthroplasty has become the treatment of choice.
The aim of this study was to investigate the mineralization patterns of both joint partners of the glenohumeral joint by means of CT-OAM and to compare them with each other. The material consisted of 57 shoulder specimens. To evaluate a correlation between age and localization of subchondral mineralization maxima, the Chi-square test correlation test was applied.
Forty-nine glenoid cavities (86 %) showed a bicentric mineralization distribution pattern with anterior and posterior maxima, only 8 glenoid cavities (14 %) revealed a monocentric mineralization pattern with anterior maxima. Forty-five humeral heads (79 %) showed a bicentric distribution pattern with anterior and posterior maxima, 12 humeral heads (21 %) could be classified as monocentric with a centro-posterior pronounced maximum (fig.3).
Herewith we could demonstrate that stress distribution in both joint partners of the glenohumeral joint is inhomogeneous and characteristically bicentric due to the physiological incongruity. Monocentric mineralization patterns can result as a cause of age-related loss of incongruity.

Density and strength distribution in the human subchondral bone plate of the patella

The aim of this study was to map the strength distribution of the human patella and correlate it to the subchondral bone plate density obtained by means of computed tomography osteoabsorptiometry (CT-OAM).
Measurements were performed at 34 standardized points on each patella. The mineralization patterns of the subchondral bone plate of 20 patellae

were displayed with the help of CT-OAM. False-coloured distribution patterns for our measurements were generated. The mechanical strength was determined at the same points by indentation testing.
We showed that neither the density nor the mechanical strength is distributed homogeneously but exhibited regular, reproducible distribution patterns which mirror long-term stress distribution in articular surfaces. A direct correlation was found between both parameters in the subchondral bone plate. The correlation of density and mechanical strength makes CT-OAM a valuable tool to assess and monitor changes in vivo.

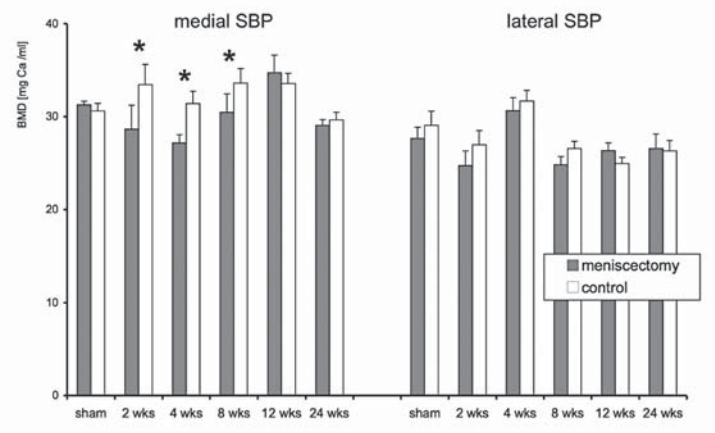

Fig. 1: BMD (mean ± SD) of the medial and lateral subchondral bone plate in all groups. As early as 2 weeks after meniscectomy there is a significant decrease in the calcium concentration. From the 12th postoperative week onwards the difference is no longer discernible (* p\0.05 versus control).

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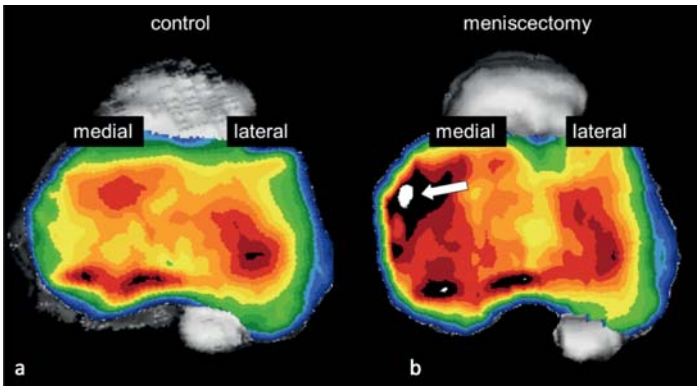

Fig. 2: Illustrative example of subchondral mineralization distribution in the tibial joint surface. The various colors represent different levels of Hounsfield units (HU). **a)** On the lateral tibial joint surface on the non-operated left side there was one centrally located density maximum, on the medial tibial joint surface two density maxima, one of them was located ventrally and another dorsally. **b)** 24 weeks postoperatively a shift of the ventrally located density maximum to the margin (arrow) was observed.

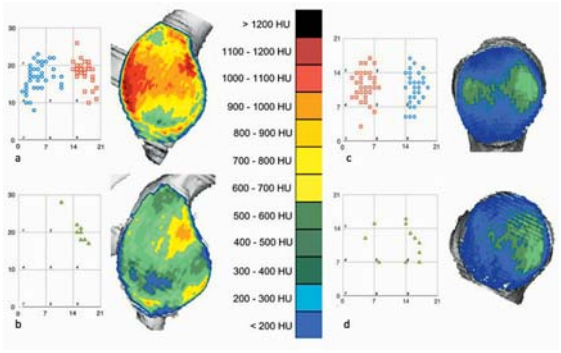

Fig. 3: Summary chart of all maxima in bicentric (a) and monocentric (b) glenoid cavities (anterior is on the right, posterior is on the left) and of all maxima in bicentric (c) and monocentric (d) humeral heads (anterior is on the left, posterior is on the right) each of them compared with a typical example of the mineralization distribution.

Heart Failure

Cardiac Progenitor Cells

Receptor Tyrosine Kinases

Cell Adhesion

Myocardial Remodeling

Myocardial Research

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Cardioprotection and regeneration

Using *in vitro* cellular and *in vivo* mouse models of ischemic, metabolic and cancer therapy-related cardiomyopathy, our research aims at an improved understanding of cardiac homeostasis and its response to disease. In particular, we explore molecular mechanisms governing viability and regenerative potential of the myocardium by studying cues cardiac cells receive from their microenvironment, and how the cells are affected by these cues. The ultimate goal of our research is to advance therapeutic strategies for cardioprotection and regeneration.

One major focus of our laboratory is on the interaction of cardiac cells with their surrounding matrix. Specifically, we seek to identify novel adhesion-dependent regulators of cardiomyocyte and cardiac progenitor cell function, and to delineate their role in post-ischemic myocardial remodeling. Previous studies from our laboratory provided novel insights into how the abundantly expressed laminin-dependent adhesion molecule β1-integrin mediates cardiomyocyte hypertrophy and survival (Häuselmann, 2011; Rosc-Schlüter, 2012), which could be relevant for the control of the cardiac growth response and for cardiac tissue preservation upon injury. Besides cell survival, maintenance and restoration of cardiac cellularity require a delicate balance between proliferation and differentiation of cardiac precursor cells. We recently found that adhesion of cardiac progenitor cells and myoblasts to matrix proteins affects the intracellular distribution, stability and activity of Yes-associated protein (YAP, Fig. 1), thereby regulating proliferation and cell cycle progression of cardiac precursor cells. Adhesion-induced cell cycle regulation may represent an important step in the process of new cardiomyocyte formation, in particular at the site of cardiac injury, where matrix proteins are upregulated and cell density is low. Current efforts are focusing on the precise mechanisms and *in vivo* implications of YAP-associated cardiac progenitor cell regulation and on the identification of a possible molecular switch to gain control over cardiac cell cycle. Eventually, results from these studies could help improve regeneration of the injured heart.

Another major topic of our research is to understand how fms-like tyrosine kinase 3 (Flt3) and its ligand (FL), an early-acting hematopoietic cytokine, affect cardiac myocytes and progenitor cells, and their significance to cancer therapy-induced cardiotoxicity and ischemic heart disease. Flt3 is a receptor tyrosine kinase expressed on hematopoietic progenitor cells and a regulator of progenitor cell proliferation and differentiation. Flt3 belongs to the cancer kinome and high levels and/or activity-enhancing mutations of Flt3 are present in acute myeloid leukemia cells, turning Flt3 into a major target of tyrosine kinase inhibitors (TKIs). TKIs can cause cardiomyopathy, but the underlying mechanisms are incompletely understood. We recently identified Flt3 as a cytoprotective system in the ischemic heart. Specifically, we demonstrated that Flt3 activation with recombinant FL protects cardiomyocytes against oxidative stress-induced apoptosis *in vitro* and *in vivo* and improves post-myocardial infarction remodeling and function in mice (Pfister, 2013). We further identified cardiac fibroblasts as source of myocardial FL and found enhanced FL-secretion in response to hypoxia (Fig. 2). In addition, we demonstrated that cardiac progenitor cells express Flt3 and also secrete FL, raising the possibility that FL/Flt3 acts as an intrinsic para- and/or autocrine system in the heart. In fact, preliminary data support the hypothesis that intrinsic Flt3-signaling plays a role in the regulation of cardiac progenitor cells. Further studies delineating the role of intrinsic Flt3-signaling in the heart and its implications with regard to cancer-therapy related cardiotoxicity and other types of heart disease associated with oxidative stress are ongoing.

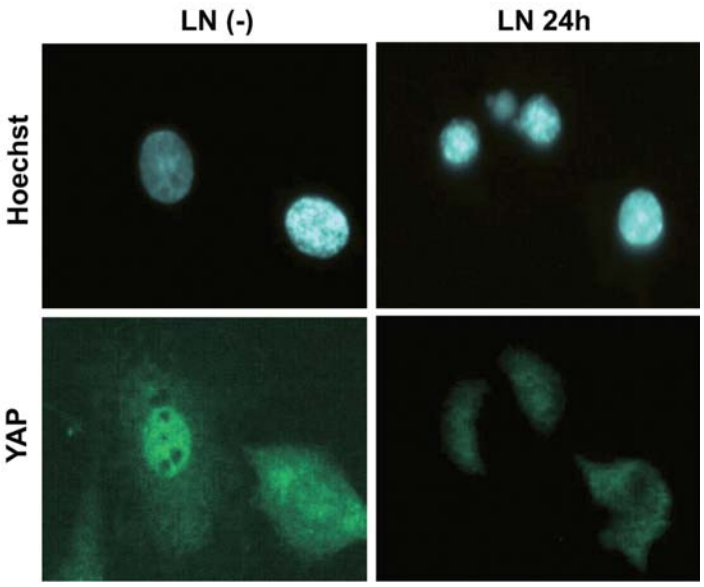

Fig. 1: Laminin (LN) induces redistribution and degradation of Yes-associated protein (YAP) in cardiac progenitor cells. YAP: Yes-associated protein (green); Hoechst: nuclear counterstaining (blue)

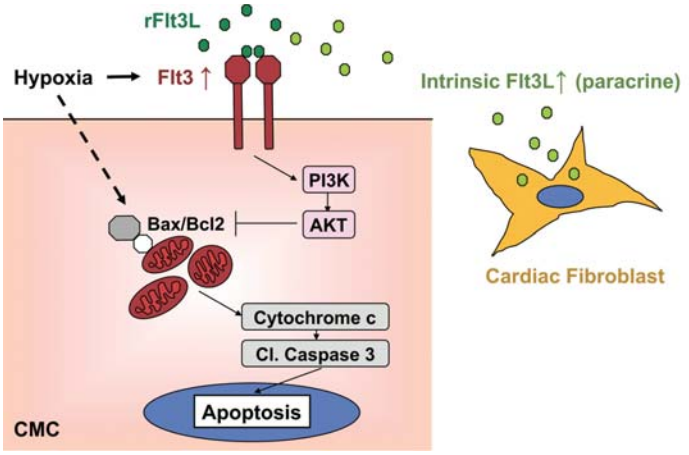

Fig. 2: Proposed model of Flt3-mediated cardioprotection. Flt3: fms-like tyrosine kinase 3 receptor; Flt3L: Flt3 ligand; rFlt3L: recombinant Flt3L; CMC: cardiomyocyte; cl.: cleaved, in press.

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Transducible Artificial Transcription Factor

Endothelin

Neurodegeneration, Mitochondria

Meningothelial Cells

Optic Nerve

Ocular Pharmacology and Physiology

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Understanding and treating optic nerve degeneration

Degeneration of retinal ganglion cells (RGCs) is the underlying cause for vision loss associated with a variety of diseases among them glaucoma, dominant optic atrophy and others affecting in the order of 100 million patients worldwide. Neurodegenerative stress such as mitochondrial dysfunction and associated oxidative stress as well as insufficient ATP production are at the heart of RGC degeneration. These neurodegenerative stress conditions can be brought about by genetic mutation or individual predisposition together with environmental factors. But also pathophysiological conditions such as ischemia due to dysregulated perfusion or optic nerve compartmentalization constitute stress conditions leading to the death of irreplaceable RGCs.
Our work focused on understanding basic mechanisms leading to neurodegeneration in connection to pathophysiologically relevant stress such as optic nerve compartmentalization, oxidative stress or mitochondrial dysfunction. Furthermore, to translate insight about dysregulated ocular perfusion and its connection to degenerative eye disease back into the clinic, the development of novel therapeutics in the form of transducible artificial transcription factors to influence retinal perfusion and to treat associated diseases was at the center of our work.

The optic nerve microenvironment
The optic nerve is a part of the central nervous system and as such protected by meninges and cerebrospinal fluid forming a specific microenvironment. Meningothelial cells (MECs) are the cellular component of the meninges and based on clinical observation we hypothesize that MECs are involved in pathophysiological processes in the optic nerve especially during optic nerve compartmentalization. Our recent studies revealed that MECs are a part of the central nervous system immune response and are involved in cytokine release and phagocytic clearance of bacteria and apoptotic cells.

Mitochondrial maintenance and degeneration of neuronal cells
Maintenance of mitochondrial function is a multi-tiered process involving protein degradation, autophagic removal of dysfunctional mitochondrial subunits, and ultimately mitochondria-dependent programmed cell death, but also regulation of mitochondrial dynamics. We studied the role of the mitochondrial ubiquitin ligase MARCH5 known to be involved in the regulation of mitochondrial fission and the degradation of proteins associated with amyotrophic lateral sclerosis and Joseph-Machado disease. We found that inhibition of MARCH5 function prevented mitochondrial fragmentation and apoptosis in cell models for elevated pressure, oxidative stress and Aβ-induced neurodegeneration (Figure 1). Our data further confirms the mitochondrial involvement in neurodegeneration and establishes modulation of mitochondrial fission potentially through inhibition of MARCH5 as potential therapeutic strategy for the treatment of neurodegenerative disorders.

Transducible artificial transcription factors
Regulating gene expression at will holds great promise for the treatment of many diseases. Advances in the understanding of zinc finger based natural transcription factors allow now for the generation of artificial transcription factors (ATFs) capable of targeting any human gene with high selectivity (Figure 2). Depending on the transcriptionally active protein domain incorporated into such ATFs, up- as well as down-regulation of gene expression is achievable. However, for ATFs to be useful as therapeutics, delivery to the site of action needs to be considered. By employing protein transduction technology further improved by us, we achieved efficient delivery of active

ATFs to the nuclear compartment. Medically relevant genes targeted by us using transducible ATFs include a modulator of mitochondrial morphology involved in optic nerve degeneration and ETRA and ETRB coding for the endothelin receptors A and B, respectively (Figure 3). We envision using these ATFs for the treatment of endothelin-dependent ocular blood flow dysregulation and optic nerve degeneration. First experimentation in animal models to assess distribution and efficacy of ETRA-specific ATFs in the ocular compartment are underway.

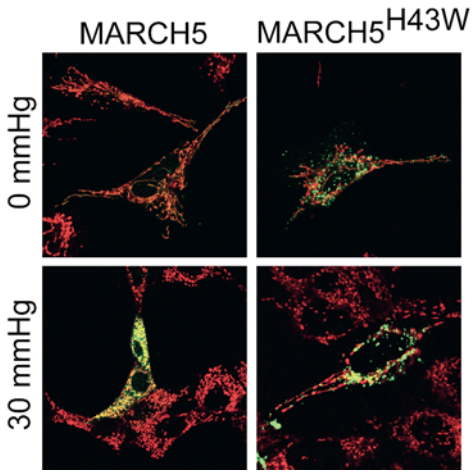

Figure 1: Inactivation of MARCH5 inhibits pressure induced mitochondrial fragmentation. Neuronal cells (RGC5) transfected with MARCH5 or dominant-negative MARCH5H43W were incubated under ambient (0 mmHg) or glaucoma-mimicking (30 mmHg) elevated pressure conditions. Cytochrome c staining was used to reveal mitochondrial morphology (green – MARCH5; red – cytochrome c).

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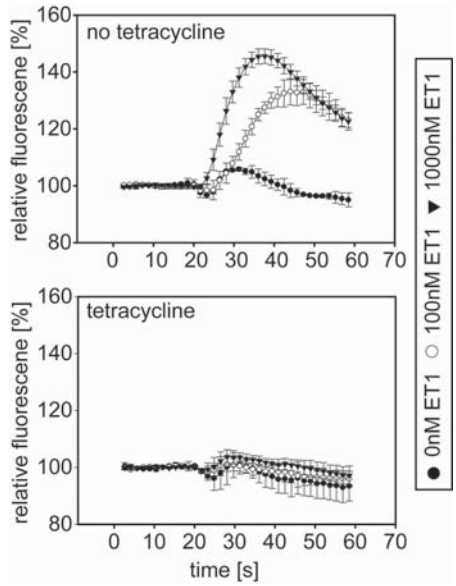

Figure 2: ETRA-specific ATF blocks endothelin dependent calcium signaling. 293 FlpIn-TRex cells containing ETRA-specific ATF under the control of a tetracycline-inducible promoter were induced for 24 hours with tetracycline (lower panel) or left untreated as control (upper panel). Intracellular calcium flux was measured following stimulation with 0, 100, or 1000 nM endothelin (ET-1) using an automated fluorescence plate reader (FlexStation 3, Molecular Devices). Please note the complete loss of ET-1 dependent calcium signaling in cells expressing ETRA-specific ATF compared to control cells.

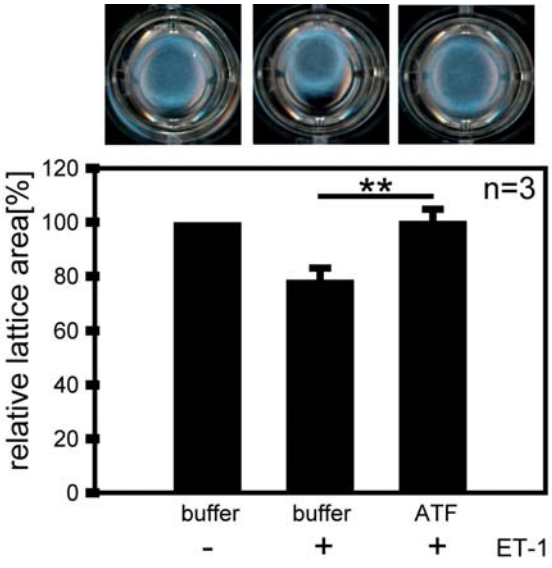

Figure 3: ETRA-specific ATF prevents smooth muscle cell contraction. Primary human uterine smooth muscle cells (hUtSMCs) were embedded into a collagen matrix to from lattices. Lattices were treated with buffer or ETRA-specific transducible ATF protein for 72 hours before treatment with 0 or 100 nM ET-1. Lattice area was measured after 8 hours and normalized to buffer treated lattices not induced with ET-1. Please note the marked difference in area between treated and control lattices consistent with a block of ET1 dependent hUtSMC contraction following ATF treatment.

Inflammation

Fibroproliferation

Human Primary Lung Cell Cultures

Signalling Pathways

Airway Remodelling

Pulmonary Cell Research

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Cell differentiation in chronic inflammatory lung diseases

Chronic inflammatory lung diseases (asthma, COPD, fibrosis) are increasing worldwide. None of the diseases can be cured, only the symptoms can be controlled by anti-inflammatory and muscle relaxant drugs. The pathogenesis of chronic inflammatory lung diseases is not well understood as the existing theory of deregulated immune response cannot explain most pathologies sufficiently.

Asthma affects more than 235 million people (www.who.int/mediacentre/factsheets/fs307/en) and is a lifelong disease. Asthma is not curable, only symptoms can be controlled. Environmental triggers, mainly allergens trigger 55% of all asthma attacks. The remaining 45% are due to stress, exercise, or changes in temperature, humidity or dust content. In 1922, asthma was described as increased thickening of airway wall smooth muscle bundles. After decades of studying the role of the immune response in asthma the idea that altered smooth muscle function is a major asthma cause has been revived in the past two decades. Destroying smooth muscle cells by heat in patients with severe asthma kept them symptom free for 5 years. However, this form of bronchoscopic therapy can only be applied to few asthma patients. To better understand the origin of asthma we isolate human diseased and non-diseased smooth muscle cells from bronchoscopic biopsies to study and investigate the conditions leading to the asthmatic phenotype. Together with our colleagues from Australia, Canada, Greece and Netherlands we observed that asthmatic smooth muscle cells produce: (i) pro-inflammatory Wnt-proteins, and (ii) short forms of hyaluronic acid. Furthermore, the cells produce less of anti-inflammatory semaphorin 3a. We also provided data indicating that the mechanism controlling the translation of the cell differentiation controller C/EBP-alpha from mRNA to protein is impaired in asthma and can be down-regulated by house dust mite allergen. Why this translation impairment is specific for C/EBP-alpha is still unclear.

COPD was the 4th most frequent cause of death worldwide in 2008 (www.who.int/mediacentre/factsheets/fs315/en) with increasing prevalence. COPD cannot be cured, only the symptoms can be controlled and progression might be slowed down by anti-inflammatory and muscle relaxing drugs. In high income countries cigarette smoking is the major cause of COPD. In low-income countries the exposure to dust, smoke, and ashes (open fire cooking, heating) are independent causes. Exposure to risk factors early in life (pregnancy, first 6 years) increases the susceptibility to develop COPD later. The mechanisms that lead to COPD are unclear and involve the immune response as well as tissue forming cells of the small airways. Using human diseased versus non-diseased lung cells we have shown, that other cellular pathologies as in asthma can be found in cells of COPD patients, including: (i) deregulated C/EBP translation control by cigarette smoke, (ii) faster degradation of hyaluronic acid contributing to inflammation, and (iii) lower response to the anti-remodelling action of steroids and beta2-agonists, which are the most prescribed drugs in COPD therapy. Our translational COPD studies are embedded in large clinical trials.

Lung Fibrosis is a rare but devastating non-malignant disease of the lung. Bronchiolitis obliterans (BO) develops as a severe complication following allogeneic stem cell transplantation. We have been one of the few groups worldwide being able to isolate and maintain epithelial cells and fibroblasts from fibrosis and BO patients. In collaboration with the University of Bern we have recently shown that the differentiation of epithelial cells isolated from human lung tissue is different from that of non-diseased lungs. Our data indicates that IPF lungs contain larger numbers of pluripotent cells which display an epithelial phenotype, but can differentiate into a large variety of other cell types. Beside our studies to understand the role of these cells in fibrosis and BO we use these rare cells to investigate beneficial effects of novel anti-fibrotic drugs.

Connection to Clinical Practice

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Translational research in inflammatory lung diseases

The clinical research is supported by a SNSF professorship and focused on large cohorts of patients with COPD, selected groups of patients with asthma, lung fibrosis and bronchiolitis obliterans (GvHD of the lung) following allogeneic stem cell transplantation. Risk factor analyses are performed and new biomarkers tested for predictors for exacerbations and survival of COPD patients. The clinical setting of a close collaboration of pulmonologists with other clinical specialists (thoracic surgeons, hematologists, pathologists etc) and basic researchers as well as the close location of the clinic of pneumology and the DBM give us the unique opportunity to culture cells from the human lung of patients with specific lung diseases. Human epithelial cell cultures, fibroblasts and bronchial smooth muscle cell cultures are taken from bronchoscopic biopsies and immediately processed. This approach allows to identify pathophysiological pathways of the diseased cells and to test new medications on a cellular level. Components of tissue remodelling are studied as well as the influence of allergic and non allergic stimuli. In parallel to the translational research projects numereous investigator driven noncommercial randomised studies are performed to optimise patients safety during bronchoscopy in patients with and without COPD. Under the lead of the Basel clinical research team collaboration with groups in Germany, Italy, Spain, France, the Netherlands, Belgium, UK, Serbia and Greece have been established.

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Neutrophil Extracellular Traps (NETs)

Pregnancy

Preeclampsia

Inflammation

Rheumatoid Arthritis

Prenatal Medicine

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Role of neutrophil NETs in pregnancy and auto-inflammatory conditions (rheumatoid arthritis)

The previous focus of our lab was on the analysis of cell-free DNA, liberated by dying cells into the circulation. Our specific aim was to use this for the development of non-invasive tests for prenatal diagnosis, by exploiting the finding that during pregnancy, the placenta releases DNA of fetal origin into the maternal circulation. By the use of sophisticated genome sequencing approaches this approach has successfully made the transition from bench to clinic, and is being offered commercially by a number of providers e.g., Sequenom, Verinata, Natera etc [1].

For this reason we sought a new research focus and have turned our attention to a rather pivotal question that still remained, namely, what was the source of this maternal cell-free DNA and why was it elevated in certain conditions like preeclampsia?

A hint of what the source of the maternal cell-free DNA could be was provided by a fascinating discovery in 2004, which described that upon activation circulatory neutrophils could expel their nuclear DNA into the extracellular environment, where it could serve as a sticky trap to ensnare and kill microorganisms. Consequently these structures were called NETs or neutrophil extracellular traps.

Prompted by these findings we investigated whether such events occurred in preeclampsia, and were startled to determine that NETs could be triggered in isolated neutrophils by treatment with inflammatory placental debris as found in preeclampsia. We also detected large numbers of NETs directly in the intervillous space in preeclamptic placentae [2].

Since preeclampsia is a highly inflammatory condition, we were curious whether such events occurred in other auto-inflammatory disorders e.g. rheumatoid arthritis (RA). In collaboration with Prof. P. Hasler, KSA, Aarau, we observed that the levels of cell-free DNA were much higher in serum samples than plasma, and that this was significantly different to the same analysis performed on healthy control samples [3]. As this difference was not evident in rapidly processed EDTA plasma samples, it implied that during the clotting process during serum generation, a population of cells was liberating their DNA into the extracellular milieu in an increased amount. We postulated that these could be neutrophils undergoing NETosis.

Recently we were able to confirm that this suspicion via the detection of NETs associated neutrophil granular components in serum samples, as well as in culture supernatants of isolated neutrophils *(Chowdhury et al., submitted)*.

These studies furthermore implicated enhanced NETosis in the underlying aetiology of RA, in that the presence of the deiminating enzymes PAD2 and 4 on these extracellular structures may drive the generation of auto-antibodies against citrullinated peptides (ACPA).

We are now focusing our attention on the regulation of NETosis in normal pregnancy, and how this is dysregulated under a number of different complications *(Giaglis et al., in preparation)*.

In a collaborative study with Prof. M. Bühler, FMI, we concluded our analysis of the role of miRNA in regulating trophoblast differentiation. This study indicated that the expression of miR-455 and miR-210 was sensitive to hypoxic conditions and their abundance was enhanced in preeclamptic placentae. Bioinformatic analysis of predicted targets suggested that MUC1 could be regulated by miR-455-3P, a feature we confirmed by gene transfections assays. These experiments also linked HIF2A in this regulatory pathway *(Lalevee et al., in preparation)*.

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Connection to Clinical Practice

Development of new markers to detect preeclampsia

A major obstetrical concern is that no reliable diagnostic aids exist to identify pregnancies at risk for preeclampsia, as well as a number of other complications. In past experiments we examined whether the maternal plasma proteome can be examined by quantitative isobaric labeling (iTRAQ) mass spectrometric approaches. In order to evaluate the suitability of such an approach we first examined pregnancies bearing a fetus with trisomy 21, as this involved the sole addition of a single chromosome, and as such was not as complex as multifactorial conditions such as preeclampsia. This study indicated that this approach was feasible, as we detected quantitative changes in concentration of known screening markers such as betaHCG.

In a subsequent study we extended this study and examined 1st trimester maternal plasma samples from pregnancies at risk for preeclampsia, in which we also detected potentially new screening biomarkers, as well as confirming changes in currently used ones. In order to extend the scope and variety of potential biomarkers, we examined for changes in gene expression in placental samples obtained from normal healthy deliveries and those affected by preeclampsia.

It is now our intention to establish a large biobank to test the efficacy of our newly found biomarkers more rigorously. In addition, we will determine whether we can enhance the specificity and sensitivity of current screening tests, for the detection of late onset preeclampsia. We will also determine whether proteomic approaches can be used to develop new biomarkers for preterm labour or gestational diabetes.

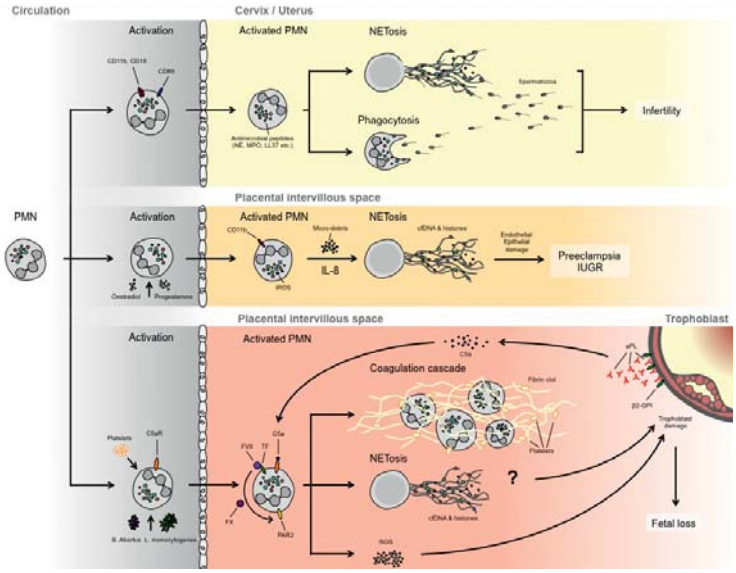

Fig. 1: Hahn, S., I. Hosli, and O. Lapaire, Non-invasive *prenatal diagnostics using next generation sequencing: technical, legal and social* challenges. Expert Opin Med Diagn, 2012. **6**(6): p. 517-28.

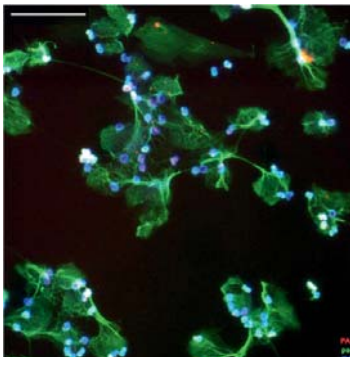

Fig. 2: Gupta, A.K., et al., *Induction of neutrophil extracellular DNA lattices by placental microparticles and IL-8 and their presence in preeclampsia.* Hum Immunol, 2005. **66**(11): p. 1146-54.

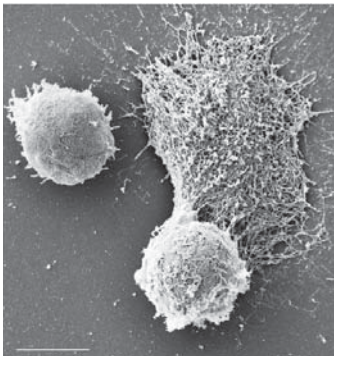

Fig. 3: Zhong, X.Y., et al., *Increased concentrations of antibody-bound circulatory cell-free DNA in rheumatoid arthritis.* Clin Chem, 2007. **53**(9): p. 1609-14.

Selected Publications

- Gupta, A.K., Joshi, M.B., Philippova, M., Erne, P., Hasler, P., Hahn, S., and Resink, T.J. (2010). Activated endothelial cells induce neutrophil extracellular traps and are susceptible to NETosis-mediated cell death. FEBS letters *584,* 3193-3197.
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- Kolla, V., Jeno, P., Moes, S., Lapaire, O., Hoesli, I., and Hahn, S. (2012). Quantitative proteomic (iTRAQ) analysis of 1st trimester maternal plasma samples in pregnancies at risk for preeclampsia. Journal of biomedicine & biotechnology *2012,* 305964.
- Lapaire, O., Grill, S., Lalevee, S., Kolla, V., Hosli, I., and Hahn, S. (2012). Microarray screening for novel preeclampsia biomarker candidates. Fetal diagnosis and therapy *31,* 147-153.

T-cadherin

Atherosclerosis

Cancer

Growth Factor RTKs

Signal Transduction

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T-cadherin and tissue homeostasis

Cadherins comprise a family of cell-cell adhesion proteins critical to architecture and function of tissues in developing and adult organisms. T-cadherin (T-cad) is peculiar in structure: it lacks transmembrane and cytosolic domains and is membrane-anchored via a GPI moiety, implying distinct functions and molecular circuitry. We have hypothesized that the "functional predestination" of T-cad is the control of tissue architecture through both "guiding" navigation of moving structures or segregation of functional tissue compartments and "guarding" integrity of functionally connected tissue layers. T-cad expression is altered in cardiovascular disorders and cancers. We focus on delineating T-cad-dependent cellular functions and signal pathway utilization in vascular and cancer cells, with the broader goal being to define basic biological mechanisms underlying T-cad-mediated control of tissue homeostasis.

T-cadherin in the vasculature
T-cad expression is upregulated on vascular smooth muscle cells (SMC) and endothelial cells (EC) during atherosclerosis and restenosis. Using in vitro and in vivo approaches we have previously identified angiogenic and survival functions for T-cad in EC. Relevant signal effectors include PI3K/Akt/mTOR, GSK3β, β-catenin, p38MAPK and RhoA/Rac GTPases and membrane molecular adaptors include Grp78, ILK and integrin β3. T-cad is shed from activated/damaged EC as a component of microparticles (MP), which via homophilic-based interactions can serve local/distal protective signaling functions during conditions of endothelial injury or dysfunction. These studies support T-cad upregulation as a modulator of survival/reparative behavior of EC in cardiovascular disorders. However, sustained T-cad up-regulation in EC can have deleterious consequences of promoting endothelial insulin resistance (Fig. 1). One explanation for the ability of T-cad to impact insulin signaling is that its adaptor recruitment activates signaling responses that converge with the insulin-insulin receptor (IR)-dependent pathway at the level of common intracellular targets. Alternatively, T-cad may directly increase IR pathway activity through T-cad/IR co-association in lipid raft domains. Control of the IR signal cascade by T-cad represents a novel cadherin-based signaling pathway at the crossroads of vascular and metabolic disorders. Current investigations address the contribution of T-cad to SMC (patho)biology.

T-cadherin in cancer
T-cad has been implicated in cancer progression primarily on the basis of genetic and epigenetic studies. We apply multidisciplinary in vitro and in vivo experimental approaches to understand the cellular functions and molecular mechanisms of action of T-cad in tumour biology. Immunohistochemical analysis of human skin showed that decrease/loss of T-cad in squamous cell carcinoma (SCC) tumours occurs in association with acquisition of the invasive/malignant phenotype (Fig. 2A). In vitro and in vivo investigations show that T-cad loss in SCC promotes cell elongation, cell cluster disorganization, motility and invasive/metastatic potential (Fig. 2B and C), effects which are due to enhanced EGFR pathway activity. T-cad gain or loss respectively recruit or release EGFR from lipid raft domains, suggesting that T-cad acts as a negative auxiliary regulator of EGFR in SCC. We postulated that modulation of EGFR activity by T-cad could be a regulatory mechanism common to other RTKs. Using several cancer cell lines including prostate and colon carcinoma cells we found that T-cad regulates activity of both EGFR and IGF-1R and their cross talk. This is relevant to evolution of drug resistance.

Concluding remarks

Modulation of growth factor receptor tyrosine kinase activity and cross-talk may be a common mechanistic principle underlying T-cad-dependent control of vascular and epithelial (tumour) cells behavior (Fig. 3). T-cad dysfunction carries consequences for receptor complementarity and cell migration, proliferation, invasion, differentiation and polarity, which are key determinants of vascular (dys)function/remodeling and of tumour progression/metastasis.

Fig. 1

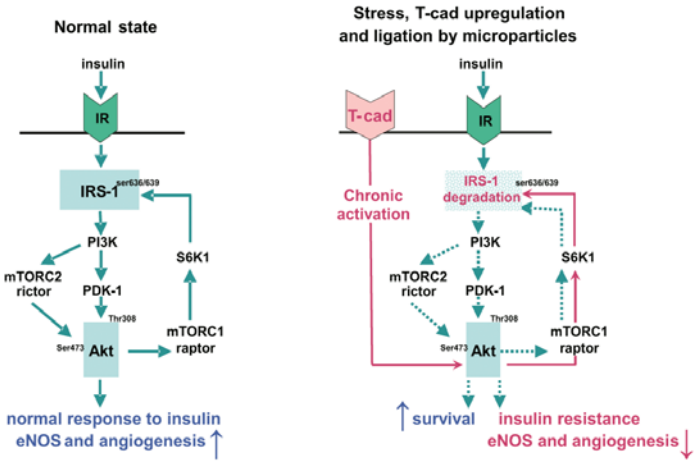


Fig. 2

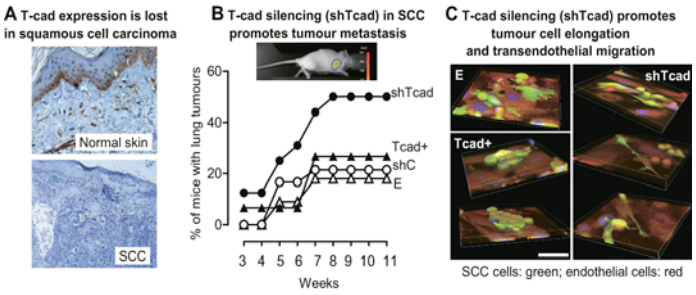
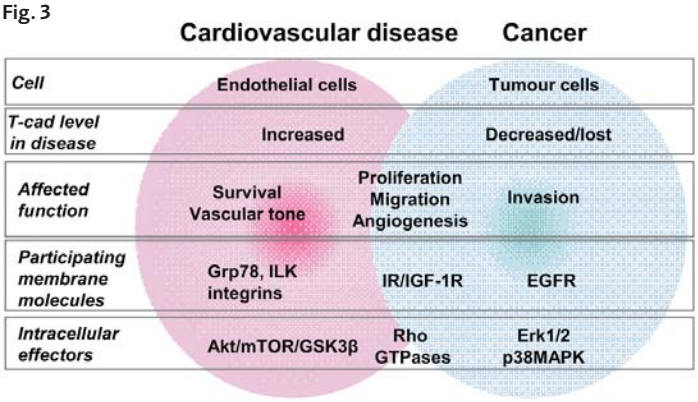


Fig. 3



Connection to Clinical Practice

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Detection of early atherosclerosis and the vulnerable patient

Atherosclerosis is clinically silent long before plaque rupture and ensuing cardiovascular events. Detection of preclinical atherosclerosis and the shift from "indolent" to acute ischemic disease has great clinical benefit, yet remains a diagnostic challenge. Atherosclerosis profiling using a multimarker diagnostic paradigm comprising physical characteristics and lesional composition of vessels, endothelium function, plasma biomarkers of endothelial damage/dysfunction (ED) and inflammatory status and specific relationships between these parameters could improve risk stratification of patients and determination of treatment measures. We have compiled a wide-ranging clinical data base on a large cohort of study subjects (including healthy individuals, patients without cardiovascular risk factors, and patients with different stages of atherosclerosis defined on the basis of angiographic and IVUS data) and a corresponding bank of plasma samples and blood leukocyte isolates for biomarker analysis. We have established links between vascular shear stress and coronary vessel calcification and between ED and morphology of coronary atherosclerotic plaques. We have also demonstrated that levels of plasma T-cad correlate with ED, invoking T-cad as a biomarker of early atherosclerosis. Currently we are examining the link between oxidative processes in the vessel and initiation of atherosclerosis, endothelial dysfunction and plaque burden by exploring the potential use of oxidized phospholipid species as disease biomarkers.

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- Kyriakakis, E., Maslova, K., Philippova, M., Pfaff, D., Joshi, M.B., Buechner, S.A., Erne, P., and Resink, T.J. (2012). T-Cadherin is an auxiliary negative regulator of EGFR pathway activity in cutaneous squamous cell carcinoma: impact on cell motility. The Journal of investigative dermatology 132, 2275-2285.
- Pfaff, D., Philippova, M., Kyriakakis, E., Maslova, K., Rupp, K., Buechner, S.A., Iezzi, G., Spagnoli, G.C., Erne, P., and Resink, T.J. (2011). Paradoxical effects of T-cadherin on squamous cell carcinoma: up- and down-regulation increase xenograft growth by distinct mechanisms. The Journal of pathology 225, 512-524.
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- Philippova, M., Pfaff, D., Kyriakakis, E., Buechner, S.A., Iezzi, G., Spagnoli, G.C., Schoenenberger, A.W., Erne, P., and Resink, T.J. (2013). T-cadherin loss promotes experimental metastasis of squamous cell carcinoma. European journal of cancer 49, 2048-2058.
- Philippova, M., Suter, Y., Toggweiler, S., Schoenenberger, A.W., Joshi, M.B., Kyriakakis, E., Erne, P., and Resink, T.J. (2011). T-cadherin is present on endothelial microparticles and is elevated in plasma in early atherosclerosis. European heart journal 32, 760-771.

Hematopoietic Stem Cells

Cancer Stem Cells

Developmental Pathways

Xenograft Models

Zebrafish

Stem Cells and Hematopoiesis

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Stem cell pathways in development and oncogenesis

Stem cell pathways in development and oncogenesis

Molecular pathways directing stem and progenitor cell development during embryogenesis (e.g. SCL, MLL, AML1/RUNX1) can reactivate expression in adult cells and contribute to their malignant transformation. We have previously identified the BMP-WNT-CDX-HOX signaling pathway as an essential regulator of developmental hematopoiesis and later on demonstrated involvement of CDX genes in human leukemia. Gene expression arrays performed on CDX2-modified leukemic cells confirmed HOX genes as targets but also revealed the zink finger transcription factor EVI1 as a putative downstream molecule.

Ecotropic viral integration site 1 (EVI1) in leukemia

EVI1 has been intensively studied in acute myeloid leukemia (AML) where high EVI1 expression, detectable in ca. 10% of patients predicts an adverse clinical outcome. Ca. 20% of the high EVI1 adult AMLs present rearrangements at the chromosomal locus 3q26 harboring the EVI1 gene, however in all other cases the upstream regulatory mechanisms driving EVI1 expression are not yet fully understood. Associations with translocations involving 11q23 (the chromosomal region harboring the mixed lineage leukemia, MLL gene) or deletions of 7q- have been reported in subsets of patients.
We recently reported that EVI1 also expresses in ca. 10% of pediatric acute lymphoblastic leukemias (ALL). Knockdown of EVI1 expression enhanced apoptosis in response to conventional ALL chemotherapies and suppressed leukemogenesis in murine xenotransplantion models. We are currently investigating the molecular targets and regulation of EVI1 in leukemia and other putative mechanisms by which EVI1 influences disease aggressiveness (e.g. by regulating homing properties and the interactions between leukemic cells and their niches).

EVI1 regulates developmental hematopoiesis

In parallel to our studies in leukemia, we explore the role of EVI1 during hematopoietic development in zebrafish and in vitro differentiating human induced pluripotent stem (iPS) cells. EVI1 regulates embryonic myelopoiesis and HSC development while not affecting primitive erythropoiesis. Mechanistically, EVI1 exerts its effects partly through induction of the downstream gene GATA2. Currently we are exploring putative additional EVI1 targets and mechanisms by which EVI1 affects hematopoietic development.

The role of embryonic stem cell proteins in cancer stem cells

There is increasing evidence that solid tumors harbor so-called cancer stem cells (CSCs) responsible for disease initiation, maintenance, metastasis and relapse after conventional anti-tumor therapies. We hypothesized that the embryonic proteins SOX2 and OCT4, which regulate stemness in ESCs and participate in the reprogramming of somatic cells to pluripotent stem cells also induce CSCs. In line, we could show that enhanced SOX2 expression associates with stemness, disease aggressiveness and therapy resistance in ovarian cancer. Furthermore, SOX2 expression was associated with the presence of lymph-node metastasis in early-stage breast carcinoma patients, suggesting that SOX2-expressing cells have increased invasive and metastatic potential, both properties linked to CSC identity. Current research in the lab uses a newly developed GFP-tagged lentiviral reporter system to isolate and characterize SOX2-expressing cells, with the overall aim of identifying druggable CSC targets.

Using zebrafish xenografts as a tool for studies on cancer biology
Several studies report the feasibility of xenotransplanting human tumor cells into zebrafish embryos and adult fish. This model provides a unique opportunity to monitor tumor-induced angiogenesis, invasiveness, and response to a range of treatments in vivo and in real time. Data collected in our laboratory suggest that human ALL and ovarian carcinoma cell lines as well as primary cells can be xenotransplantated into fish embryo and give rise to tumors. Currently we use zebrafish xenotransplant assays for investigating CSC identity in limiting dilution assays and for analyzing interactions between tumor cells and their environment.

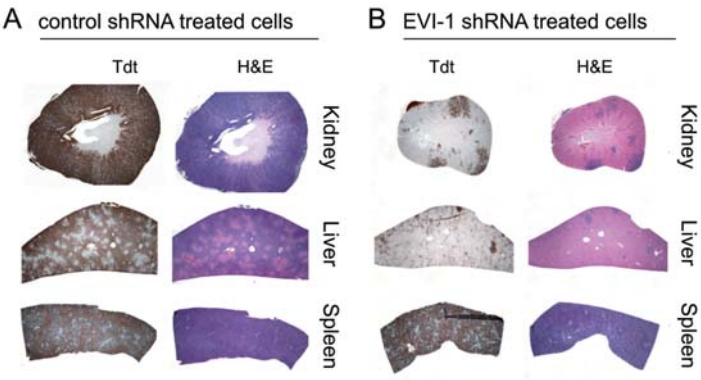

Fig. 1: *EVI1* knockdown suppresses the leukemogenic potential of human acute lymphoblastic leukemia (ALL) cells transplanted into NSG mice.
10^5 NALM-16 cells treated with control-shRNA (A) or *EVI1*-shRNA lentiviruses (B) were injected in 6 to 8 weeks old immune compromised NSG- (NOD/SCID/IL-$2Rg^{null}$) mice via tail vein injection. Histophathological analyses performed 8 weeks after injection revealed strongly diminished leukemic infiltration in mice transplanted with *EVI1*-knockdown versus control cells. Shown are representative sections of murine organs (kidney, liver and spleen) analyzed by hematoxylin & eosin staining and TdT expression at 12.5 magnification.

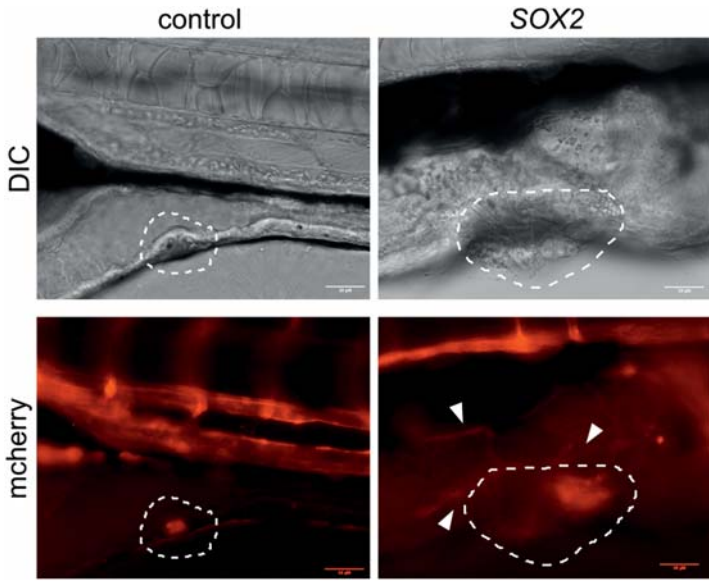

Fig. 2: Xenotransplantation assays of human tumor cells using zebrafish embryo as a host organism.
Control (left) and *SOX2*-overexpressing (right) human serous ovarian cancer cells from the Caov-3 cell line generated by treatment with lentiviruses carrying the GFP-protein as a selection marker were injected into the yolk of 48 hpf (hours post fertilization) zebrafish larvae. Zebrafish adaptive immune response has not been established at this time-point, therefore the xenotransplantation procedure does not require immune suppression. Shown are tumors derived from 10 transplanted human cells as examined 3 days after transplantation. Please note that *SOX2*-overexpressing cells generate tumors of larger size compared to control cells (circled areas, upper panel) and induce robust neovascularization (highlighted by white arrowheads in the lower panel). To visualize vessel formation, injections were performed into transgenic zebrafish embryos expressing mCherry under the control of an endothelial specific marker (red staining, lower panel).

Connection to Clinical Practice

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Analysis of EVI1 regulation and prognostic value in ALL, and analysis of SOX2 in gynecological tumors

In order to investigate the potential clinical relevance of our findings in experimental models, the expression of EVI1, SOX2 and potential downstream targets will be investigated in primary leukemia and ovarian cancer cells derived from patient samples and correlated with clinical and histopathological parameters as applicable.

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– Bareiss PM, Paczulla A, Wang H, Schairer R, Wiehr S, Kohlhofer U, Rothfuss OC, Fischer A, Perner S, Staebler A, et al. SOX2 Expression Associates with Stem Cell State in Human Ovarian Carcinoma. Cancer Res. 2013;73:5544-5555.
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– Konantz M, Balci TB, Hartwig UF, Dellaire G, André MC, Berman JN, Lengerke C. Zebrafish xenografts as a tool for in vivo studies on human cancer. Ann N Y Acad Sci. 2012;1266:124-137.
– McKinney-Freeman S, Cahan P, Li H, Lacadie SA, Huang HT, Curran M, Loewer S, Naveiras O, Kathrein KL, Konantz M, et al. The transcriptional landscape of hematopoietic stem cell ontogeny. Cell Stem Cell. 2012;11:701-714.
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Cartilage Repair

Bone Repair

Stem Cells

Bioreactors

3D Culture Models

Engineered Stromal Tissues

Tissue Engineering

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Engineering 3D skeletal tissue models and grafts

Our ultimate goal is to generate cellular grafts to repair cartilage and bone tissues, as well as complex osteochondral lesions. Beyond a potential clinical use as implants, the engineered constructs also represent invaluable 3D model systems to study progenitor cell differentiation and tissue development. The main scientific questions are related to (i) the functionality of human mesenchymal cells (mature, progenitor, stem cells) for bone and cartilage repair, (ii) the effect of specific chemical and physical environmental factors on skeletal tissue engineering, and (iii) the interaction of different cell types during ex vivo tissue morphogenesis. The projects, at the interface between fundamental research and clinical translation, bring together the competences of biologists, engineers and surgeons. Beyond national (SNSF) and industrial programs, research is funded in the context of European consortia, which favor a strong international networking of the group.

Main recent achievements:

1. Bone organ engineering. After establishing the capacity of human bone marrow-derived mesenchymal stromal cells to generate bone tissue through an endochondral ossification process, we further developed and characterized an upscaled, 3D scaffold-based model that displays morphological, phenotypic, and functional features of a "bone organ" (Fig. 1). In collaboration with the lab of Prof. M. Manz at the University Hospital Zurich, we determined that the frequency of hematopoietic stem and progenitor cells in the ectopic ossicle is comparable to native bones, and the marrow from the ossicles is capable of reconstituting multilineage long-term hematopoiesis in lethally irradiated mice. Generating bone grafts through endochondral ossification provides an innovative approach to tackle fundamental and translational aspects of bone morphogenesis and regeneration, besides enabling a controlled investigation and manipulation of hematopoietic stem cell niches in physiological and pathological conditions.

2. Cellular graft manufacturing in bioreactors. Different non-invasive methods using sensors have been developed for the monitoring and control of culture parameters and cartilage graft quality using an automated bioreactor-based manufacturing system. Implementation of these controls is essential to perform streamlined graft manufacturing in a regulatory compliant way for clinical applications (Fig. 2). In order to further improve the previously developed integrated system for commercialization, the spin-off "Cellec Biotek AG" was founded in 2011. Together with Cellec and 7 other partners, we coordinate the EU-Project BIO-COMET aiming at the development of a controlled, automated perfusion bioreactor to manufacture human cartilage grafts of 30mm diameter to be used in a multicenter clinical trial for treatment of articular cartilage defects in the knee of up to 5cm^2. The foreseen automated process includes the digestion of a small cartilage biopsy, cell seeding on and expansion in a 3D scaffold and subsequent engineering of a cartilage graft.

3. Engineering 3D culture models. The direct seeding, expansion and differentiation of freshly isolated, primary human cells within the pores of 3D scaffolds using perfusion-based bioreactor systems, bypassing the typical monolayer cell expansion, was shown to maintain the tissue regeneration capacity of various human progenitor cells. Recently, we demonstrated that the 3D co-culture of endothelial/mesenchymal cells from adipose tissue generates complex vascular structures in vitro, allowing to engineer clinically relevant-sized, pre-vascularized constructs with enhanced engraftment and bone tis-

sue formation capacity (Fig. 3). Adipose-derived cells were similarly used to pre-vascularize skin substitutes, resulting in improved engraftment and functional performance in animal skin defect models (collab. with Prof. E. Reichmann, Zurich). Engineered 3D stromal environments are also used as advanced models to study other cell types, including tumor cells (collab. with Prof. G. Spagnoli), thymic epithelial cells (collab. with Prof. G. Holländer), osteoclastic and hematopoietic cells.

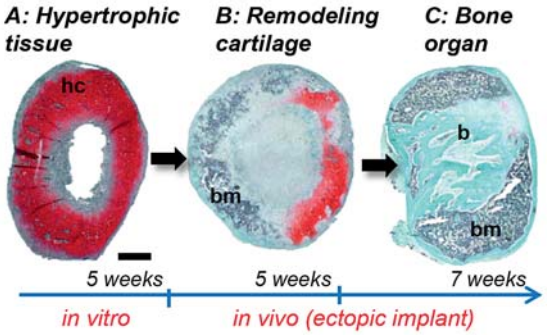

Fig. 1: Development of an engineered endochondral bone organ. Engineered hypertrophic cartilage (hc) templates based on human mesenchymal stromal cells undergo extensive remodeling into bone (B) and bone marrow (bm) upon ectopic implantation into nude mice. A: Safranin O, B & C: Masson's trichrome. Bar = 1 mm

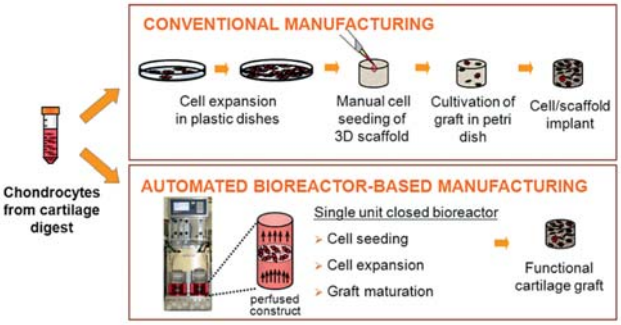

Fig. 2: Conventional tissue engineering processes are based on labor-intensive manual cell culture methods, which possess risks of contamination, high operator variability, limited scale-up potential, & high costs. Bioreactor-based platforms can overcome such limits.

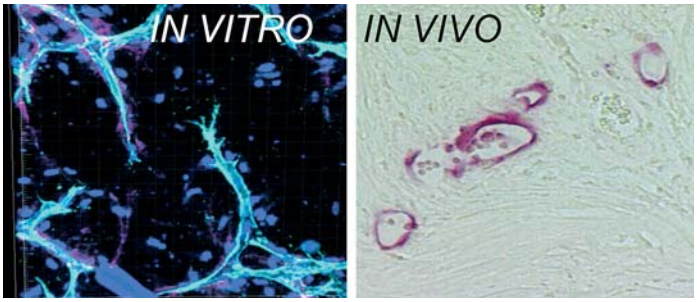

Fig. 3: Left: Confocal microscopy picture of vascular structures built by human adipose tissue-derived cells (staining: CD31 (endothelial marker, in light blue), collagen type IV (basement membrane marker, in pink) and DAPI (nuclear marker, in dark blue)). Right: Immunohistochemical staining against human endothelial cell marker CD34 (in red) identifies the presence of functional human blood vessels in implanted engineered tissues, functionally connected with the host (rat) vasculature.

Connection to Clinical Practice

Prof. Dr. Marcel Jakob, Prof. Dr. Dirk J. Schaefer
Department of Surgery

Engineered skeletal tissue grafts in trauma, orthopaedic and reconstructive plastic surgery

The main goal is the translation of engineered cellular implants into specific surgical procedures and reconstructive indications. Currently targeted clinical applications:

Trial 1. Use of engineered nasal cartilage for reconstruction of the alar lobule of the nose following tumour resection. A phase I clinical trial (5 patients) has been completed in February 2013. (PD Dr. M. Haug, Dr. I. Fulco)

Trial 2. Intra-operative transplant of adipose tissue-derived cells to enhance humeral fracture healing in osteoporotic patients. The study is currently recruiting patients (5 patients out of 20 treated) (Dr. A. Mehrkens, Dr. A.M. Müller, Dr. F. Saxer, Dr. S. Schreiner, Dr. P. Studer)

Trial 3. Use of nasal chondrocyte-based engineered cartilage for the treatment of articular cartilage defects in the knee after traumatic injury. The study is currently recruiting patients (5 out of 10 treated) (Dr. M. Mumme, PD Dr. M. Arnold (Bruderholz), Dr. T. Schwamborn (CrossKlinik), Dr. G. Pagenstert, Dr. R. Largo)

Additional clinical translational projects:

- Bioreactor-based manufacturing of nasal cartilage grafts for the reconstruction of articular cartilage defects in the knee after traumatic injury (Dr. L. Iselin)
- Engineered hyperthropic cartilage implants for the treatment of non-unions via endochondral ossification (PD Dr. C. Jaquiery, PD Dr. S. Schaeren)
- Engineered vascularized bone grafts for bone reconstruction in critical cases and conditions (Dr. A. Kämpfen, Dr. L. Tchang, Dr. R. Osinga)
- Engineered osteochondral grafts for complex joint reconstruction (Dr. M. Barandun, Prof. V. Valderrabano, Dr. C. Candrian, PD Dr U. Studler, Prof O. Bieri)

Selected Publications

- Scotti C et al., (2013) Engineering of a functional bone organ through endochondral ossification. *Proc. Natl Acad Sci USA* 110:3997-4002
- Di Maggio N et al., FGF-2 maintains a niche-dependent population of self-renewing highly potent non-adherent mesenchymal progenitors through FGFR2c. *Stem Cells* 30:1455-1464
- Santoro R et al., (2012) Bioreactor based engineering of large-scale human cartilage grafts for joint resurfacing. *Biomaterials* 31(34):8946-52
- Acharya C et al., (2011) Enhanced chondrocyte proliferation and mesenchymal stromal cells chondrogenesis in coculture pellets mediate improved cartilage formation. *J Cell Physiol* 227:88-97
- Gueven S et al., (2011) Engineering of large osteogenic grafts with rapid engraftment capacity using mesenchymal and endothelial progenitors from human adipose tissue. *Biomaterials* 32,5801-5809

DBM Focal Area Oncology

Focal Area Coordinators

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The major goal of this focal area is to support and expand research in the field of molecular and clinical oncology in Basel. In particular, we aim at bridging the gaps between basic, translational, and clinical oncology research ongoing at the University of Basel and the biotech and pharmaceutical industry in the Basel area. Ultimately, the focal area should enforce collaborative efforts and common projects between various research groups, research institutes and pharmaceutical industry and between different disciplines. An added value is seen in innovative projects that eventually pay off by being transferred to a clinical setting. This research program relies critically on the participating individuals' enthusiasm and initiatives.

The Focal Area Oncology is currently led by Professor Gerhard Christofori, leader of the Tumor Biology Group at the DBM, and Professor Christoph Rochlitz, head of Clinical Oncology at the University Hospital. The program focuses on two major areas: first to support basic, translational, and clinical research by either generating additional positions or opportunities for oncology research by hosting new recruitments, such as SNSF Assistant Professors within the DBM. In 2012, Professor Richard Herrmann retired from his position as Head of Clinical Oncology at the University Hospital and as co-leader of the DBM Oncology Program. We thank him for his continuous enthusiasm and support of the program and for never ceasing to emphasize the importance of clinical observations and questions for basic research. The responsibilities for patient-oriented clinical oncology research have now been transferred to his successor Professor Christoph Rochlitz. We are also very happy to see Professor Alfred Zippelius being appointed Research Professor of Clinical Oncology in 2013 to strengthen the transition between basic and clinical research.

The second focus of the Focal Area Oncology is to increase communication between the various researchers, clinicians and pharmaceutical company representatives in Basel and to foster scientific exchange and technological collaboration. Towards this goal, one-day symposia are organized to offer platforms for the discussion of research progress and for the exchange of ideas. Many members of the DBM Oncology Program are also engaged in the Basel Signaling Alliance, a center of excellence at the University of Basel, and they have been organizing two high-impact international research conferences on signaling and cancer: "TOR, PI3K and Akt - 20 years on" in 2011 and "Membrane dynamics in physiology and disease" in 2012. In addition, outstanding international cancer researchers are invited to present lectures within the "DBM Oncology Program Seminars", and impromptu guest seminars complete the seminar activities of the research program. These communication activities have led to highly successful collaborations and research networks, notably beyond the borders of institutes and pharmaceutical companies.

Many of our efforts within the DBM Oncology Program have been part of international and national research initiatives that cover innovative approaches to cancer research and treatment, including research on cancer genetics and genetic instability, cancer epigenetics, angiogenesis and metastasis, signal transduction, cancer stem cells, tumor immunology, and novel therapeutic regimen. In the years to come, we need to further enforce scientific exchange between basic and patient-oriented research. In particular, we need to facilitate the identification of clinical problems for the design of appropriate and innovative basic research approaches and, on the other hand, to improve on the rapid translation of basic research results into clinical application.

Brain Tumor

Glioma Development

Tumor Invasion

Cancer Genetics

Biomarker

Brain Tumor Biology

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Glioma development: from biomarker identification to molecular mechanisms

Gliomas are among the deadliest malignancies, with a median survival varying between few months for the most frequent malignant grade IV glioblastoma (GBM), to over 20 years for diffuse low-grade glioma (LGG). Gliomas progress by invading adjacent brain tissue. The main goal of the Laboratory of Brain Tumor Biology is to understand mechanisms underlying tumor cell invasion. This involves the identification of biomarkers, genetic regulators, signaling networks and molecular effectors of tumor cell invasion that can ultimately be targeted to control glioma progression.
Through an active exchange between clinics and research laboratory, we are directly collecting resected glioma biopsies that are used for genotyping and *ex vivo* cell culture. In parallel, we are entering personal; clinical; imaging, histopathological and molecular annotations to construct a comprehensive glioma patient database. This information is useful for stratifying gliomas into molecular subsets and allowing further identification of biomarkers that may reveal novel glioma pathways.

IDH mutations in low-grade gliomas

Four major genetic alterations have identified in LGG: *IDH* mutations (*IDH*mut) with *MGMT* promoter methylation (*MGMT*met) in 80% LGG, with additional *TP53* mutation, mainly in astrocytoma, or 1p/19q allelic loss, mostly in oligodendroglioma, each occurring in nearly 25% LGG and mutually exclusive. While IDH normally catalyzes dehydrogenation of isocitrate into α-ketoglutarate (αKG), IDH mutants catalyze αKG conversion into 2-hydroxyglutarate (2HG). Accumulation of 2HG leads in turn to impaired DNA demethylation, including of *MGMT*.
We have analyzed the impact of these alterations in a retrospective study of >200 LGG. Molecular parameters were more accurate survival predictors than histology ($P<0.001$). The co-segregation of *IDH*mut and *MGMT*-met ($P<0.001$) was associated with favorable outcome for overall survival (HR=0.34, $P=0.003$), while the triple combination *IDH*mut, *MGMT*met and 1p/19q loss was even more (HR=0.19, $P<0.001$), and the combination of *IDH*mut, *MGMT*met and TP53 nuclear immunopositivity was a risk factor for malignant transformation (HR=2.76, $P=0.048$) (Fig. 1).

3q26 genomic alterations in glioblastomas

We observed earlier an association between glioma invasion and *SOX2* expression. *SOX2* gene is located on 3q26, a region that also contains the glioma oncogene *PIK3CA*, and *MFN1* and *OPA1*, 2 genes involved in mitochondria fusion and hypothesized for a possible function in glioma cell invasion. The purpose of this project is to investigate a role for these respective genes in glioma cell invasion.
Inactivation of *SOX2*, *MFN1* and *OPA1* promoted glioma cell migration and invasion, pointing to a possible link between mitochondrial fusion and cell motility (Fig. 2). Copy number assays of the four 3q26 genes on 68 resected GBM DNA revealed the highest deletion frequency for *OPA1* (29%). Surprisingly, *SOX2* showed frequent gain (35%), suggesting that SOX2 function for maintaining glioma cell stemness may dominate over a role in inhibiting invasion. We are currently testing whether effectors of mitochondrial fusion MFN1 and OPA1 are under the transcriptional control of SOX2.

* left during report period

Characterization of invasion pathways in neural stem cells and tumor cells

There is increasing evidence suggesting that brain tumors originate form neural stem cells (NSCs). NSCs proliferate, self-renew and differentiate into different brain cell types, but are normally not invasive and show limited migration.

We were able to induce normal rat embryonic NSC invasion by supplying Fibroblast Growth Factor 2 (FGF2) and Bone Morphogenetic Protein 4 (BMP4). Invasion upon FGF2+BMP4 treatment was accompanied by strong migration capacity (Fig. 3). In this combination, twelve upregulated genes such as *SPARC*, *podoplanin* and *Tenascin-C* were also found to be strongly present in resected GBM tissue biopsies. This model may therefore help to identify invasion pathways to be targeted for controlling brain tumor invasion.

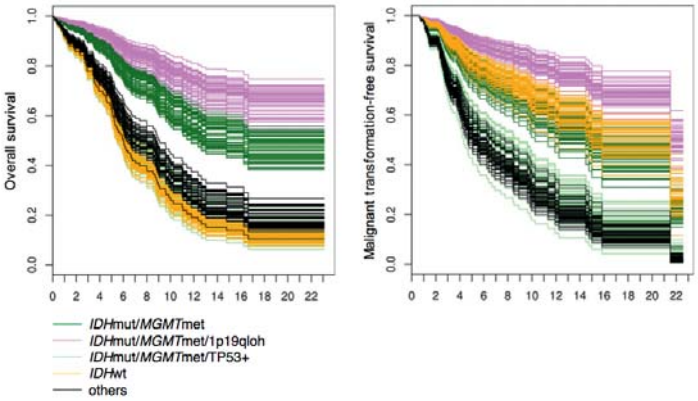

Fig. 1: Low-grade glioma patient survival based on IDH/MGMT molecular stratification.
Cox proportional hazard survival curves of imputed datasets.

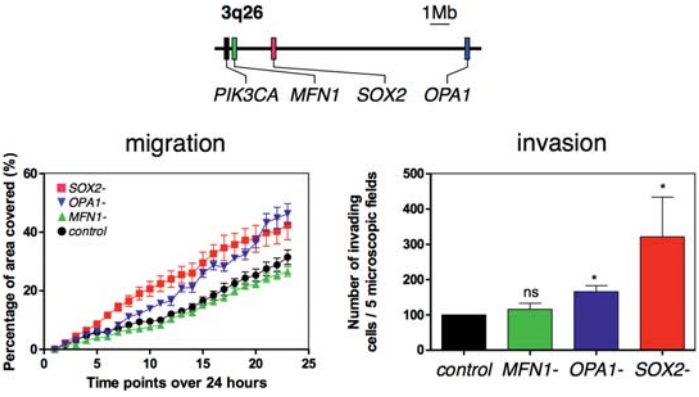

Fig. 2: The human 3q26 region in glioma cell invasion.
Color code for genes conserved throughout the figure. **top.** Genomic map of 3q26. **bottom.** Wound healing assay (left) and Boyden chamber invasion assay (right) of U373 GBM cells with lentivirus shRNA-mediated gene inactivation.

Selected Publications

- Leu S, von Felten S, Frank S, Vassella E, Vajtai I, Taylor E, Schulz M, Hutter G, Hench J, Schucht P, Boulay JL & Mariani L (2013). *IDH/MGMT*-driven molecular classification of low-grade glioma is a strong predictor for long-term survival. *Neuro Oncol* 15:469-79
- Sailer MH, Gerber A, Tostado C, Hutter G, Cordier D, Mariani L & Ritz MF (2013). Non-invasive neural stem cells become invasive in vitro by combined FGF2 and BMP4 signaling. *J Cell Sci* 126:3533-40
- Vassella E, Vajtai I, Bandi N, Arnold M, Kocher V & Mariani L (2011). Primer extension based quantitative polymerase chain reaction reveals consistent differences in the methylation status of the *MGMT* promoter in diffusely infiltrating gliomas (WHO grade II–IV) of adults. *J Neurooncology* 104:293-303
- Ochsenbein A, Schubert F, Vassella E & Mariani L (2011). Quantitative analysis of O6-methylguanine DNA methyltransferase (MGMT) promoter methylation in patients with low-grade gliomas. *J Neurooncology* 103:343-351
- Kim YH, Lachuer J, Mittelbronn M, Paulus W, Brokinkel B, Keyvani K, Sure U, Wrede K, Nobusawa S, Nakazato Y, Tanaka Y, Vital A, Mariani L & Ohgaki H (2011). Alterations in the RB1 pathway in low-grade diffuse gliomas lacking common genetic alterations. *Brain Pathology* 21:645–651

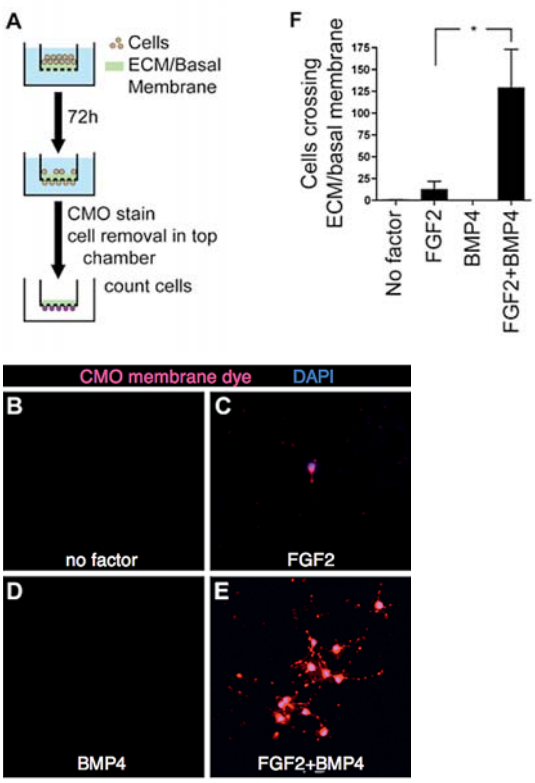

Fig. 3: FGF2 and BMP4 stimulate NSC invasion.
A. Principle of Boyden chamber invasion assay. **B-E.** Invading rat embryonic NSCs collected in lower Boyden chamber after exposure to FGF2 or/and BMP4 *in vitro*. **F.** Quantitation of invading cells.

Inflammation

Cancer

Lipid Signaling

Phosphoinositide 3-kinase (PI3K)

Cell Migration

Cell Growth

Cancer- and Immunobiology

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Lipid signaling in cancer and inflammation – targeting complexity

The immune system is essential for host defense, but has to operate in delicate balance: a multitude of genetic and environmental influences can lead to chronic inflammation, allergy, autoimmune disease, and tissue remodeling. Immune cells also take a central role in metabolic disease and the progression of cancer. Lipid modifying enzymes, such as the members of the phosphoinositide 3-kinase (PI3K) family, have been shown to control cellular activation states, and have been identified as promising drug targets.

Using genetic and pharmacological approaches, we have previously demonstrated key roles of so-called class I PI3K isoforms in innate immune cells, tumors and stroma. Simplified, class IA PI3K are activated by growth factor- and cytokine receptors, while the only class IB PI3K, PI3Kγ, operates downstream of G protein-coupled receptors (GPCRs). Here, ligand binding to GPCRs liberates Gβγ subunits from trimeric G-proteins, which recruit PI3Kγ to the plasma membrane. Among other recent findings, novel mechanisms of PI3Kγ control, and the role of PI3Kγ in allergy and obesity were the focus of our recent studies.

In allergy, cross-linking of the immunglobulin E (IgE) receptor (FcεRI) by IgE/allergen aggregates triggers the release of histamine and pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) from tissue resident mast cells. While histamine increases vascular permeability, TNF-α activates closeby endothelia, which then display cell adhesion molecules to facilitate the recruitment of mast cell precursors (Fig. 1). As described in Collmann *et al*. (2013), FcεRI signalling is relayed via PI3Kγ, and genetic ablation of PI3Kγ or PI3Kγ inhibitors blunt mast cell responses. Moreover, we could demonstrate that mast cell recruitment, but not the degranulation of tissue mast cells correlated with the severity of anaphylactic responses. Amending drug application protocols to this finding, orally applied PI3Kγ inhibitor doses could by reduced by a factor of 30, as compared to doses used in protocols targeting tissue mast cell degranulation.

In Walser *et al*. (2013), we could finally establish a mechanistic link between FcεRI and the activation of PI3Kγ: protein kinase Cβ (PKCβ), activated downstream of FcεRI, was found to phosphorylate the catalytic subunit of PI3Kγ (p110γ) on Ser582 in the so-called helical domain. This phosphorylation enhanced PI3Kγ activity, but also decoupled PI3Kγ from GPCR signalling, due a phosphorylation-induced dissociation of a PI3Kγ adapter subunit (p84). Interestingly, the phosphorylation site on PI3Kγ revealed a molecular switch that seems to be conserved between oncogenic forms of PI3Kα and phosphorylated PI3Kγ: in PI3Kα, mutations in the helical domain weaken the inhibitory interaction of the PI3Kα catalytic subunit (p110α) with the p85 regulatory subunit, and lead to the constitutive activation of the lipid kinase.

Metabolic disorders and obesity are accompanied with chronic low-grade inflammation (metabolic inflammation), which augment cardiovascular risk factors. In this respect, PI3Kγ promotes early steps in the generation of atherosclerotic lesions in murine models of atherosclerosis. As demonstrated in (Fougerat *et al.*, 2008), inhibition of PI3Kγ activity attenuated plaque formation, which was linked to the hematopoietic cell linage. Mechanistically, loss of p110γ decreased macrophage and T-cell infiltration into the intima. Once atherosclerotic lesions progress to narrowing of blood vessels through stenosis, smooth muscle migration has been reported to be potentiated by PI3Kγ-dependent signals (Fougerat *et al.*, 2012).

The obesity-associated chronic low-grade inflammation has been suggested to be the main cause of progressing insulin resistance leading to the initiation of type II diabetes in obese patients. It was also proposed that metabolic inflammation impacts energy balance during the development of obesity. We have recently found that loss of functional PI3Kγ leads to a major improve-

ment of insulin sensitivity in mice kept on a high fat diet. Obesity-dependent macrophage infiltration into adipose tissue was attenuated in p110γ null animals, and macrophage markers and inflammatory cytokine profiles were reduced in white adipose tissue (Becattini *et al.*, 2011).

An interesting outcome of this study was the observation that p110γ$^{-/-}$ mice on a high fat diet accumulated substantially less fat mass than wild type mice, while calorie intake and non-adipose tissue mass was unaffected. The difference in body weight increase could be linked to an increased thermogenesis in p110γ null animals, which was triggered by lipid kinase-dependent and independent pathways. Moreover, the lean phenotype accompanying increased thermogenesis in p110γ null mice was independent from PI3Kγ activity within the hematopoietic compartment, as not the genotype of transplanted bone marrow, but the PI3Kγ status of the host determined energy expenditure and oxygen consumption (Fig. 2, Becattini *et al.*, 2011; Wymann & Solinas, 2013).

To match the increasing complexity of receptor- and cell-specific, and subcellular lipid signaling, the ESF-funded project "Tracking of Phosphoinositide Pools – Key Signaling Components in Cell Migration and Polarisation", short TraPPs, aimed to provide novel tools to dissect PI3K signaling. The TraPPs program was concluded 2012 with the EuroMEMBRANE International Conference, "Membrane dynamics in physiology and disease" in Basel (Wymann & Simons, 2013), and is the source of novel chemical biology tools (Erhart *et al.*, 2013; Wymann & Schultz, 2012; Wymann & Wenk 2011).

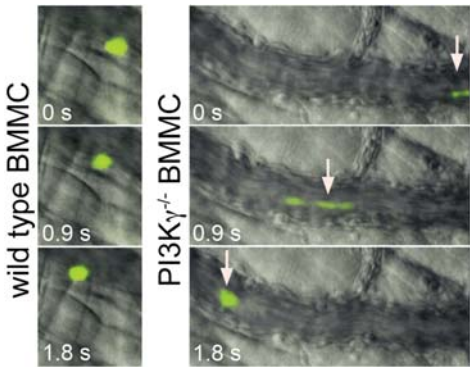

Fig. 1: The *in vivo* adhesion of fluorescently labeled bone marrow-derived mast cells (BMMC, shown in green) to endothelia was investigated in cremaster muscle blood vessels. Endothelia were activated by the injection of TNF-α to simulate an inflamed endothelial state. The left panel illustrates the capacity of wild type mast cells to adhere to endothelia, while cells lacking functional PI3Kγ (PI3Kγ$^{-/-}$) in the right panel are non-adherent. This is due to the abrogation of the GPCR → PI3Kγ → integrin activation signaling chain, and blunts the replenishment of tissue mast cells. As described in Collmann *et al.* 2013, targeting PI3Kγ-dependent cell recruitment is an efficient way to attenuate an allergic response, which involves PI3Kγ function at the level of mast cell precursors and tissue mast cells. In the latter, a connection of the high affinity IgE receptor (FcεRI) was shown to implicate a novel link between PKCβ and PI3Kγ (Walser *et al.*, 2013).

Connection to Clinical Practice

PI3K – moving towards therapy

Phosphoinositide 3-kinase (PI3K) is considered to be a promising drug target. PI3K kinase inhibitors have advanced to phase III clinical trials, and available drug-like small molecules include a wide variety from PI3K isoform-specific inhibitors to molecules targeting PI3K-related kinases such as the mammalian target of rapamycin (mTOR). Emerging from a collaboration with Prof. Dr. B. Giese (Dept. Chemistry, University of Basel, now at University of Fribourg), PIQUR Therapeutics AG was recently funded as a Spin-off of the University of Basel to explore targeted therapies in oncology. This effort profits from expertise available on site, which is reflected by the engagement of many members of the University of Basel (e.g. Prof. Dr. R. Herrmann, CMO; Prof. Dr. M.N. Hall, advisory board; Prof. Dr. A. Pfaltz, CTI collaboration; and more). Based on positive results of regulatory toxicology studies, PIQUR expects clinical trails to begin in 2014 (piqur.com).

Selected Publications

- Walser, R., Burke, J. E., Gogvadze, E., Bohnacker, T., Zhang, X., Hess, D., Kuenzi, P., Leitges, M., Hirsch, E., Williams, R. L., Laffargue, M., and Wymann, M. P. (2013). PKCbeta Phosphorylates PI3Kgamma to Activate It and Release It from GPCR Control. PLoS Biol 11, e1001587.
- Collmann, E., Bohnacker, T., Marone, R., Dawson, J., Rehberg, M., Stringer, R., Krombach, F., Burkhart, C., Hirsch, E., Hollingworth, G. J., Thomas, M., and Wymann, M. P. (2013). Transient targeting of phosphoinositide 3-kinase acts as a roadblock in mast cells' route to allergy. J. Allergy Clin. Immunol. 2013; 132:959-68.
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- *Becattini, B., *Marone, R., Zani, F., Arsenijevic, D., Seydoux, J., Montani, J. P., Dulloo, A. G., Thorens, B., Preitner, F., *Wymann, M. P., and *Solinas, G. (2011). PI3Kgamma within a nonhematopoietic cell type negatively regulates diet-induced thermogenesis and promotes obesity and insulin resistance. Proc. Natl. Acad. Sci. U S A 108, E854-E863. *equal contribution.
- Wymann, M. P. (2012). PI3Ks – Drug Targets in Inflammation and Cancer. In Subcell Biochem 58, Balla, T., M. P. Wymann, and J. D. York, eds., Springer, pp. 111-181.

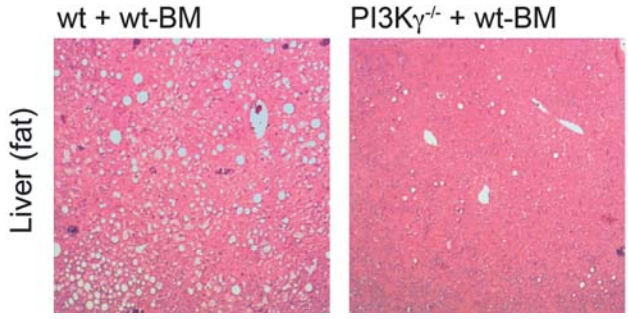

Fig. 2: The loss of PI3Kγ functions modulates energy metabolism. On a high fat (HF) diet, PI3Kγ null mice display a higher thermogenesis and burn more calories, which results in a reduced body weight and fat mass, while food intake and lean body mass remain unaffected (Becattini *et al.*, 2011). Chimeric mice were generated by the reconstitution of wild type (wt) and PI3Kγ null (PI3Kγ$^{-/-}$) bone marrow (BM) into irradiated wild type or PI3Kγ$^{-/-}$ recipients. After BM grafting, mice were kept for 20 weeks on high fat diet: wild type mice then develop a prominent liver stenosis, while PI3Kγ null mice do not. As shown in the right panel, this phenotype develops independent of the BM genotype, as PI3Kγ null recipients of wild type BM are protected against the development of a fatty liver.

Anti-Tumor Immunity

T Cell Response

Antibodies

Tumor Micro-Environment

Clinical Cancer Research

Tumor Invasion

Cancer Immunology & Biology

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* left during report period

Immune modulation and cancer: implications for novel cancer therapies

1. Cancer Immunotherapy: Harnessing the potential of anti-tumor immunity

It is increasingly appreciated that cancers are recognized by the immune system, and under some circumstances, the immune system may control or even eliminate tumors. Only recently, this concept has been reinvigorated by large clinical trials, demonstrating improved overall survical and, importantly, durable responses in a subset of patients in a way not seen with many targeted therapies and cytotoxic agents. Of particular note, the latter may also modulate immune responses and augment host immunity. For example, selected agents increase the immunogenicity of dying cancer cells, inhibit the function of locally immuno-suppressive populations such as myeloid derived suppressor cells or trigger DC maturation. These findings raise the possibility that such agents might be effectively combined with immunotherapy to induce potent anti-tumor immune responses, destroy therapy-resistant tumor cell variants selected upon anti-cancer therapy, and, ultimately, improve clinical outcomes. We investigate mechanisms of anti-tumor immunity in a variety of different mouse models including syngeneic and genetically modified tumor models engineered to carry mutations in genes known to be involved in human cancer. Of note, the latter models resemble the human disease both at the genetic and phenotypic levels. These models also provide the platform to experimentally perturb the tumor microenvironment by different anti-tumor agents; currently, their immunostimulating effects are poorly defined. The aim of our research is to improve our understanding of the immuno-modulating capacities of cytotoxic anti-cancer therapies and pave the way for a rationale design of treatment algorithms combining cytotoxic anti-tumor agents with immunotherapy.

2. Molecular mechanisms of tumor invasion

Our group has shown before that invasion of cancer cells is not only possible by single cell migration after epithelial-mesenchymal transition (EMT), but also by collective migration of cell sheets. The small glycoprotein podoplanin is a marker for collective invasion in squamous cell cancers such as skin, head and neck, lung and cervical SCC. We are looking at stromal factors (including those provided by the immune system and angiogenic cells) that may trigger podoplanin upregulation and collective invasion. By analysing serial biopsies from patients before, during and after therapy for squamous cell cancers, we analyze specific signalling pathways involved in collective migration. In addition, we test the druggability of these pathways in tumor models in vivo.

3. Development of anti-cancer strategies in early clinical trials

The focus lies on the investigation and development of treatment strategies, targets and delivery platforms in early trials in medical oncology. In collaboration with the Clinical Research Center (CCRC) at our division, we have programs ongoing to create a pipeline of agents that can move into the clinic. In translational projects, we aim at defining predictors of therapeutic responses and at understanding the mechanism of treatment responses and resistance. In addition, we define novel tumor antigens by analyzing the autoreactive antibody repertoire. The clinical programs include cancer vaccines, immune modulatory drugs, monoclonal antibodies, and nanoparticles such as immunoliposomes. In collaboration with the Department of Radiology and Nuclear Medicine (Prof. Wild), a program is centred on radiopeptides against peptide receptors. In addition, to optimally develop novel anti-cancer agents, in particular immunotherapeutics, in-vitro assays are performed to study how

these compounds modulate human effector populations in freshly excised tumor tissue, thus faithfully mimicking the situation found in cancer patients. This program is performed in collaboration with the Department of Thoracic Surgery (Prof. Lardinois), Department of Gynecology (Prof. Heinzelmann) and Pathology (Dr. Savic).

Selected Publications

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Colorectal Cancer
Microenvironment
T Cells
Monocytes
Chemokines
Icrobial Stimuli

Cancer Immunotherapy

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Tumor-host interactions in human colorectal cancer

Colorectal cancer (CRC) is a major public health problem and the second leading cause of cancer mortality in industrialized countries. Conventional staging systems, based on TNM assessment, do not precisely predict clinical outcome. Improved prognostic markers are required to identify patients at risk of disease relapse. Several tumor cell-associated markers have been proposed and possible applications in routine clinical practice are currently being investigated. During the last decade, tumor-associated stroma was also shown to play active roles in CRC progression. In particular, tumor infiltration by specific populations of immunocompetent cells has been recognized to be significantly associated with favorable clinical outcome irrespectively of tumor stage, thus possibly representing a superior prognostic factor. Mechanisms leading to recruitment of these cell populations and underlying their effects on survival remain, however, to be clarified. Furthermore, the modulation of functional activities of stromal cells by microbial products derived from gut flora has not been evaluated so far.
We are interested to investigate immune-mediated mechanisms underlying the beneficial role played by specific tumor infiltrating lymphocyte and monocyte subsets in human CRC. Understanding the complex network of tumor-host interactions in CRC may allow the identification of novel prognostic biomarkers and potential new therapeutic targets.

Prognostic relevance of cancer stem cells or immunocompetent cells in CRC
We have analyzed the prognostic relevance of cancer stem cell (CSC) markers, including CD133, CD44, CD166, ALDH-1 and EpCAM, and molecules identifying specific immunocompetent cells, such as TIA-1, CD16, PDL-1, and MPO, on tissue micro-arrays including 1420 primary CRC. Whereas expression of CSC markers is not per se predictive of poor prognosis, infiltration by TIA-1+ CD8+ T cells, CD16+ or MPO+ myeloid cells is significantly associated to prolonged patient survival. Thus, tumor-associated stroma efficiently predicts clinical outcome.

Impact of tumor-associated stromal cells on CRC progression
We have characterized phenotypes and function of tumor-associated stromal cells (TASC) in primary CRC. TASC resembled bone marrow-derived mesenchymal stromal cells and similarly enhanced invasiveness of CRC cells in vitro and in vivo, by releasing proangiogenic factors and by promoting epithelial-to-mesenchymal transition.

Role of CRC infiltrating IL-17-producing T cells
Ongoing studies concern the analysis of CRC-infiltrating IL-17-producing T -helper cells (Th17). Infiltration by IL-17+ cells, although not per se predictive of improved survival, strongly correlates with that of clinically relevant CD8+ T cells, MPO+ and CD16+ myeloid cells. We found that Th17-derived chemokines contribute directly or indirectly to the recruitment of these beneficial cell populations into tumor tissues.

Characterization of tumor-infiltrating monocyte subsets in CRC
We have recently observed that in healthy donors distinct peripheral blood monocyte subsets, identified by differential expression of CD16 and CD14, differentially expand specific T cells, including IFN-γ-producing T helper cells (Th1), Th17 and regulatory T cells (Tregs), also depending on presence or absence of microbial stimuli. Since in CRC, tumor-infiltrating CD16+ myeloid cells, possibly including monocytes, are associated to Th17 cells, we are now investigating the ability of CRC-infiltrating monocyte subsets to expand different T cell subsets upon exposure to gut flora-derived microbial stimuli.

Chemokines promoting the recruitment of clinically relevant cells into CRC tissues

A more recent project aims at elucidating chemotactic factors promoting CRC infiltration by immunocompetent cells associated to good prognosis. Several inflammatory chemokines were found to be overexpressed in CRC as compared to healthy tissues. Expression of certain chemokines correlated with that of specific cell markers, such as CD8, IFN-γ, Foxp3, and CD16. We are currently evaluating chemokine receptor profiles of circulating and tissue infiltrating T and myeloid cells in CRC patients.

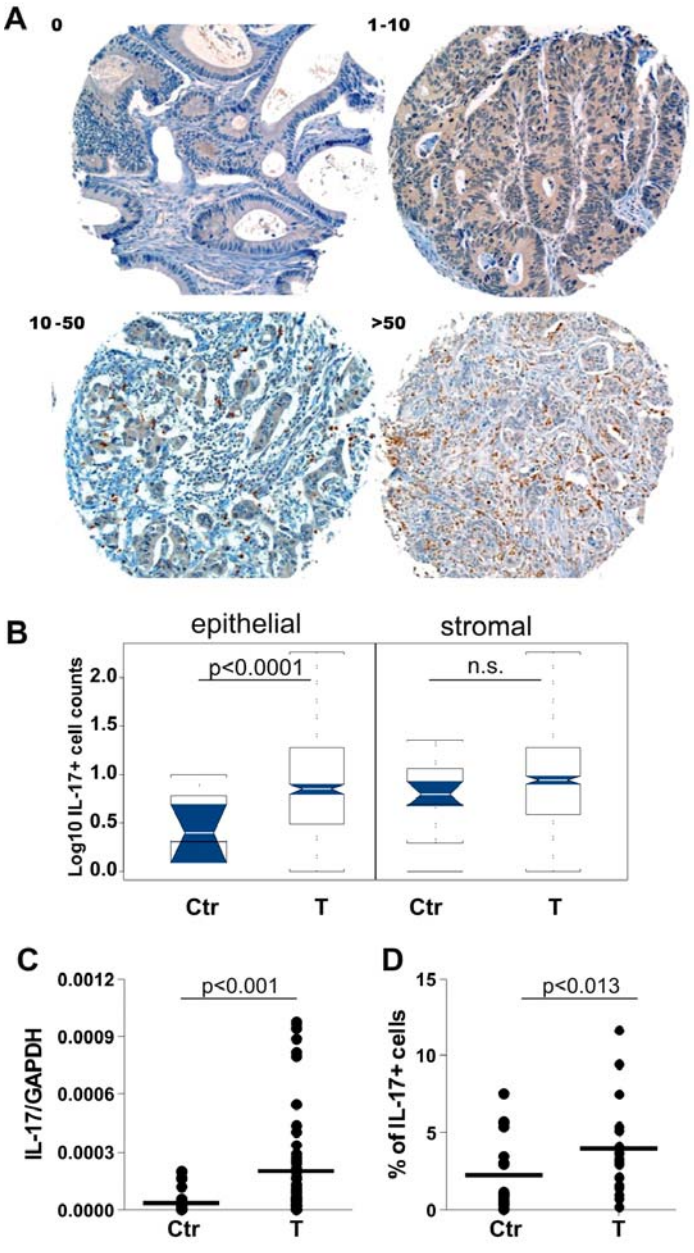

Fig. 1: TMA staining showing infiltration by IL-17+ cells in CRC. Numbers of IL-17+ range from 0 to >50 cells/punch (**A**), and they are increased in CRC samples as compared to healthy colonic tissues (Ctr) (**B–D**).

Connection to Clinical Practice

Prof. Dr. Daniel Oertli
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Immunotherapeutic intervention in human colorectal cancer

The Cancer Immunotherapy group is part of the Institute of Surgical Research and Hospital Management of the University Hospital Basel, directed by Prof. Michael Heberer. Our lab is closely connected to the Department of Surgery of the University Hospital Basel, led by Prof. Daniel Oertli. Several surgeons, including young doctors in training, have been involved in the planning and development of our research projects. Our ultimate goal is the identification of novel targets for immunotherapeutic intervention in colorectal cancer.

Furthermore, we have established a collaborative network with the surgical units of other Swiss hospitals, including Kantonsspital Olten (directed by Prof. Markus Zuber), Kantonsspital Baden (Prof. Thomas Kocher), Kantonsspital Aarau (Prof. Walter Marti), Kantonsspital St. Gallen (Dr. Michel Adamina), and Ospedale Civicio di Lugano (Prof. Raffaele Rosso), ensuring regular access to clinical samples.

We have also established a proficient collaboration with the Institute of Pathology of the University of Basel. The availability in this unit of the tissue-microarray technology has allowed the rapid evaluation of the clinical relevance of putative novel prognostic markers on large cohorts of patients. Furthermore, the mutual exchange of specific know-how has resulted in the generation of significant synergies.

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Cell Migration
Neurite Outgrowth
Local mRNA Translation
Rho GTPases
Spatio-temporal Signaling
FRET-based Biosensors

Cell Migration and Neurite Outgrowth

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Spatio-temporal regulation of cell signaling during cell migration and neuronal differentiation

The ability of vertebrate cells to directionally migrate is critical to development, the immune response and wound healing, and its regulation is compromised in pathologies such as metastatic cancer and vascular disease. The capacity of neurons to directionally extend neuronal processes is crucial for the proper wiring of the brain. Both processes take advantage of a tight spatio-temporal control of cytoskeletal and adhesion dynamics, with signaling events that operate on length and time scales of single microns and tens of seconds. One current limitation is that these biologically relevant scales are not accessible with traditional biochemical and cell biological approaches. We are broadly interested in different signaling networks regulating the two processes mentioned above with the focus to design and implement novel technologies to grasp their spatio-temporal dynamics at relevant biological scales.

Genetically-encoded biosensors to measure signaling events in time and space
We have devised a novel toolkit to rapidly construct genetically-encoded, fluorescence resonance energy transfer-based biosensors for a wide variety of signaling molecules. Our approach enables to visualize micrometric signaling domains that fluctuate of time scales of tens of seconds. By example, the GTPase RhoA is specifically activated at the tip of F-actin bundles in neuronal growth cone filopodia (Figure 1) or at the leading edge of migrating fibroblasts. Rac1 and Cdc42 are activated at overlapping but distinct regions within the growth cone. This degree of precision cannot be matched by any biochemical measurement. In the case of the MAP kinase ERK, the biosensor revealed signaling noise within a population of cells, which was not previously accessible using western blot-based measurements cell population averages. Previous work has proposed that duration of the pERK signal in response to different growth factors regulate cell fate decision such as differentiation or proliferation. We observe that these growth factor-induced signaling responses are extremely heterogeneous when analyzed at the single cell level (Fig.2). This explains the phenotypic "fate" noise observed in a population of cells: a given growth factor will not lead to homogeneous proliferation or differentiation within the cell population, but rather a mix of multiple behaviours. Thus, our biosensors provide a novel approach to understand signaling dynamics at relevant biological scales.

Local mRNA translation during neurite outgrowth
We have performed a genome-wide screen for mRNAs enriched within neuronal growth cones. We have found that the MKK7 mRNA, which encodes a MAPKK for JNK, is locally translated within the growth cone. This leads to specific activation of JNK within the neurite, where it regulates microtubule bundling necessary for robust neurite outgrowth (Figure 3). This provides a spatio-temporal signaling mechanism to specifically couple JNK signaling to regulation of microtubules, and to uncouple it from regulation of cellular stress.

Spatio-temporal signaling programs during neuronal guidance
We are currently studying a large signaling network of 220 neurite-localized proteins that regulate the cytoskeleton, identified using a proteomic approach. siRNA-mediated knockdown of these proteins only leads to very subtle phenotypes that can only be grasped using timelapse imaging of

the neurite outgrowth process. For that purpose, we have combined high content live cell imaging, computer-vision based image and statistical analyses, and identified a number of regulatory networks regulating neurite initiation, extension, branching, collapse, etc. This emphasizes the need of a system biology approach to understand these complex networks.

Fibroblast cell migration. We have identified a highly persistent fibroblast migration mode. We observe that specific cytoskeletal structures act as a spatial organizer, that allows to constantly polarize the cell and to specify different subcellular zones involved in membrane protrusion or tail retraction. We have identified a leading edge-localized, collision sensor, that allows to sense when two migrating cells encounter each other.

Fig. 1

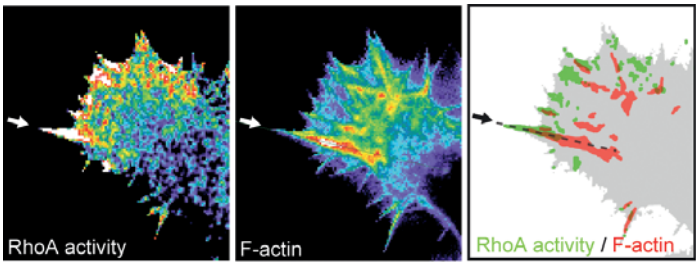

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Fig. 2

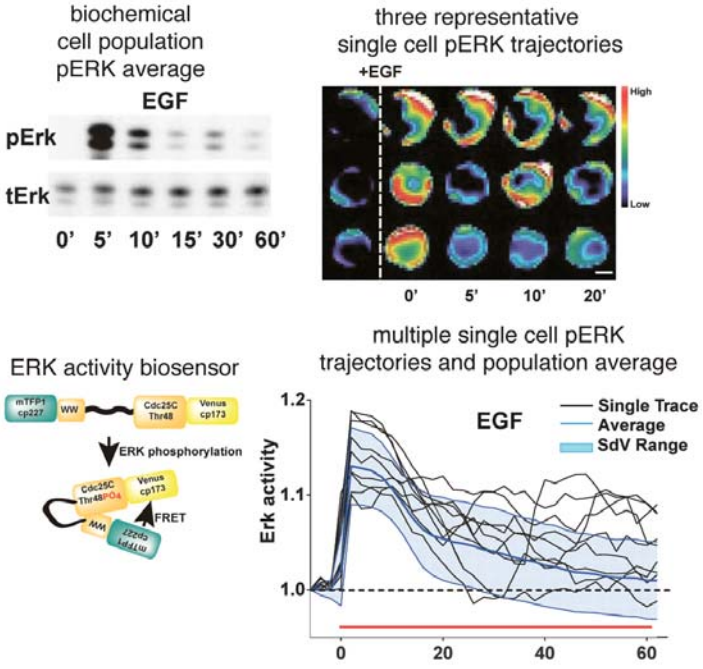
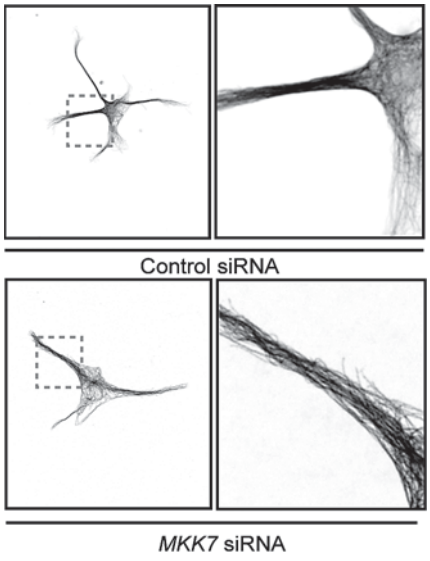


Fig. 3



Acute Leukemia

Molecular Genetics

Mouse Models

Therapeutic Targeting

Childhood Leukemia

G. von Meissner Foundation

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Dissection of molecular alterations underlying acute leukemia to develop novel therapeutic strategies

Acute myeloid leukemia (AML) is the product of a limited number of functionally cooperating genetic alterations of which mutations that lead to constitutive kinase signaling, and mutations of transcription factors or chromatin modifiers are among the most prevalent (Fig.1). Our research aims to understand the molecular mechanisms underlying these genetic alterations to define novel therapeutic strategies. In the past, we have shown that the uncontrolled activity of PIM protein kinases contributes to proliferation and survival of leukemia/lymphoma cells and characterized several small molecule PIM kinase inhibitors with anti-cancer potential (Brault et al., 2012). Unexpectedly, we found that PIM1 regulates homing and migration of leukemic stem cells by modification of the CXCR4 chemokine surface receptor that is highly expressed on AML blasts. PIM1 phosphorylates the intracellular tail of CXCR4 predominantly at Serine-339 leading to increased recycling of the receptor and enhanced cell migration. Recently, we were able to demonstrate that phosphorylation of CXCR4-Serine339 is important for mobilization of leukemic cells and of prognostic significance in AML (Brault et al., 2013)(collaboration with Alexandar Tzankov & Alicia Rovo, UHB).

In more than 50% of AML patients the leukemic cells harbor chromosomal translocations that often lead to the expression of fusions genes which encode for epigenetic transcriptional regulators like mixed lineage leukemia 1 (MLL1) or nuclear receptor set domain proteins (NSD1) which are hallmarks of aggressive AML with poor prognosis. Using a retroviral bone marrow expression and transplant model we identified meningioma 1 (MN1) as a collaborating oncogene for acute leukemia induced by the MLL-ENL fusion (Liu et al. 2010). In order to address how the cellular origin of these fusions might affect the outcome of the disease, we have established transgenic mouse lines allowing the conditional activation of several MLL fusions at a defined stage of hematopoiesis. So far, our results suggest that activation of the leukemogenic driver oncogene in hematopoietic stem cells leads to a significantly more aggressive disease than induction in more differentiated myeloid progenitor cells (collaboration with Antoine Peters, FMI).

In contrast to protein kinases it was for a long time thought that transcriptional regulators could not be pharmacologically controlled. However, improved structural molecular analysis resulted in the identification of several small molecules selectively interfering and blocking the activity of transcriptional regulators. Gene transcription is influenced by local loosening of the chromatin through reversible modification of the histone proteins. These "histone marks" are recognized by transcriptional (co)-regulators by distinct structural motifs including bromodomains (BRD). Resolving the structure of most existing BRDs allowed the identification of selectively interacting small molecules with potent anti-leukemic activity (Picaud et al., 2013)(collaboration with Stefan Knapp, SGC, Oxford).

Leukemogenic fusions often function in large multi-protein complexes, e.g. the activity of MLL-fusions is dependent on the interaction with several adapter proteins like the lens epithelial-derived growth factor (LEDGF) (Fig. 2). Interestingly, LEDGF is also known as being essential for integration of the HIV-virus into host chromatin. In collaboration with Zeger Debyser (KU, Leuven), we have identified the domains of LEDGF that are essential for its interaction with MLL-fusions. Overexpression of small LEDGF-derived peptides was able to disrupt the complex and to impair MLL-mediated transformation in cell lines as well as in mouse leukemia models. These observations initiated a currently ongoing screen for small molecules that might be able to imitate these anti-leukemic effects (Mereau et al., 2013).

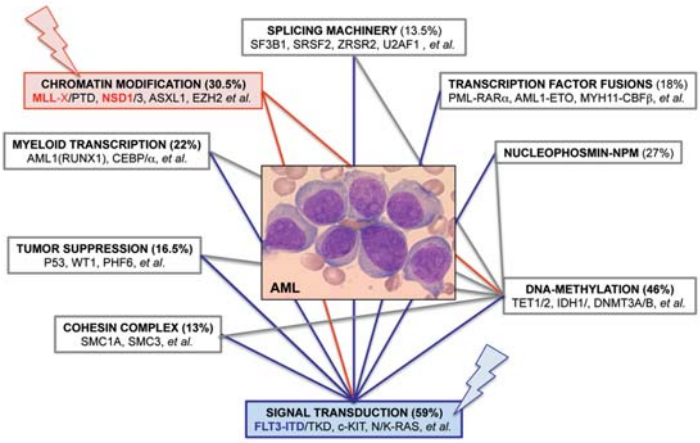

Fig. 1: Molecular genetics of acute myeloid leukemia (AML)
AML is the product of a limited number of driver mutations (e.g. fusion genes, point mutations or deletions) in chromatin modifiers, transcription factors, regulators of the splicing machinery, tumor suppressors, members of the cohesion complex, and regulators of DNA methylation. Alterations in these pathways often occur in combination with mutated signaling mediators of which the constitutively activated FLT3-ITD receptor tyrosine kinase is among the most prevalent. Our lab is particularly interested in leukemogenic alterations of chromatin modifiers such as mixed lineage leukemia 1 (MLL1) or the nuclear receptor interacting SET domain proteins (NSD1-3).

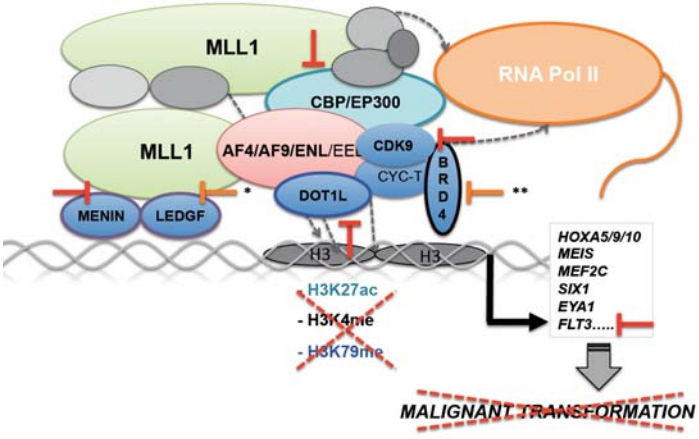

Fig. 2: Targeting protein-protein interactions in the MLL-fusion complex
MLL-fusions mediate a leukemogenic gene expression program in large dynamically composed multi-protein complexes (as simplified in this schema). Disruption of critical protein-protein interactions and/or blocking enzymatic functions is currently explored as novel therapeutic avenue for MLL-fusion driven acute leukemia. We have dissected critical interactions of the lens-derived epithelial growth factor (LEDGF) with menin and MLL (*Mereau et al., 2013), and demonstrated the anti-leukemic activity of small molecules blocking the bromodomain of BRD4 (**Picaud et al., 2013).

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- Brault, L., Menter, T., Obermann, E.C., Knapp, S., Thommen, S., Schwaller, J*., and Tzankov, A*. (2012). PIM kinases are progression markers and emerging therapeutic targets in diffuse large B-cell lymphoma. British Journal of Cancer 107, 491-500.
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Ovarian Cancer, Carcinosarcoma

Cervical Cancer

Vulva Melanoma

Breast Cancer

Endometriosis

Tumor Marker

Malignant Transformation Drug Resistance

Transcriptomics and Glycomics

Gynecological Cancer Research

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Gynecological cancers and biomarker research

Ovarian cancer is the fifth most common cause of death from all cancers in women and the leading cause of death from gynecological malignancies. The overall prognosis is poor due to the lack of reliable screening tools (e.g. tumor markers), the heterogeneity of the disease and the unknown origin of this cancer. Our research focus (both basic and translational) covers the genetic origin of gynecological cancers, in particular ovarian/tubal/peritoneal cancers, the molecular biology of the development of these cancers, and the identification of diagnostic/prognostic predictors and therapeutic targets. Our research is translational and utilizes modern high-throughput technologies and our previously established cohort of 800 Swiss and 800 Australian healthy and gynecological cancer patients, which is linked to a large biobank and comprehensive clinicopathological data. We have moved from Sydney, where we have worked in the Lowy Cancer Centre (University of New South Wales) for the past five years, and joined the DBM in July 2012.

Anti-glycan antibodies. Glycobiology is a relatively new and continuously growing field in cancer research and believed to foster the understanding of cancer development and the identification of novel tumor markers. We were the first research group who used a printed glycan array (PGA) approach to screen blood and ascites of a Swiss cohort of 250 patients for diagnostic anti-glycan antibodies (AGA). The results showed that naturally occurring AGA to P_1, a glycosphingolipid, discriminate healthy women from ovarian cancer patients with a sensitivity and specificity comparable to that of the current tumor marker CA125 (Jacob et al., 2012a). These findings were validated and proofed method- (Pochechueva et al., 2011a; Pochechueva et al., 2011b) and cohort-independent (manuscript submitted). The validation technology, our in-house designed multiplex suspension array (SA), is based on fluorescently-labeled polystyrene microspheres as solid support for unique carbohydrate ligands and combines the advantages of flow-cytometric multiplex SA and advanced carbohydrate chemistry. This technology allows to profile and to detect naturally occurring tumor associated anti-glycan antibodies simultaneously in human serum. Preliminary data also indicate that it is the IgM-subtype of the AGA identified in the PGA that discriminates between healthy individuals and cancer patients.

We are currently investigating the significance and biological function of our top candidate P_1 in ovarian cancer: Is P_1 glycosphingolipid expressed specifically on the surface of ovarian cancer cells (both tissue and cell lines)? Can naturally occurring AGA directed against P_1 antigen be isolated from ascites and do these AGA bind to naturally expressed P_1 present on ovarian cancer cell lines? Is P_1 implicated in malignant transformation and other cellular processes such as proliferation, adhesion, migration, and invasion? We also investigate the molecular mechanism(s) underlying the synthesis of P_1, with particular focus on expression regulation of *A4GALT* (encodes the key glycosyltransferase in of P_1 synthesis), the processes leading to the presence of P_1 on the cell surface, and the potential of P_1 as a prognostic/diagnostic marker and as a therapeutic target, for instance for anti-P_1-antibody drug conjugates. We are also aiming to optimize the current version of glycan-based SA in order to maximally reduce experimental background and exclude false-positive/negative results due to antibody cross-reactivity, as well as to bring it closer to clinics. Polyethylene glycol (PEG) modifications will be applied to the molecular construct on bead surface and PEG-modified beads are expected to exhibit reduced binding to off-target antibodies.

Endometriosis and drug resistance. Age, family history of gynecological cancer, obesity and reproductive history are among the risk factors for ovarian cancer. There is evidence that patients with endometriosis, a benign, chronic and estrogen-dependent disease in women of reproductive age,

have an increased risk for ovarian cancer, suggesting that ovarian cancer and endometriosis may share some functional relationship. Mutations in *PTEN, p53, KRAS,* and only recently *ARID1A* are suspected to play a role in the malignant transformation of endometriosis towards endometrioid and clear cell ovarian cancers. We have recently shown in a TMA-study that ARID1A expression is lost in ovarian endometrioses (Samartzis et al., 2012, Mod Pathol). However, more studies are needed to learn whether loss of ARID1A may be diagnostic factor for risk of malignant transformation. Innate and – in particular – acquired (during treatment; *e.g.* with cisplatin) drug resistance, is a major obstacle in ovarian cancer therapy. An ongoing project (collaboration with the Lowy Cancer Research Centre, Sydney) addresses the efficacy of novel polyarsenic-adamantane compounds in DNA repair-deficient, drug resistant ovarian cancer cells. Our preliminary results indicate that these compounds retain efficacy in these resistant cells. These promising compounds are currently tested in a mouse model.

Wnt-signaling pathway. In recent studies we identified the Wnt-signaling pathway, a pathway essential for the development, differentiation, polarity, migration, adhesion and survival, to be involved in ovarian cancer development. We found that expression of secreted frizzled related protein 4 (SFRP4), a Wnt signaling antagonist, is lost in ovarian cancer patients and is strongly linked with survival (Jacob et al., 2012b). I addition, SFRP4 is involved in epithelial to mesenchymal transition (EMT) (Ford et al., 2013, PLoS ONE). Recently, we demonstrated that the non-canonical Wnt ligand, Wnt5a, is upregulated in the same cohort of epithelial ovarian cancer patients and that with stronger expression patients had shorter relapse-free and disease-specific survivals (manuscript submitted). We are currently investigating Ror2, a recently described and evolutionary conserved receptor tyrosine kinase, structurally related to the Frizzled receptors and assumed to act as the specific receptor for Wnt5a.

Candidate gene approaches. Modern high-throughput technologies for transcriptomics permit the analysis of the expression of thousands of genes in one experiment within a specific biological system. This allows the profound insight into the heterogeneous genetic background of a disease and, combined with the consideration of clinicopathological parameters of patients, the identification of novel candidate tumor markers as well as new diagnostic, prognostic and therapeutic targets. Our previously investigated markers include HE4 (Fig. 1) (Jacob et al., 2011) and GAS6 (Bühler et al., 2013, Biomed Res Int). Using our previously constructed tissue microarrays we are currently examining by immunohistochemistry the protein expression of MELK, a serine/threonine protein kinase, CXADR, a member of the immunoglobulin superfamily and a component of vertebrate tight junctions, and CD47, a widely expressed cell surface receptor that serves as a counter-receptor for signal regulatory protein-α and as a receptor for the secreted protein thrombospondin-1.

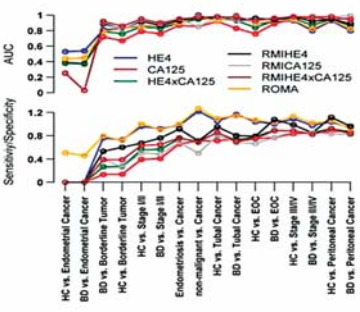

Fig. 1: Diagnostic performance of currently used biomarker for ovarian cancer (Swiss cohort)
Summarized ROC values. AUC and ratio of sensitivity divided by specificity summarized for each binary classifier. Each colored line presents ROC for an individual model. Comparisons sorted by the ratio sensitivity/specificity of CA125 (red line). Ratio of 1 where sensitivity and specificity are equal (gray line); healthy control (HC); benign disease (BD).

Connection to Clinical Practice

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Clinicopathological projects: breast cancer, vulva melanoma, MMMT endometrium and SCC cervix

The significance of **elastosonography,** a non-invasive diagnostic tool in **breast cancer,** in the differentiation between benign and malignant findings is unclear. Respective strain ratios show that the average ratio for malignant were 2-fold higher but not statistically significant. The strain ratio is thus an unsuitable indicator to decide in favor or against an invasive diagnostic assessment. Atomic force microscopy of biopsies show that tumor tissue is less stiff than benign, suggesting this nanomechanical "fingerprint" as diagnostic value (Plodinec et al. 2012, Nat Nanotech). Our retrospective study of patients with adnexal mass detected by US (1998-2012) revealed that a **risk of malignancy index** (RMI) of < 200 indicates a high probability that adnexal mass is not invasive ovarian cancer. RMI calculation is an inexpensive, reliable, and easy-to-use tool for management of adnexal masses and triage for surgery. Comparison of **MMMT of endometrium** (E) and endometrium showed differences in clinicopathological characteristics, survival, and response to platinum/anthracycline-based chemotherapy (was superior to platinum/taxol). A **vulva melanoma** (VM) study with a literature review and cKIT immunohistochemistry in 33 Australian VM patients revealed cKIT expression as independent survival predictor.

Selected Publications

- Jacob, F., Goldstein, D. R., Bovin, N. V., Pochechueva, T., Spengler, M., Caduff, R., Fink, D., Vuskovic, M. I., Huflejt, M. E., and Heinzelmann-Schwarz, V. (2012a). Serum antiglycan antibody detection of nonmucinous ovarian cancers by using a printed glycan array. International Journal of Cancer 130, 138-146.
- Jacob, F., Meier, M., Caduff, R., Goldstein, D., Pochechueva, T., Hacker, N., Fink, D., and Heinzelmann-Schwarz, V. (2011). No benefit from combining HE4 and CA125 as ovarian tumor markers in a clinical setting. Gynecologic Oncology 121, 487-491.
- Jacob, F., Ukegjini, K., Nixdorf, S., Ford, C. E., Olivier, J., Caduff, R., Scurry, J. P., Guertler, R., Hornung, D., Mueller, R., et al. (2012b). Loss of secreted frizzled-related protein 4 correlates with an aggressive phenotype and predicts poor outcome in ovarian cancer patients. PloS One 7, e31885.
- Pochechueva, T., Jacob, F., Goldstein, D. R., Huflejt, M. E., Chinarev, A., Caduff, R., Fink, D., Hacker, N., Bovin, N. V., and Heinzelmann-Schwarz, V. (2011). Comparison of printed glycan array, suspension array and ELISA in the detection of human anti-glycan antibodies. Glycoconjugate Journal 28, 507-517.

Neuropsychiatric and Neurodevelopmental Disorders

Hereditary Colorectal Cancer

Genotype-phenotype Correlations

Chromosome Abnormalities

Human Genomics

New Group since 2013

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Molecular genetic analysis of hereditary colorectal cancer syndromes, neuropsychiatric and neurodevelopmental phenotypes

The major goal of our research group is to identify the molecular (genetic) basis of human diseases and use this knowledge to understand the disease-causing molecular mechanisms. The group has a long-standing focus on hereditary colorectal cancer syndromes and has recently also included genetically complex neuropsychiatric disorders and developmental delay as research subjects.

Hereditary colorectal cancer syndromes

Our group predominantly focused on Familial adenomatous polyposis (FAP) and Lynch syndrome during the reporting period. We identified APC germ line mosaicism in two unrelated patients with classical polyposis coli in whom neither full Sanger sequencing nor gene dosage analysis on leucocyte-derived DNA could identify a pathogenic *APC* mutation. Using the protein truncation test (PTT), a technique largely replaced by DNA-sequencing, we were able to identify two novel, pathogenic *APC* alterations present in a mosaic state, at blood levels (1-15%) below the detection limits of conventional Sanger sequencing consequently allowing carrier testing in both families (Fig. 1). The findings demonstrate the value of the PTT in identifying mosaic mutations in apparently *APC* mutation negative FAP patients with *de novo* classical polyposis and the need to keep it within the diagnostic repertoire for *APC* mutation analysis (Necker et al., 2011).
Whether or not breast cancer is part of Lynch syndrome, an autosomal dominant cancer predisposition caused by mutations in DNA mismatch repair (MMR) genes, is a heavily debated issue. In 92 Swiss female MMR mutation carriers we observed that in contrast to endometrial and ovarian cancer, which occurred significantly more often and at younger age in mutation carriers (median 50.5 and 49.0 years; $P < 0.00001$), overall cumulative breast cancer incidence closely mirrored the one in the Swiss population (56.5 years). We found that 6 (85.7%) of seven breast cancer specimens available for molecular investigations displayed the hallmarks of MMR deficiency. Combined with data from the literature, MSI was present in 26 (70.3%) of 37 and altered MMR protein expression in 16 (72.7%) of 22 breast cancer specimens from proven MMR mutation carriers, strongly suggesting that MMR deficiency plays a pivotal role for breast cancer development in Lynch syndrome (Buerki et al., 2012).

Neuropsychiatric disorders

We have systematically sought after genetic risk factors for common neuropsychiatric disorders (schizophrenia, bipolar disorder, major depression) by performing genome-wide association studies (GWAS). Our GWAS of bipolar disorder is a successful example of this strategy (Cichon et al., 2011). The strongest association signal was identified at the gene *neurocan (NCAN)*, located on chromosome 19 (Fig. 2). The gene is highly expressed in cortical and hippocampal areas in mice, regions previously implicated in bipolar disorder in a variety of neuropsychological, neuroimaging, and postmortem studies. *NCAN* expression peaks during embryonal development and significantly drops after birth. The gene product obviously plays a crucial role in adhesion and migration of neuronal cells (Cichon et al., 2011). In follow-up experiments, we performed genotype-phenotype correlations and explored the behavioural phenotype of *Ncan* knock-out mice *($Ncan^{-/-}$)*. Our results

strongly suggest that the genetic risk variant in *NCAN* impacts on mania symptoms in humans (Miró et al., 2012).

In further follow-up work, we could show that genetic variation in NCAN not only influences the risk of developing bipolar disorder but also schizophrenia (Mühleisen et al., 2011). These results suggest that there is a stronger genetic overlap between these two common psychiatric disorders than previously thought. In fact, recent collaborative studies (including our patient and control samples) that systematically looked for shared risk variants between five different common psychiatric disorders found that a relatively high proportion of specific risk variants confers a risk to different phenotypes, i.e. show pleiotropic effects (Cross-Disorder Group of the Psychiatric Genetics Consortium, 2013).

Neurodevelopmental delay

In this clinically oriented research part, genotype-phenotype correlations as well as disease gene identification studies were performed in patients and families recruited in our clinical service (e.g. Filges et al., 2011).

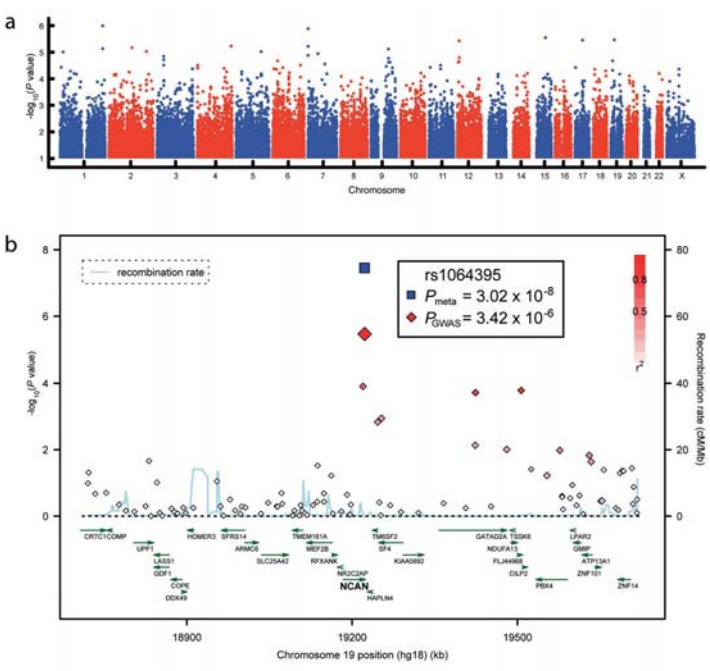

Fig. 2: Results of a GWAS for bipolar disorder. (a) Manhattan plot showing a genome-wide overview of association results for SNPs. The x-axis depicts all the whole genome from chromosome 1 to X. The y-axis shows the negative decadic logarithm of the p-value for each tested SNP (b) Regional association plots of the strongest associated region around the gene NCAN on chromosome 19. The most associated marker from the GWAS (enlarged red diamond) is centered in a genomic window of 1 Mb (hg18, RefSeq genes). The p-value of the same marker is given after inclusion of all replication samples (blue square). The linkage disequilibrium strength (r2) between the sentinel SNP from the GWAS and its flanking markers is demonstrated by the red (high) to white (low) color bar. The recombination rate (cM/Mb; second y-axis) is plotted in blue, according to HapMap-CEU.

Selected Publications

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- Jaeger, E., Leedham, S., Lewis, A., Segditsas, S., Becker, M., Cuadrado, P.R., Davis, H., Kaur, K., Heinimann, K., Howarth, K., et al. (2012). Hereditary mixed polyposis syndrome is caused by a 40-kb upstream duplication that leads to increased and ectopic expression of the BMP antagonist GREM1. Nat Genet *44,* 699–703.
- Cichon, S., Mühleisen, T.W., Degenhardt, F.A., Mattheisen, M., Miró. X., Strohmaier, J., Steffens, M., Meesters, C., Herms, S., Weingarten, M., et al. (2011). Genome-wide association study identifies genetic variation in neurocan as a susceptibility factor for bipolar disorder. Am J Hum Genet *88,* 372-381.
- Cross-Disorder Group of the Psychiatric Genomics Consortium (2013). Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. Lancet *381,* 1371-1379.
- Filges, I., Shimojima, K., Okamoto, N., Rothlisberger, B., Weber, P., Huber, A. R., Nishizawa, T., Datta, A. N., Miny, P. and Yamamoto, T. (2011). Reduced expression by SETBP1 haploinsufficiency causes developmental and expressive language delay indicating a phenotype distinct from Schinzel-Giedion syndrome. J Med Genet *48,* 117-122.

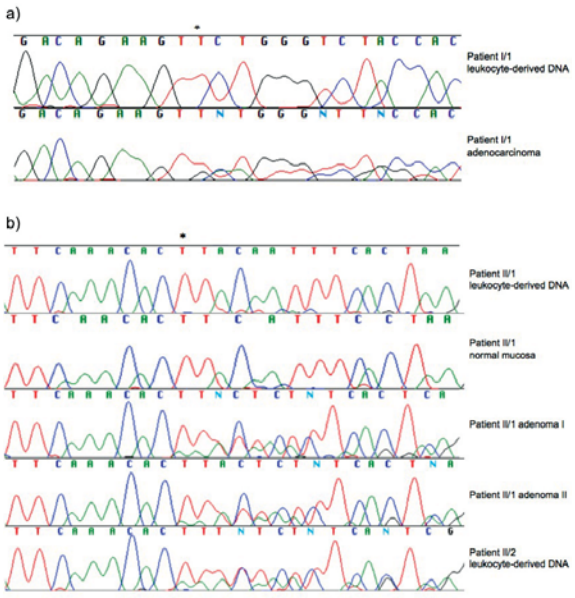

Fig. 1: Results from conventional Sanger sequencing of APC exon 15c in different tissues from (A) patient I/1 and (B) patient II/1 and his daughter, patient II/2. The asterisks denote the start of (A) the five base pair insertion c.2715_2716insGAAGT and (B) the four base pair deletion c.2802_2805delTTAC, respectively.

(Epi)Genome Maintenance

DNA Damage

DNA Repair

DNA Methylation

DNA Demethylation

Molecular Genetics

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Genome and epigenome maintenance in development, aging and disease

Reactive agents of endogenous and environmental origin pose a continous threat to the integrity of genomes. They have a potential to chemically modify the DNA, thereby altering its coding properties and promoting genetic mutation. Such "damage" to DNA, however, does not only occur through random chemical reactions but also by the action of enzymes, in wich case the purpose is to specifically increase genetic variance or alter cell fate determining epigenetic signatures, i.e. DNA methylation. Modifications of either kind occur thousands of times in our DNA every day and need to be controlled if the genetic and epigenetic makeup of cells is to be maintained. We explore biological processes that enforce stability to the structure and function of the genome. Our objective is to provide a thorough understanding of the mechanisms involved and the consequences of their dysfunction for cell identity, transformation and cancer.

Genetic and epigenetic maintenance by DNA base excision repair

A main focus of our recent work has been the clarification of the biological function of the DNA repair enzyme "Thymine DNA Glycosylase" (TDG). TDG first caught our attention because of its ability to hydrolyze thymine or uracil from T•G and U•G DNA mismatches. These mismatches arise frequently in genomic DNA by deamination of cytosine (>U) or 5-methylC (>T) and, unless repaired, will generate C>T mutations, the most prevalent nucleotide change found in human cancers. By its enzymatic activity, TDG is implicated in the antimutagenic repair of these mismatches, but this function has never been corroborated by genetic evidence.

We have been pursuing various approaches to unravel the biological function of TDG and made important discoveries along the way. Through protein interaction studies, we found that SUMO-conjugation is required for full functionality of TDG and thereby established a novel mechanistic paradigm for coordination of DNA repair processes. Through genetic work, we learned that base excision by TDG contributes critically to the DNA toxicity of the chemotherapeutic drug 5-FU and were able to elucidate the underlying molecular mechanism. A breakthrough in understanding TDG function, however, came with the finding that TDG is essential for mouse embryonic development. We showed that this unexpected phenotype reflects a role of TDG in controlling DNA methylation dynamics in differentiating cells, rather than a defect in mutation avoidance, thus expanding the biological function of DNA repair from the genetic to an epigenetic level. The underlying mechanisms became evident with recent discoveries by others of proteins (TET1-3) capable of oxidatizing 5-methylC to 5-formyl- and 5-carboxylC in DNA, both of which are substrate for base excision by TDG. The role of this TET-TDG axis of active DNA demethylation in the establishment of cell identity and in carcinogenesis is subject of current research.

Cancer epigenetics

Aberrant CpG methylation contributes to tumorigenesis by dysregulating of the genome. Exactly why, how and when DNA methylation changes arise during carcinogenesis is unknown. We aim to identify genetic and environmental conditions controlling DNA methylation stability in human tissue and assess the underlying molecular mechanisms. Using the colon and its cancers as a model, we examined the colorectal mucosa of healthy individuals for the presence of cancer-prone methylation changes. We found that aberrant gene promoter methylation arises in an age-, locus- and gender-specific manner. The drift in gene promoter DNA methylation occurs with variable rates across the genome and concerns a variety of genes controlling key pathways

of carcinogenesis. Notably, we could show that lifestyle factors modulate the rate of DNA methylation drift at cancer-relevant genes; e.g. Aspirin use suppressed, while a high BMI increased promoter hypermylation, the same way as they modulate colorectal cancer risk. These findings provided an epigenetic paradigm for how the environment modulates cancer risk. The underlying molecular pathways as well as the predictive value of the targets of methylation drift are subject of currently investigation.

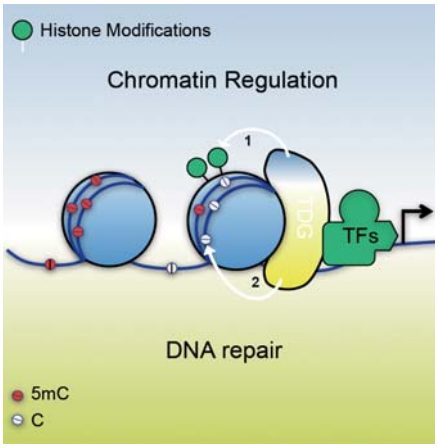

Fig. 1: TDG dependent DNA excision repair controls epigenetic states through DNA demethylation.

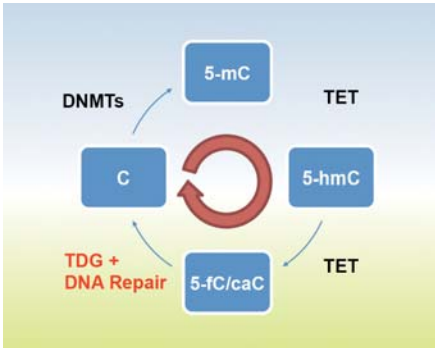

Fig. 2: TDG and TET hydroxylases cooperate in cyclic DNA methylation and active oxidative demethylation at CpG di-nucleotides in the genome. TDG excises 5-fC and 5-caC, thereby initiating excision repair incorporating an unmethylated C. 5-mC, 5-methylcytosine; 5-hmC, 5-hydroxymC; 5-fC, 5-formylC; 5-caC, 5 carbocylC.

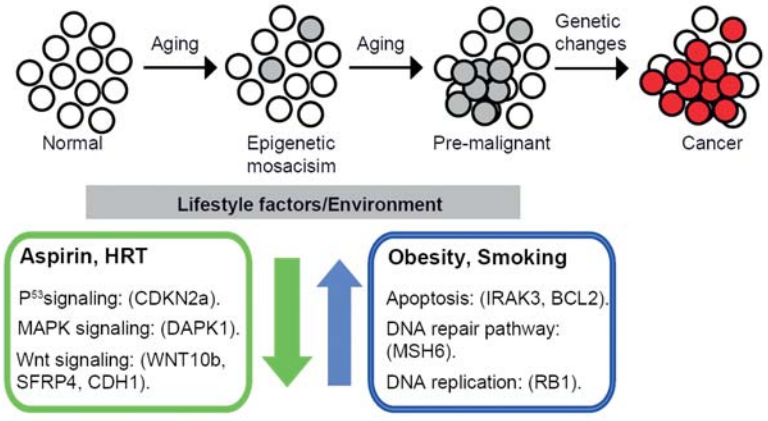

Fig. 3: Lifestyle factors modulate the rate of DNA methylation drift in the aging colonic mucosa and, by inference, early events of colorectal carcinogenesis.

Selected Publications

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- Fritsch, O., Burkhalter, M. D., Kais, S., Sogo, J. M., and Schär, P. (2010). DNA ligase 4 stabilizes the ribosomal DNA array upon fork collapse at the replication fork barrier. DNA Repair (Amst) *9*, 879-888.
- Cortazar, D., Kunz, C., Selfridge, J., Lettieri, T., Saito, Y., Macdougall, E., Wirz, A., Schuermann, D., Jacobs, A. L., Siegrist, F., Steinacher, R., Jiricny, J., Bird, A., and Schär, P. (2011). Embryonic lethal phenotype reveals a function of TDG in maintaining epigenetic stability. Nature *470*, 419-423.
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- Jacobs, A., and Schär, P. (2012). DNA glycosylases: in DNA repair and beyond. Chromosoma *121*, 1-20.

Cancer
Immune Response
Immunotherapy
Tumor Associated Antigens
Tumor Microenvironment
Translational Oncology

Oncology Surgery

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Cancer-immune system interactions: between active antigen specific immunotherapy and the analysis of tumor microenvironment

The molecular characterization of a large number of tumor associated antigens (TAA) and the results of a wealth of active, antigen specific immunotherapy clinical trials provide ample proof of principle that cancers can be targeted by patients' own immune response to measurable clinical benefit. However, emerging evidence suggests that tolerance to TAA, limiting the extent of specific immune responsiveness and tumor microenvironmental conditions intrinsic to cancer tissues might undermine, at least in part, the clinical effectiveness of the induction of tumor specific immune responses.
Based on this background our research group addresses translational oncology projects aimed at the development of innovative clinically relevant cancer immunotherapy protocols and at the characterization of intratumoral environment in a variety of cancers.
During the past fifteen years we have developed an active, antigen specific treatment platform based on the use of recombinant vaccinia virus (rVV) of own design and construction.
A rVV encoding gp100 280-288, Melan-A/MART-1 27-35 and tyrosinase 1-9 HLA-A0201 restricted epitopes from melanoma associated differentiation antigens and CD80 and CD86 co-stimulatory molecules has been used in the treatment of stage III/IV melanoma in phase I/II clinical trials showing safety and promising clinical results. To decrease the intrinsic vector immunogenicity, possibly hindering the induction of transgene specific immune responses, we have now constructed a rVV encoding the Herpes simplex virus derived ICP47 protein, which blocks TAP mediated antigen processing while leaving unaltered the presentation of antigenic peptides directly delivered within the endoplasmic reticulum (ER). A rVV encoding ICP47 and ER targeted TAA epitopes proved to be superior in the induction of tumor specific immune responses in cells from donors with high vaccinia specific immune responsiveness. On the other hand, considering that CD40 triggering by helper T cells promotes the presentation of MHC class I restricted epitopes by activating a variety of antigen presenting cells (APC), we have constructed a series of rVV encoding CD40 ligand (CD154). Our previous studies underline that cancer/testis TAA are expressed in high (>30%) percentages of cases in tumors of high epidemiological relevance, including lung, skin cancers, and urothelial malignancies. Therefore, we have constructed a rVV encoding a multiplicity of HLA-class I restricted cancer/testis TAA epitopes together with CD80 and CD154. "In vitro" data are consistent with a high capacity of this reagent to induce specific immune responsiveness.
Our studies on the prognostic relevance of specific tumor microenvironment features are conducted in collaboration with the Institute of Pathology of the University of Basel and focus, in particular, on colorectal cancer (CRC) and prostate cancer (PCA). Regarding CRC we could show that, similarly to a majority of solid malignancies, natural killer (NK) cell infiltration of CRC is poor and devoid of prognostic significance. In contrast, CRC infiltration by CD16+/CD11b+ myeloid cells appears to be surprisingly associated with good prognosis. Since CRC infiltration by myeloperoxidase producing cells is also associated with improved overall survival, these data may suggest that cells of the granulocytic lineage might possess antitumor functions in CRC.
On the other hand, PD-1/PD-L1 interaction is currently being targeted by therapeutic monoclonal antibodies to prevent T cell "exhaustion". However, we could show that while CD8+ T cells infiltrating CRC are by and large PD-1-, expression of PD-L1 by tumor cells is paradoxically associated with good prognosis.

Interestingly, in PCA the expression of pro-inflammatory cytokine genes appears to be detectable in early stages of the disease, to extents significantly higher than in benign prostatic hyperplasia.
Taken together these data underline the specificities inherent in anticancer immune responsiveness in different cancer types and anatomical districts and suggest that therapeutic interventions should be tailored accordingly.

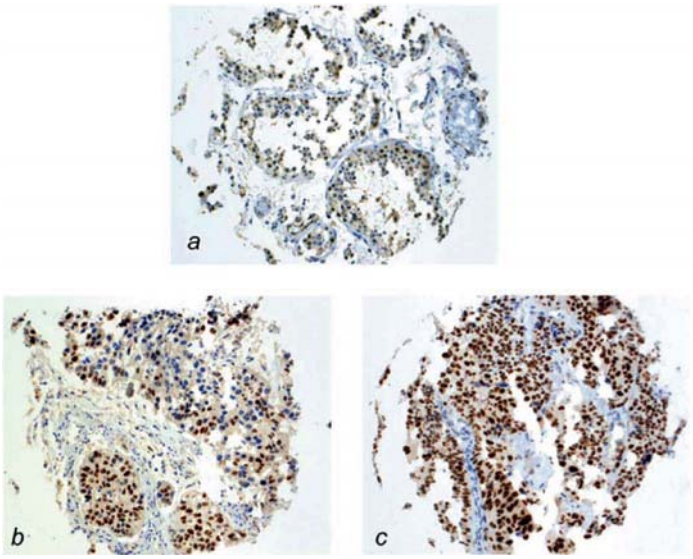

Fig. 1: MAGE-A10 expression in spermatogonia and cancer cells. Sections from healthy testis (panel A) and urothelial carcinomas (panels B and C) were stained with MAGE-A10 specific 3A11 monoclonal antibody. Positive nuclear staining was detectable in healthy spermatogonia and cancer but not interstitial cells. Evidence of MAGE-A10 expression may be detectable in a fraction of tumor cells (panel B) or in a large majority of them (panel C).

Connection to Clinical Practice

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Translational science in surgical oncology

The "Oncology" lab of the Institute for Surgical Research and Hospital Management within the Department of Biomedicine, is closely connected with the surgical clinics of the University Hospital. Young doctors perform their MD dissertations in our lab and surgeons at different levels of their education and clinical careers spend periods ranging between six months and two years in the lab. This close interaction has enormously facilitated the access to clinical specimens and information, the development of translational projects and the planning of clinical trials.
Within this frame, a critical role is also played by the interaction with the Institute of Pathology, maximizing clinical information and providing the indispensable view "from the tumor side" required for a realistic approach to cancer immunotherapy. In particular the tumor microarray technology developed within this Institute allows a rapid evaluation in a multitude of clinical specimens of working hypotheses emerging from basic immunology and cancer research.

Selected Publications

– Raafat N, Sadowski-Cron C, Mengus C, Heberer M, Spagnoli GC, Zajac P. (2012). Preventing vaccinia virus class-I epitopes presentation by HSV-ICP47 enhances the immunogenicity of a TAP-independent cancer vaccine epitope. Int J Cancer 131:E659-669.
– Mengus C, Schultz-Tater E, Coulot J, Kastelan Z, Goluza E, Coric M, Spagnoli G, Hudolin T. (2013). MAGE-A10 cancer/testis antigen is highly expressed in high grade non muscle invasive bladder carcinomas. Int J Cancer 132:2459-63.
– Droeser R, Hirt C, Viehl CT, Frey DM, Nebiker C, Huber X, Zlobec I, Eppenberger-Castori S, Tzankov A, Rosso R, et al. (2013). Clinical impact of programmed cell death ligand 1 expression in colorectal cancer. Eur J Cancer (2013) 49, 2233-2242.
– Droeser RA, Hirt C, Eppenberger-Castori S, Zlobec I, Viehl CT, Frey DM, Nebiker C, Rosso R, Zuber M, Amicarella F, et al. (2013). High myeloperoxidase positive cell infiltration in colorectal cancer is an independent favorable prognostic factor. PLosOne 8(5):264814.
– Le Magnen C, Bubendorf L, Rentsch CA, Mengus C, Gsponer J, Zellweger T, Rieken M, Thalmann GN, Cecchini M, Germann M. et al. (2013). Characterization and clinical relevance of ALDH bright populations in prostate cancer. Clin Cancer Res. 2013; 19:5361-5371.

Angiogenesis

Cancer

EMT

Lymphangiogenesis

Metastasis

Signal Transduction

Tumor Biology

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Molecular dissection of tumor angiogenesis, lymphangiogenesis, and metastasis

The vast majority of cancer deaths are due to metastasis. One major objective of our research is the identification and characterization of the molecular pathways underlying malignant tumor progression and metastasis formation; these pathways may be potential targets for innovative cancer therapies. In particular, we focus on the contribution of tumor angiogenesis and lymphangiogenesis to tumor progression and on the molecular mechanisms underlying the transition from benign neoplasia to malignant cancers and the metastatic dissemination of tumor cells. In addition to tumor cell lines in vitro, we employ transgenic mouse models of tumorigenesis to determine causal connections between the expression of particular genes and tumor progression and metastasis in vivo.
The development of malignant tumors is in part characterized by a tumor cell's capability to overcome cell-cell adhesion and to invade surrounding tissue by a process referred to as epithelial-mesenchymal-transition (EMT). EMT underlies the conversion of epithelial, differentiated cells to mesenchymal, migratory and invasive cells. In the past years, we have learned that EMT occurs in multiple stages and is regulated by sophisticated molecular networks regulating the expression of a large number of protein- and miRNA-encoding genes. Notably, we have identified several transcription factors that act as master regulators not only in the initiation and execution of the morphogenic process of EMT but also in providing survival signals to cancer cells and thus allowing cancer cells to seed and grow metastases in distant organs. We investigate the direct target genes of these transcription factors and their functional contribution to tumor metastasis. These transcription factors bear many hallmarks of stem cell functions and may also define "cancer-initiating cells" which are able to seed metastasis. Transcriptional control is also studied in the context of the epigenetic regulation of gene expression, such as histone modifications and DNA methylation, and their role in maintaining a cancer cell's plasticity to undergo EMT and to revert back to a differentiated cell (MET). Finally, we assess the role of miRNAs and lncRNAs and their target genes in the regulation of EMT and metastatic dissemination. With these experimental approaches we aim at the identification of the master regulators of EMT and metastasis and we plan to scrutinize their potential as therapeutic targets for preventing metastatic disease.
In a second line of research, we investigate the molecular pathways underlying the development of evasive resistance to targeted cancer therapy. We employ a number of mouse models to study the pathological, physiological and molecular consequences of therapies targeting tumor angiogenesis and malignant tumor progression. In particular, we use cell biological, biochemical and bioinformatical analysis to delineate the molecular pathways allowing cancer cells to escape from targeted therapy. Moreover, we employ various transgenic mouse models for the design and testing of innovative cancer therapies, either based on anti-angiogenic strategies or by directly targeting cancer cells. For example, in collaboration with Prof. Christoph Rochlitz, Dr. Andreas Wicki, and Dr. Christoph Mamot, Clinical Oncology, University Hospital Basel, we have tested immunoliposomes that are designed to target the tumor vasculature. Finally, in collaboration with pharmaceutical companies we are investigating the efficacy and biological consequences of various anti-angiogenic and anti-metastatic cancer treatments.

* left during the report period

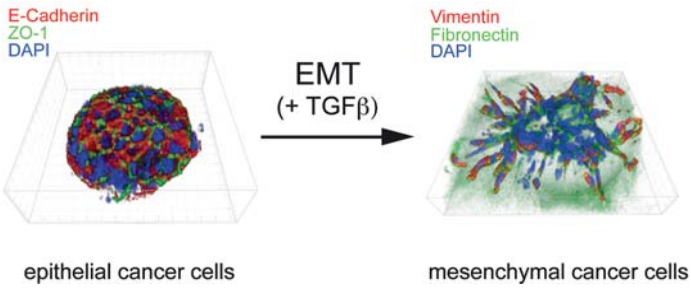

Fig. 1: Changes of invasive properties of breast cancer cells during TGFβ-induced EMT. Epithelial breast cancer cells derived from a breast tumor of a MMTV-Polyoma Middle T transgenic mouse (Py2T) were cultured in a three-dimensional Matrigel matrix and induced by TGFβ to undergo an epithelial-mesenchymal transition (EMT). Structures were grown for 6 days, and stained directly in Matrigel with antibodies against epithelial E-cadherin and ZO-1 or against mesenchymal vimentin and fibronectin. Immunofluorescence images were acquired by confocal microscopy, and three-dimensional reconstructions of confocal imaging stacks are displayed.

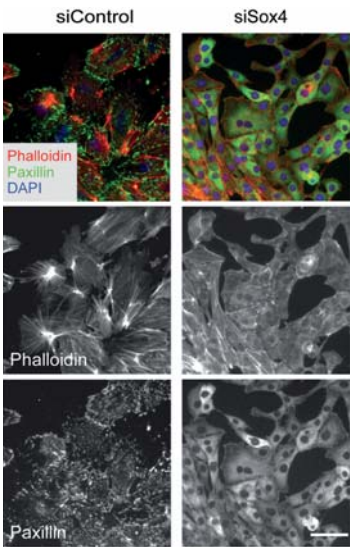

Fig. 2: Sox4 is required for TGFβ-induced EMT. NMuMG normal murine mammary gland epithelial cells were depleted of Sox4 expression by the transfection of specific siRNA (siSox4) and then treated with TGFβ for 4 days to induce EMT. EMT-induced stress fibers (SF) and focal adhesions (FA) were visualized by staining with phalloidin (SF) and paxillin (FA) and immunofluorescence microscopy (left panels). DAPI was used to visualize nuclei. Note that in the absence of Sox4 stress fibers and focal adhesions failed to form. Size bar, 50μm.

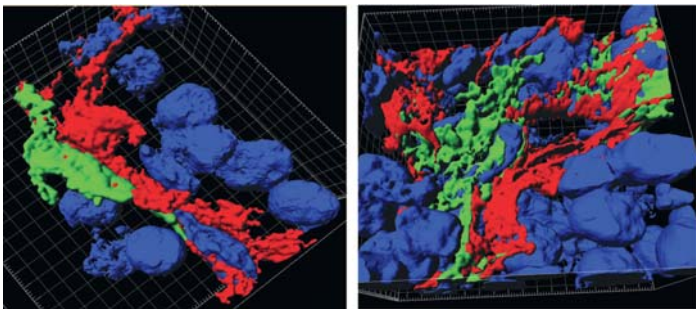

Fig. 3: Confocal three-dimensional reconstruction of pericytes (green) binding to tumor endothelial cells (red) in pancreatic β-cell tumors of Rip1Tag2 transgenic mice. Note that transgenic expression of angiopoietin-1 in tumor cells induces maturation of tumor vessels, while transgenic expression of angiogepoietin-2 prevents vessel maturation. Blue = nuclei of endothelial cells, pericytes and tumor cells.

Connection to Clinical Practice

Dr. Andreas Wicki, Prof. Dr. Christoph Rochlitz, Prof. Dr. Alfred Zippelius, Prof. Dr. Markus Heim
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Targeted therapy and evasive resistance

Angiogenesis is a key process in tumor progression. In the past years, therapeutic approaches against the vascular endothelial growth factors (VEGF), VEGF receptor-2 signaling axis have been designed and are now in routine clinical use. However, an efficient and longstanding targeting of tumor-associated endothelial cells has not been achieved. In collaboration with Prof. Christoph Rochlitz, Dr. Andreas Wicki, and Dr. Christoph Mamot we have employed anti-VEGFR2 antibodies covalently linked to pegylated liposomal doxorubicin (PLD) to specifically ablate tumor-associated endothelial cells in several transgenic and transplantation mouse models of cancer. Anti-VEGFR2-targeted immunoliposomes loaded with doxorubicin (anti-VEGFR2-ILs-dox) have proven superior in therapeutic efficacy to several other anti-angiogenic approaches and, hence, may provide a novel and promising anti-cancer strategy for patient treatment.

The development of resistance to targeted cancer therapy (evasive resistance) has appeared a major obstacle in patient care. In collaboration with Prof. Alfred Zippelius and Prof. Markus Heim, we have begun to establish patient-derived xenotransplanted (PDX) mouse models of head and neck squamous carcinoma and hepatocellular carcinoma that recapitulate evasive resistance by employing serial biopsies from patients undergoing targeted therapy. We aim at the establishment of mouse models that recapitulate the development of evasive resistance to targeted therapies and that can be used for molecular, biochemical and genomic analysis of the processes underlying evasive resistance and to test first alternative therapies to overcome evasive resistance.

Selected Publications

- Fagiani, E., Lorentz, P., Kopfstein, L., and Christofori G. (2011) Angiopoietin-1 and 2 exert antagonistic functions in tumor angiogenesis yet both induce lymphangiogenesis. Cancer Res. 71, 5717-5727.
- Yilmaz, M., Maaß, D., Tiwari, N., Waldmeier, L., Schmidt, P., Lehembre, F., and Christofori, G. (2011) Dlx2 protects from TGFβ-induced cell-cycle arrest and apoptosis. EMBO J. 30, 4489-4499.
- Waldmeier, L., Meyer-Schaller, N., Diepenbruck, M. and Christofori, G. (2012) Py2T murine breast cancer cells, a versatile model of TGFβ-induced EMT in vitro and in vivo. PLoS One 7, e48651.
- Tiwari, N., Meyer-Schaller, N., Arnold, P., Pachkov, M., van Nimwegen, E., and Christofori, G. (2012) Klf4 is a transcriptional regulator for genes critical for EMT, including Mapk8 (Jnk1). PLoS One 8, e57329.
- Tiwari, N., Tiwari, V.K., Waldmeier, L., Balwierz, P.J., Arnold, P., Pachkov, M., Meyer-Schaller, N., Schübeler, D., van Nimwegen, E., and Christofori, G. (2013) Sox4 is a master regulator of epithelial-mesenchymal transition (EMT) by controlling Ezh2 expression and epigenetic reprogramming. Cancer Cell 23, 768-783.

DBM Focal Area Immunology

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We live in a hostile environment, where we are continuously facing insults that can disrupt our physical integrity. Immunology is dedicated to understanding the various facets of interactions between host and environment, whether they are hostile or not. Equally important, immunologists aim at defining the mechanisms that maintain internal homeostasis in the body. Reflecting the broad spectrum of the field, our Focal Area (FA) Immunology brings together a great variety of research groups with projects touching upon many of these fundamental aspects.

The FA Immunology of the Department of Biomedicine (DBM) currently comprises 21 research groups and offers a dynamic platform for exchange among them. This is best exemplified by the weekly immunology seminars and fruitful collaborations among the research groups. Whereas some groups concentrate their effort on developmental aspects of the immune system, others are dedicated to the study of how immune responses are regulated. The impact of the groups' research goes beyond the specific field of immunology and is addressing basic principles in biology and medicine likewise. Hence, the research topics in this FA range from clinical and experimental immunology, through diabetes research and hepatology, to transplantation nephrology and virology.

A major goal of the FA Immunology is to understand the immunological aspects of diabetes, specifically the pathogenesis of diabetic nephropathy and islet inflammation in type 2 diabetes. Additional clinical and translational questions drive research groups that study the immune response against fungal and other infections. Other groups investigate how primary immunodeficiency and autoimmunity are interlinked; the role of natural killer cells in controlling infection and disease relapse in transplant recipients, and how autoantibodies targeting the complement factor C1q impact the disease course of systemic lupus patients. Other important questions tackled in the FA Immunology relate to the study of interaction characteristics between immune system and chronic viral infection. This research is centered on basic aspects defining immune mechanisms conferring protection or triggering autoimmunity, as well as clinical issues such as testing of viral tropism or virus evolution under antiviral therapy.

This wide variety of basic and translational immunological research activities within this FA is strongly linked to clinical medicine of the University Hospitals and other institutions in Basel and worldwide – making the FA Immunology a dynamic hot spot for biomedical research.

Complement

Autoantibodies

Systemic Lupus Erythematosus

Clinical Immunology

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The pathogenic role of autoantibodies against complement C1q and complement MBL and in human diseases

Systemic lupus erythematosus (SLE) is the archetype of a systemic autoimmune disease. However, the causes and pathogenic mechanisms of SLE are still not fully understood. A major hypothesis of the pathogenesis of SLE assumes that the disease is driven by a defective clearance of dead and dying (apoptotic) cells. In the context of an altered clearance, these apoptotic cells could become antigenic and initiate an autoimmune response. The complement system has been shown to play an important role in the clearance of apoptotic cells and the deficiency of one of the early components of the classical pathway of complement is strongly associated with the development of SLE. However, most SLE patients have no primary complement deficiency. In contrast, hypocomplementemia in SLE patients is a secondary event and most often associated with antibodies against the first component of the classical pathway of complement (C1q). As we and others have shown, autoantibodies against C1q (anti-C1q) strongly correlate with renal flares in SLE patients. Our studies suggest that the occurrence of anti-C1q in SLE patients is necessary but not sufficient for the development of severe lupus nephritis. It is possible that anti-C1q interfere with the normal function of the complement system including the clearance of apoptotic cells. As we could show, anti-C1q specifically target C1q when bound to the surface of early apoptotic cells. More recently, we could identify a major linear epitope on the C1q molecule targeted by anti-C1q. A diagnostic ELISA using this peptide was shown to be more specific and more sensitive than a conventional anti-C1q assay for the detection of active nephritis in SLE patients. However, the role of anti-C1q in other diseases is not yet established and the suspected pathogenic mechanism of anti-C1q remains to be elucidated. Furthermore, the importance of regular anti-C1q measurements as a clinical follow-up marker in SLE patients is not yet established. Therefore, our group aims to further examine the pathological role and the clinical relevance of anti-C1q antibodies in a double approach based on experimental studies of anti-C1q and clinical studies of patients with SLE. In the experimental studies we want to understand i) the consequences of the binding of anti-C1q for the complement system and for phagocytic cells, ii) the origin of anti-C1q that potentially are initiated by molecular mimicry, and iii) the binding of von Willebrand Factor (vWF) to C1q since anti-C1q were found to have a striking sequence homology with vWF.

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Connection to Clinical Practice

In our clinical studies we are analysing the role of anti-C1q as an important follow-up parameter in SLE patients but also the role of other autoantibodies and serum cytokines as biomarkers of the disease.
Independent from anti-C1q studies, we are studying the role of complement split products (i.e. activation parameters) and complement mannan-binding lectin (MBL) in clinical settings. MBL is strongly related to C1q and has been shown to play an important role in the defence against infectious agents. More recent studies suggest that MBL also binds to apoptotic cells and plays a pro-inflammatory role in experimental settings of ischaemia-reperfusion injury. The high frequency of functional MBL deficiency in the general population (about 25%) predestinates MBL for clinical studies investigating its role in human diseases.

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- Osthoff M, Katan M, Fluri F, Schuetz P, Bingisser R, Kappos L, Steck A, Engelter ST, Müller B, Christ-Crain M, Trendelenburg M. Functional deficiency of mannose-binding lectin is associated with smaller infarction volume and favorable outcome in ischemic stroke patients. PLoS ONE 2011; 6(6): e21338.
- Koenig K, Groeschel I, Perinova SS, Tesar V, Eisenberger U, Trendelenburg M. Serum cytokine profile in patients with active lupus nephritis. Cytokine 2012; 60: 410-416.
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Hematopoiesis

Stem Cells

T Cell Development

B Cell Development and Autoimmunity

Developmental and Molecular Immunology

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Molecular mechanisms guiding hematopoietic cell development

The role of FLT3L in hematopoietic development
All cells of the blood are derived from hematopoietic stem cells (HSC's). However the molecular mechanisms that drive the differentiation of these into various hematopoietic cells is still poorly understood. Now we have analyzed the role of FLT3L in this process by transgenic over-expression. Our analyses revealed that, FLT3L transgenic mice displayed a dramatic expansion of dendritic and myeloid cells, leading to splenomegaly and blood leukocytosis (Figure 1). Bone marrow myeloid and lymphoid progenitors were significantly increased in numbers but retained their developmental potential. Furthermore, transgenic mice developed anemia together with a reduction in platelet numbers. FLT3L was shown to rapidly reduce erythrocyte numbers when injected into wild-type mice, indicating a direct negative role of the cytokine on erythropoiesis. We conclude that FLT3L acts on multipotent progenitors in an instructive way, inducing their development into myeloid/lymphoid lineages while suppressing their megakaryocyte/erythrocyte potential (Figure 2). (A manuscript describing this has been submitted)

An epigenetic profile of early T cell development
Cellular differentiation of the T cell branch of the immune system begins with the hematopoietic stem cell, which undergoes a series of stages characterized by progressive restriction in multipotency and acquisition of specific lineage identity.
At the molecular level, the restriction of cell potential, commitment and differentiation to a specific lineage is achieved through the coordinated control of gene expression and epigenetic mechanisms. Here we analyzed and compared the gene expression profiles and the genome wide histone modification marks H3K4me3 and H3K27me3 in 1) In vitro propagated hematopoietic stem cells, 2) In vitro generated and propagated pro T dells derived from these HSC's, and 3) Double positive thymocytes derived from these pro T cells upon injection of Rag deficient mice. The combined analyses of the different datasets highlighted the importance of both transcriptional and epigenetic repression in shaping the early phases of T cell development.

Autoimmunity and aging
Some autoimmune features, like production of anti-nuclear antibodies (ANA) or rheumatoid factor (RF) become abundant in elderly human beings. In most cases, these elderly remain healthy. In this report we have investigated, whether the same holds true for inbred strains of mice. We found, that almost all mice of the strain C57BL/6 (B6) spontaneously produce high titers of IgG ANA, when they reach the age of 20 months. At that time, large numbers of germinal centers are present in the spleen, IgG deposition can be seen in glomeruli of kidneys and lymphocyte infiltrates are found in the salivary glands. Despite all these signs of an autoimmune response, the mice remain healthy.
In marked contrast to B6 mice, mice of the inbred strain DBA/2 do not produce any IgG ANA's at that age. However, the F1 hybrids of these two strains (BDF1), show an intermediate incidence and lower titers of IgG ANA production, pointing out the importance of the genetic background. The ANA production is CD4 T cell dependent, since B6 mice deficient for MHC class II do not produce IgG ANA's upon ageing. Experiments with lethally irradiated mice clearly show, that it is the ageing hematopoietic environment and not the precursor cells, which determine IgG ANA production. Adult thymectomy of young B6 mice will rapidly create a situation where ANA production commences as a result of altered T cell homeostasis, mimicking the condi-

tions observed in old mice. Thus, our findings indicate that disturbed T cell homeostasis can drive the onset of some autoimmune features. Moreover, we generated over 170 IgG ANA producing hybridomas from individual old B6 and Balb/c mice. Around half of these IgG ANA's were of the IgG2A and the other half were of the IgG2B isotype. No ANA of the IgG1 isotype was found indicating that TH1 cells drive this autoantibody formation. Currently we are trying to identify the antigen(s) that trigger this autoantibody formation.

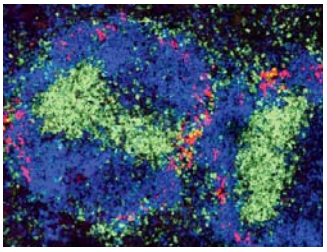

Fig. 1: Immunofluorescence of spleen section of FLT3L-Tg mouse stained for B cells (anti-IgM, green) dendritic cells (anti-CD11c, blue) and marginalzone macrophages (anti-SIGN Related 1, red)

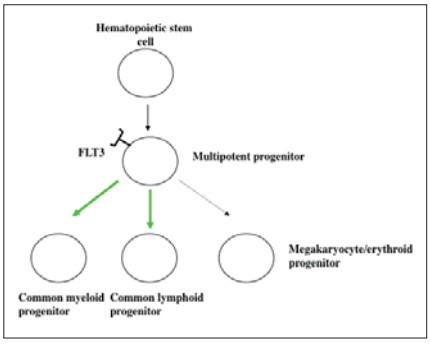

Fig. 2: Model for the instructive action of FLT3L. In the presence of excess of FLT3L MPP preferentially differentiate into CLP's and CMP's whereas their differentiation MEP's is partially blocked

Connection to Clinical Practice

Prof. Dr. Antonius Rolink
University of Basel, Department of Biomedicine

Reconstitution of the adaptive immune system

In vivo reconstitution of the T cell compartment with in vitro propagated pro T cells

Patients with severe hematological disorders like leukemia's, lymphomas and myelomas usually undergo radio- and /or chemotherapy. However, since these therapies destroy blood cell production these patients have to be subsequently transplanted with HSC's. A drawback of such a therapy is that the reconstitution of adaptive immune system and especially the T cell compartments is relatively slow and thus the patients go through a rather long phase in which they are immunodeficient.

We have now developed a stromal cell free culture system that very efficiently allows the differentiation of mouse HSC's into pro-T cells. This culture system consists of plate bound Notch ligand Delta like 4, IL-7 and SCF. Thus HSC's cultured under these conditions differentiate into cells that are equivalent to double negative 2 (DN2) cells found in the thymus and these then can be propagated (doubling times 24 – 30 hrs) in vitro for at least up to 6 months. Transplantation of these into T cell deficient mice like Rag2-/- or CD3ε-/- revealed that these cells can efficiently give rise to one wave of T cell development. The mature T cells that exit the thymus are functional since transplanted CD3ε-/- can mount a T cell dependent humoral immune response. Moreover, upon transplantation into T lymphopenic like pre-Tα deficient mice these pro-T cells are efficiently able to improve the peripheral T cell compartment.

Based on our mouse findings it is likely to assume that human pro-T cells generated a similar way upon co-transplantation with HSC's will accelerate T cell development in these patients.

Selected Publications

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- BAFF-R expression correlates with positive selection of immature B cells. Tussiwand R, Rauch M, Flück LA, Rolink AG. Eur J Immunol. 2012 Jan;42(1):206-16. doi: 10.1002/eji.201141957. Epub 2011 Dec 5.
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- Auto-reconstitution of the T-cell compartment by radioresistant hematopoietic cells following lethal irradiation and bone marrow transplantation. Bosco N, Swee LK, Bénard A, Ceredig R, Rolink A. Exp Hematol. 2010 Mar;38(3):222-232. e2. doi: 10.1016/j.exphem.2009.12.006. Epub 2010 Jan 4.

Innate Lymphoid Cell

Cytokine

Fetal

Development

Signalling

Inflammation

Developmental Immunology

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Development and function of innate lymphoid cells and lymphoid tissues

Innate lymphoid cells (ILCs) are a group of lymphocytes, which in contrast to T and B cells, lack somatically rearranged antigen receptors. They appear to originate from a common Id2-dependent progenitor cell. In analogy with Th1, Th2 and Th17 T cells, ILCs can be divided into 3 major groups: 1) IFNγ-producing ILC1, 2) IL-4, -5, and -13-producing ILC2, and 3) IL-22 and -17-producing ILC3; the latter depend on the nuclear orphan receptor RORγt. Our previous research has shed light on how a RORγt+ILC3 subset, named lymphoid tissue inducer (LTi) cells, regulates the generation of secondary lymphoid organs. Type 2 and 3 ILCs have recently become the focus of attention for their potential roles in early responses to infection, inflammation, and tissue repair (Figure 1). The identification of molecular pathways that control ILC development and function is essential for a better understanding of how ILCs may contribute to protective or inflammatory responses. Our research is focusing on 1) lineage commitment and 2) age and environment dependent regulation of ILC type 2 and 3 function in mouse and man.

Signaling pathways controlling ILC development

Our research group has a long-standing interest in investigating pathways that regulate ILC development. ILCs emerge from progenitor cells in both the foetal liver and bone marrow (BM). We have established an in vitro assay that allows the development of ILCs from either foetal liver-derived α4β7+ precursor cells, or from BM derived hematopoietic stem cells expressing retroviral Nup98HoxB4 fusion protein. Using genetically modified mouse models (loss or gain of function, reporter gene) and retroviral gene delivery models, we have gained further insights into the cytokine-driven regulation of ILCs. We have reported three critical pathways involved in the life cycle and differentiation of ILC3s and in their function to control lymphoid tissue generation (Figure 2). One is mediated by the cytokine receptor IL-7R that binds to IL-7, a survival factor for lymphocytes. The other is triggered by the engagement of TSLPR, a cytokine receptor involved in allergic inflammation and T cell differentiation. Aryl hydrocarbon receptor (AHR) ligands control postnatal c-kit expression, the third cytokine receptor involved in the expansion of ILC3s. Importantly, we could show that IL-7R and c-kit collaborate in establishing the pool of ILCs and in the generation of lymph nodes. Now, we have found that both IL-2 and Flt3L differentially regulate the number of foetal and adult ILCs.

ILCs in innate and adaptive immunity

ILC3s are important for the maintenance of mucosal barrier function and antimicrobial immunity. We found that upon IL-1β stimulation, ILC3s up-regulate MHC class II expression, express co-stimulatory molecules, secrete cytokines (e.g. IFNγ, IL-2, GMCSF) and in analogy with dendritic cells, gain the ability to present antigen to CD4+ T cells. This cognate interaction induces T-cell activation and proliferation *in vitro* while its disruption impairs T cell-mediated responses *in vivo*. In addition, we found that ILC3s can directly respond to particular Toll-like receptor ligands. Taken together, our data show that under inflammatory conditions, ILC3s can trigger T cell responses, ascribing to them a novel function, namely connecting innate and adaptive immunity.

ILCs and tissue repair

ILC3s are the main producers of IL-22, a cytokine known to be involved in protection against bacteria, as well as the regeneration of epithelial and liver cells. ILC3s can help to restore and maintain the architecture of lymphoid tissues after virus-induced injury, suggesting a key function of this subset in tissue repair. We have now developed new mouse models to study the role for ILCs in improving immune competence following irradiation, BM transplantation, and recovery from colitis. In mice with a selective increase in ILC numbers but lack of T, B and NK cells, we are analysing the impact of ILC3s on recovery from inflammatory intestinal damage, and in generating and stabilizing lymphoid niches under immune-compromised conditions.

Fig. 1

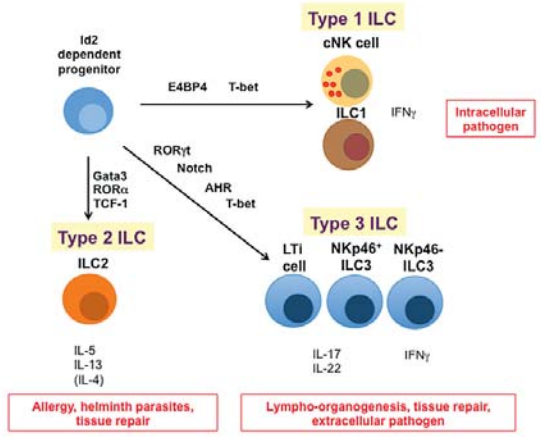
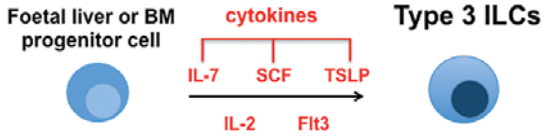


Fig. 2



Connection to Clinical Practice

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Diagnostics of cytokine levels and ILC function in preterm infants

Several clinic-pathological conditions in children are associated with the development of lymphopenia, including BM transplantation, cancer, severe infections and autoimmune diseases. In addition, preterm neonates often show marked lymphopenia and diminished plasma levels of IL-7. A leading cause of death in these children is mucosal infections and pulmonary immaturity. The maturation of the preterm lung requires a balance between beneficial tissue remodelling responses that drive proliferation while also acting to limit these responses in order to prevent fibrosis. There is strong evidence that ILCs have a role in mucosal immunity and pulmonary tissue homeostasis in man. For example, transcriptional profiling of lung ILCs identified a strong enrichment for genes that regulate immune defence and wound-healing. This suggests that ILC subsets have tissue-protecting functions. To better understand how lymphopenia and premature birth may alter protective ILC functions, the number, ratio and cytokine profile of ILCs in the peripheral blood of normal and preterm neonates needs to be explored. The ability to specifically target ILC functions could be beneficial in promoting mucosa and lung integrity. Our collaborator in the division of neonatology and infectiology/vaccinology have a strong background in lung physiology, mucosal infections and stress responses of preterm infants.

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Diabetes
Immunology
Metabolism
Glucose
Insulin
Obesity

Diabetes Research

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Shuyang Xu (MD-PhD student)

Islet inflammation in type 2 diabetes

Our research focuses on the mechanisms and therapy of decreased insulin production by the pancreatic islets in the obesity associated type 2 diabetes. In previous studies we demonstrated that the metabolic stress evoked by high glucose and saturated fatty acids (contained in animal fat) may induce death of the insulin producing beta-cells of the islets. Subsequently we identified interleukin 1 beta as a key mediator of these deleterious effects and showed that it is produce by human beta-cells in type 2 diabetes. More recently we published several additional studies supporting the concept that this mechanism leads to an inflammatory process and underlies the failure to produce sufficient amount of insulin in type 2 diabetes. On the basis of this we initiated clinical trials in patients with type 2 diabetes that vindicates this hypothesis and opens the way for a causative treatment. Furthermore we identified a new endocrine loop by showing that elevated IL-6 mediates a cross talk between insulin sensitive tissues, L cells and pancreatic islets to adapt to changes in insulin. Finally, recently we uncovered the first monogenic form of type 1 diabetes. This research has contributed to the concept that the innate immune system is part of the regulation of metabolism.

The overall goal of the present projects aim at understanding the precise role and regulation of the uncovered islet inflammation in type 2 diabetes and test therapeutic interventions.

Selected Publications

- Biason-Lauber, A., Boni-Schnetzler, M., Hubbard, B.P., Bouzakri, K., Brunner, A., Cavelti-Weder, C., Keller, C., Meyer-Boni, M., Meier, D.T., Brorsson, C., et al. (2013). Identification of a SIRT1 Mutation in a Family with Type 1 Diabetes. Cell metabolism 17, 448-455.
- Donath, M.Y., Dalmas, E., Sauter, N.S., and Boni-Schnetzler, M. (2013). Inflammation in obesity and diabetes: islet dysfunction and therapeutic opportunity. Cell metabolism 17, 860-872.
- Ehses, J.A., Lacraz, G., Giroix, M.H., Schmidlin, F., Coulaud, J., Kassis, N., Irminger, J.C., Kergoat, M., Portha, B., Homo-Delarche, F., et al. (2009). IL-1 antagonism reduces hyperglycemia and tissue inflammation in the type 2 diabetic GK rat. Proceedings of the National Academy of Sciences of the United States of America 106, 13998-14003.
- Ellingsgaard, H., Hauselmann, I., Schuler, B., Habib, A.M., Baggio, L.L., Meier, D.T., Eppler, E., Bouzakri, K., Wueest, S., Muller, Y.D., et al. (2011). Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. Nature medicine 17, 1481-1489.
- Larsen, C.M., Faulenbach, M., Vaag, A., Volund, A., Ehses, J.A., Seifert, B., Mandrup-Poulsen, T., and Donath, M.Y. (2007). Interleukin-1-receptor antagonist in type 2 diabetes mellitus. The New England journal of medicine 356, 1517-1526.

T lymphocytes
Antigen Recognition
Infection
Autoimmunity
Cancer
Vaccines

Experimental Immunology

Prof. Dr. Gennaro De Libero
Department of Biomedicine
Tumorimmunology
University Hospital Basel

Group Members
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Dr. Artjem Kalinichenko (postdoctoral fellow)
Dr. Marco Lepore (postdoctoral fellow)
Dr. Lucia Mori (project leader)
Sebastiano Sansano* (technician)
Dr. Stefano Vavassori (postdoctoral fellow)

* left during report period

T cells specific for non-peptidic antigens: role in infection, autoimmunity, tumor surveillance and immunoregulation

We study how T lymphocytes become activated during the immune response. T cells recognize as antigens peptides, lipids, and phosphorylated non-peptidic metabolites. Our research focus is the study of the human T cell populations that recognize lipid antigens presented by CD1 molecules, those that recognize small metabolites presented by MR1 molecules and those that recognize phosphorylated metabolites.
One area of investigation is the identification of lipid antigens relevant for human diseases. Lipid-specific T cells kill mycobacteria-infected cells and intracellular mycobacteria, thus exerting protective function. Therefore, the identification of mycobacterial lipid antigens is instrumental to further our knowledge and to identify novel targets of immunotherapy.
Three *Mycobacterium tuberculosis* lipids were identified with potential relevance during mycobacterial infections. We identified a new mycobacterial lipid, a di-acylated sulfotrehalose, which induces a strong specific immune response in tuberculosis patients. This lipid is generated by virulent mycobacteria and detection of specific immune response is a novel tool to detect the presence of mycobacterial latent infection. Analogs of this lipid are being evaluated as subunit vaccine in human CD1b transgenic animals generated in our laboratory.
The second lipid antigen is hexamannosylated phosphatidyl-myo-inositol (PIM6). We found that CD1e is required for PIM6 processing, thus attributing the first known function to this CD1 molecule. CD1e binds this glycolipid antigen and offers it to hydrolases and lipases for proper processing of the carbohydrate and lipid moieties. In addition, CD1e participates in loading other CD1 molecules with processed lipid antigens.
The third lipid antigen is glycerol-monomycolate. This lipid has also adjuvant properties and therefore it represents a unique molecule, which combines the two important functions of antigen and adjuvant. Combination of glycerol-monomycolate with other immunogenic lipids facilitates the induction of specific response in CD1b transgenic mice and thus this lipid may be considered an ideal subunit of lipid-based anti-mycobacterial vaccines.

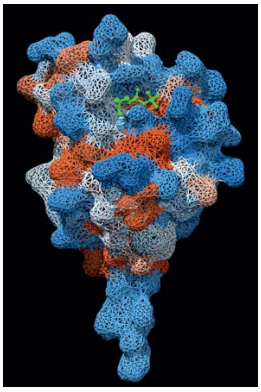
We identified the nature of self-lipids that induce selection in thymus of invariant natural killer T cells and their expansion in the peripheral lymphoid organs. These lipids are generated within peroxisomes and belong to the family of lysoplasmalogens. Animals deficient in the enzymes responsible for the peroxisomal synthesis of plasmalogens showed profound defects in iNKT cell development and peripheral expansion.

A second major topic of investigation is the population of T cells restricted to MR1 molecules. We have found an extreme oligoclonality in the circulating MAIT cells, which are MR1 restricted. Less than 5% of unique TCR Vβ sequences may account for up to 90% of the total repertoire. In addition, public sequences are very frequently shared among different individuals, thus indicating a selection bias introduced by antigenic stimulation. The comparison of MAIT cells in inflammatory disease patients showed high prevalence of activated cells within gut lesions but not in normal gut tissue.

The third main interest is the mechanism of antigen recognition by human TCR γδ cells. We have identified Butyrophilin 3A1 (BTN 3A1) as the presenting molecule to T cells expressing the TCR Vγ9-Vδ2. BTN 3A1 binds phosphorylated metabolites, which are the antigens stimulating Vγ9-Vδ2 cells, in a shallow pocket. Hydrogen bonds within the pocket position the

antigens for proper stimulation of the TCR. Antigen binding to BTN 3A1 was detected with a 1:1 stoichiometry with microbial antigens interacting with higher affinity than the endogenous ones. Importantly, BTN 3A1 is ubiquitously expressed and is not polymorphic. It also efficiently presents endogenous phosphorylated metabolites that accumulate within tumor cells and stimulate Vγ9-Vδ2 cells. Thus, BTN 3A1 represents an ideal candidate molecule to manipulate the immune response during infections and to promote tumor immunosurveillance.

Fig. 1



Selected Publications

- Vavassori, S., Kumar, A., Wan, G.S., Ramanjaneyulu, G.S., Cavallari, M., El Daker, S., Beddoe, T., Theodossis, A., Williams, N.K., Gostick, E., Price, D.A., Soudamini, D.U., Voon, K.K., Olivo, M., Rossjohn, J., Mori, L., and De Libero, G. (2013). Butyrophilin 3A1 binds phosphorylated antigens and stimulates human gammadelta T cells. Nat Immunol *14,* 908-916.
- Facciotti, F., Ramanjaneyulu, G.S., Lepore, M., Sansano, S., Cavallari, M., Kistowska, M., Forss-Petter, S., Ni, G., Colone, A., Singhal, A., Berger, J., Xia, C., Mori, L., and De Libero, G. (2012). Peroxisome-derived lipids are self antigens that stimulate invariant natural killer T cells in the thymus. Nat Immunol *13,* 474-480.
- De Libero, G., and Mori, L. (2012). Novel insights into lipid antigen presentation. Trends Immunol *33,* 103-111.
- Facciotti, F., Cavallari, M., Angenieux, C., Garcia-Alles, L.F., Signorino-Gelo, F., Angman, L., Gilleron, M., Prandi, J., Puzo, G., Panza, L., Xia, C., Wang, P.G., Dellabona, P., Casorati, G., Porcelli, S.A., de la Salle, H., Mori, L., and De Libero, G. (2011). Fine tuning by human CD1e of lipid-specific immune responses. Proc Natl Acad Sci U S A *108,* 14228-14233.
- Kyriakakis, E., Cavallari, M., Andert, J., Philippova, M., Koella, C., Bochkov, V., Erne, P., Wilson, S.B., Mori, L., Biedermann, B.C., Resink, T.J., and De Libero, G. (2010). Invariant natural killer T cells: linking inflammation and neovascularization in human atherosclerosis. Eur J Immunol *40,* 3268-3279.

Virus

Immunity

Pathogenesis

Vaccine

Alarmin

Persistent Infection

Experimental Virology

New Group since 2013

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Department of Biomedicine
Microbiology
University of Basel

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Julie Decroux (technician)
Benedict Fallet (PhD student)
Marylise Fernandez* (technician)
Dr. Susan Johnson* (postdoctoral fellow
Sandra Kallert (PhD student)
Dr. Bastien Mangeat (postdoctoral fellow)
Melissa Remy (PhD student)
Dr. Linda Terry* (postdoctoral fellow)

* left during report period

Immunity and pathogenesis in viral infection

Our research interests are centered around the intricate interplay between virus and host, with special emphasis on persistent infection. In broad terms we investigate the following aspects thereof:

- Immune protection against persistent viral infection
- Virally vectored vaccines
- Viral triggers of autoimmune disease
- Mechanisms of viral pathogenesis
- Role of alarmins in antiviral immunity

In the context of the above areas we are equally interested in both arms of adaptive immunity, i.e. T cell and B cell / antibody responses. We combine molecular virological techniques ("reverse genetics") for the engineering of infectious viruses with state-of-the-art mouse infection models and cellular immunology. Although fundamental by character, the questions addressed have immediate links to major unmet global health needs. In the mid- to long-term, this offers considerable translational potential, notably for vaccination and treatment of persistent viral diseases like human immunodeficiency virus (HIV), hepatitis B and C virus, as well as for select autoimmune disorders.

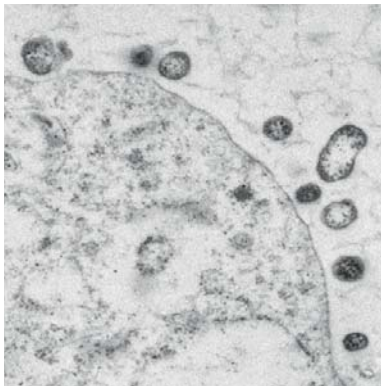

Fig. 1: Electron micrograph of lymphocytic choriomeningitis virus particles budding from a host cell

Selected Publications

- Bonilla, W.V., Frohlich, A., Senn, K., Kallert, S., Fernandez, M., Johnson, S., Kreutzfeldt, M., Hegazy, A.N., Schrick, C., Fallon, P.G., *et al.* (2012). The alarmin interleukin-33 drives protective antiviral CD8(+) T cell responses. Science *335,* 984-989.
- Flatz, L., Hegazy, A.N., Bergthaler, A., Verschoor, A., Claus, C., Fernandez, M., Gattinoni, L., Johnson, S., Kreppel, F., Kochanek, S., et al. (2010a). Development of replication-defective lymphocytic choriomeningitis virus vectors for the induction of potent CD8+ T cell immunity. Nat Med *16,* 339-345.
- Bergthaler, A., Flatz, L., Hegazy, A.N., Johnson, S., Horvath, E., Lohning, M., and Pinschewer, D.D. (2010). Viral replicative capacity is the primary determinant of lymphocytic choriomeningitis virus persistence and immunosuppression. Proc Natl Acad Sci U S A *107,* 21641-21646.
- Flatz, L., Rieger, T., Merkler, D., Bergthaler, A., Regen, T., Schedensack, M., Bestmann, L., Verschoor, A., Kreutzfeldt, M., Bruck, W., et al. (2010b). T cell-dependence of Lassa fever pathogenesis. PLoS Pathog *6,* e1000836.
- Pinschewer, D.D., Schedensack, M., Bergthaler, A., Horvath, E., Bruck, W., Lohning, M., and Merkler, D. (2010). T cells can mediate viral clearance from ependyma but not from brain parenchyma in a major histocompatibility class I- and perforin-independent manner. Brain *133,* 1054-1066.

Human Immunology

Immunometabolism

T Lymphocytes

Immunological Memory

Immediate-early ('innate-like') Response

Homing

Immunobiology

Prof. Dr. Christoph Hess
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and Division of Medical Outpatient Clinic
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Dr. Sarah Dimeloe (postdoctoral fellow)
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Warren Fingrut* (exchange student)
Marco Fischer (MD-PhD student)
Patrick Gubser* (MD-PhD student)
Gideon Hoenger (technician)
Annaïse Jauch* (Master student)
Ann-Catherine Joschko* (exchange student)
Nicolas Krähenbühl* (MD student)
Florian Marquardsen (exchange student)
Dr. Matthias Mehling* (postdoctoral fellow)
Leyla Razik (technician)
Dr. Mike Recher (postdoctoral fellow)
Friederike Wagner* (exchange student)

Immunometabolism – the metabolic basis of lymphocyte functioning

Metabolic pathway usage dictates cellular responsiveness. Our general interest is to assess how the metabolic repertoire of various human lymphocyte subsets defines their functionality. Over the last few years our specific focus was on characterizing metabolic pathways and their regulation among resting and immediate-early activated naïve and antigen-experienced human CD8 T cell subsets.

During acute viral infection, pathogen-specific naïve CD8 T cells become activated –followed by clonal expansion and differentiation into cytotoxic effector cells (1). Resolution of infection triggers the contraction of effector cell population, which is accompanied by the formation of a long-lived memory pool (2). In a highly coordinated process, memory CD8 T cells subsequently enhance host protection upon secondary infection (recall response) (2).

Naïve and memory CD8 T cells are metabolically quiescent cells, which primarily depend on oxidative phosphorylation (OXPHOS) as their energy source (3). T cell receptor (TCR)-ligation and co-stimulation of quiescent cells initiates dramatic changes in cellular metabolic pathway usage (4). Upregulation of aerobic glycolysis (Warburg effect) is an important feature of this metabolic adaptation, and a prerequisite for growth and expansion of CD8 T cells (4). The early recall phase of an immune response relies on antigen-experienced T cells that are able to acquire effector function with 'innate-like' response kinetics (5). Effector memory (EM) CD8 T cells are specialized antigen-experienced lymphocytes that traffic between blood and non-lymphoid tissues (5). EM CD8 T cells are thus ideally positioned to rapidly respond and execute effector functions at sites of infection.

The metabolic requirements that support the pivotal immediate-early memory functionality were at the center of our recent interest. A defining metabolic feature of EM CD8 T cells was their selective capacity to rapidly upregulate and sustain aerobic glycolysis following mitochondrial stress –measured as extracellular acidification rate (ECAR). EM CD8 T cells migrate to, and reside in different environmental niches (5). A high glycolytic capacity likely affords increased adaptability to changes in nutrient availability and/or stimulatory conditions. Glycolysis was also increased in memory CD8 T cells following TCR/CD28 stimulation (Fig. 1). In a PI3K-AKT pathway dependent manner, the immediate-early glycolytic phase was critical for rapid IFN-gamma production by memory CD8 T cells, including EBV-specific memory cells. Pharmacologic blockade of glycolysis during the early phase of CD8 T cell activation resulted in the diminution of IFN-gamma production, potentially via abrogation of chromatin remodeling (data not shown). Our observations indicate that memory CD8 T cells rely on CD28 signaling to sustain immediate early metabolic switch, and that CD28 co-stimulation is also important for enhanced IFN-gamma production. AKT activity and phosphorylation by upstream kinases –PI3K (pT308) and mTORC2 (pS473)– is required for stable metabolic switch and for GAPDH cytoplasmic expression (data not shown). Together, our findings identify a previously unrecognized phase of the Warburg effect and assign it a role beyond supporting effector T cell proliferation, namely in regulating the immediate-early effector response of memory CD8 T cells.

* left during report period

1. Haring JS, Badovinac VP, Harty JT. Inflaming the CD8 T cell response. Immunity 2006, 25(1): 19-29.
2. Kaech SM, Wherry EJ. Heterogeneity and cell-fate decisions in effector and memory CD8 T cell differentiation during viral infection. Immunity 2007, 27(3): 393-405.
3. Fox CJ, Hammerman PS, Thompson CB. Fuel feeds function: energy metabolism and the T-cell response. Nature reviews Immunology 2005, 5(11): 844-852.
4. Jacobs SR, Herman CE, Maciver NJ, Wofford JA, Wieman HL, Hammen JJ, et al. Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. Journal of immunology 2008, 180(7): 4476-4486.
5. Masopust D, Picker LJ. Hidden memories: frontline memory T cells and early pathogen interception. Journal of immunology 2012, 188 (12): 5811-5817.

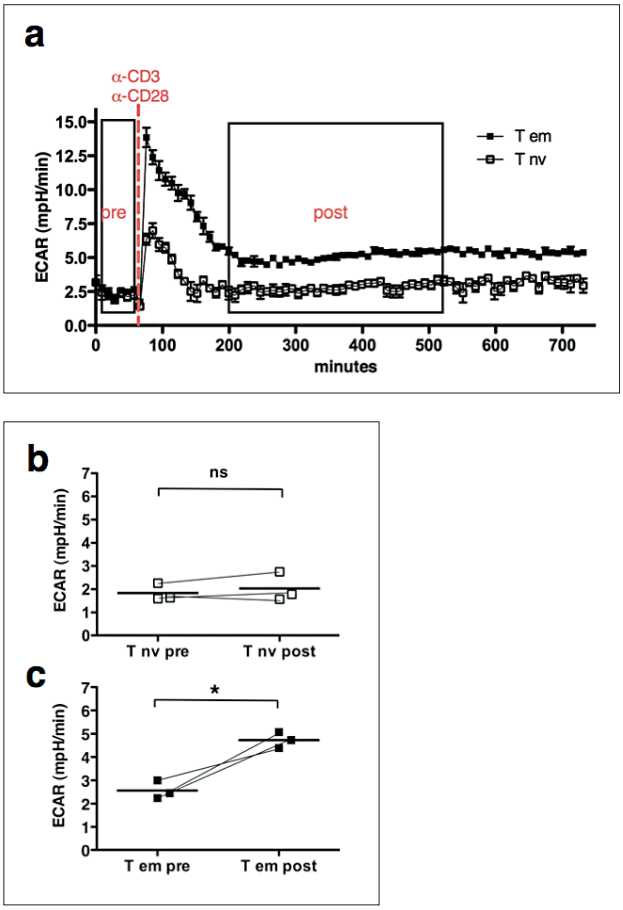

Fig. 1.: Activation induced immediate-early glycolytic switch is intrinsic to EM CD8 T cells.
Representative ECAR (**a**) of naïve (□) and EM (■) CD8 T cells following *'in-Seahorse'* activation with αCD3 (0.2 µg/mL) and αCD28 (20 µg/mL) mAb. Boxes represent ECAR values utilized for calculation of pre- and post-injection means.
Analysis of pre- and post-injection mean ECAR values of naïve (**b**) and EM (**c**) CD8 T cells (n=3 separate donors, paired two-tailed Student's *t*-test). T nv = naïve CD8 T cells; T em = effector memory CD8 T cells.

Connection to Clinical Practice

Prof. Dr. Christoph Hess
Medical Outpatient Division

Clinical studies of fingolimod; opportunities to study translational aspects of human T cell homing

In close collaboration with the Neurology Department of our hospital, translational studies were conducted assessing homing of T cells both under steady-state conditions, and upon antigenic stimulation (vaccination).
Homing of T cells is a highly regulated process mediated by an orchestrated interplay of chemokines/chemokine receptors and adhesion molecules. Acting as a functional antagonist on the S1P receptor (S1PR), the pharmacological compound fingolimod –which has shown efficacy in the treatment of multiple sclerosis (MS)– blocks the egress of T cell from lymphnodes.
By studying depletion kinetics of T cells in the blood of de novo fingolimod exposed individuals, we found that CD4 T cells diminish earlier than CD8 T cells. This suggests that –under steady state conditions– CD4 T cells enter lymphoid tissue, and thus access dendritic cells, more frequently than CD8 T cells. Differences in calculated homing frequencies between naïve and antigen-experienced T cell subsets were even more pronounced, naïve T cells homing 2-3x as often to lymphnodes as central memory cells. We further characterized immune responses to influenza-vaccine in fingolimod-treated patients and in untreated healthy controls. Counter-intuitively, vaccine-triggered T cells –in contrast to steady-state homing T cells– accumulate normally in blood despite efficient S1PR-blockade.
Together these studies provide insight into important aspects of human T cell biology, namely their recirculation properties under homeostatic conditions and upon antigenic challenge.

Selected Publications

– M. Stern, G. Opelz, B. Doehler, C. Hess. Natural Killer Cell Receptor Polymorphisms and Post-Transplant non-Hodgkin Lymphoma. Blood, 13 (2010), pp. 3960-3965.
– M. Mehling , P. Hilbert, S. Fritz, B. Durovic, D. Eichin, O. Gasser, J. Kuhle, T. Klimkait, R.L.P. Lindberg, L. Kappos, C. Hess. Antigen-specific adaptive immune responses in fingolimod-treated MS patients. Ann. Neurol., 69 (2011), pp. 408-413.
– M. Mehling, V. Brinkmann, A.-V. Burgener, P. Gubser, A.D. Luster, L. Kappos, C. Hess. Lymph node access-hierarchy and homing-frequency of human T cells derived from peripheral blood depletion-kinetics after S1P-receptor blockade. J. All. Clin. Immunol, 131 (2013), pp. 1440-1443.
– B. Durovic, O. Gasser, J. Sigle, H.H. Hirsch, M. Stern, A. Buser, C. Hess. Protection from EBV is associated with HLA-C and HLA-Bw4 variants and tonsillectomy. J. Virol., 87 (2013), pp. 6526-6529.
– P. Gubser, G.R. Bantug, L. Razik, M. Fischer, S. Dimeloe, G. Heonger, B. Durovic, A. Jauch, C. Hess. Rapid effector-memory CD8+ T cell function requires an immediate-early glycolytic switch. Nature Immunology, 14 (2013), pp. 1064-1072.

Primary Immunodeficiency

Autoimmunity

Hypogammaglobulinemia

Recombination Activating Gene

B Cells

T Cells

Immuno-deficiency

New Group starting 2014

Prof. Dr. Mike Recher
SNSF Professor
Department of Biomedicine
and Division of Medical Outpatient Clinic
University Hospital Basel

Group Members
Fabian Baldin (PhD student)
Florian Marquardsen (PhD student)
Marius Elkuch (MD Master student)

Mechanisms of primary immunodeficiencies and associated autoimmunity

Primary immunodeficiencies (PID) are a rapidly evolving group of genetically determined diseases of the immune system associated with susceptibility to infection and/or autoimmunity. To date, more than 180 different PID have been characterized.

Deficiency of the recombination activating genes (RAG) is associated with PID affecting number and function of both T and B lymphocytes. RAG-PID associated clinical phenotypes range from pediatric severe combined immunodeficiency (SCID) to adult-onset RAG-associated granulomas and autoimmune disease. While complete loss-of-function mutations lead to RAG-dependent SCID, so-called hypomorphic RAG mutations are associated with the late-onset RAG-associated diseases.

The mechanisms involved in autoimmunity and immune-dysregulation due to hypomorphic RAG-mutations are poorly defined. Autoimmunity and immune-dysregulation are also associated with RAG-independent PID, implying that PID and autoimmunity are fundamentally linked.

One main focus of the lab is to analyze in murine models how gradual RAG dysfunction impacts on immunity to infectious pathogens and at the same time to the formation of autoimmunity. This may help identifying mechanisms involved in the generation of autoimmunity in general.

– Notarangelo, L.D. (2010). Primary immunodeficiencies. J Allergy Clin Immunol *125*, S182-194.
– Niehues, T., Perez-Becker, R., and Schuetz, C. (2010). More than just SCID – the phenotypic range of combined immunodeficiencies associated with mutations in the recombinase activating genes (RAG) 1 and 2. Clin Immunol *135*, 183-192.
– Abraham, R.S., Recher, M., Giliani, S., Walter, J.E., Lee, Y.N., Frugoni, F., Maddox, D.E., Kirmani, S., and Notarangelo, L.D. (2013). Adult-onset manifestation of idiopathic T-cell lymphopenia due to a heterozygous RAG1 mutation. J Allergy Clin Immunol *131*, 1421-1423.
– Walter, J.E., Rucci, F., Patrizi, L., Recher, M., Regenass, S., Paganini, T., Keszei, M., Pessach, I., Lang, P.A., Poliani, P.L., et al. (2010). Expansion of immunoglobulin-secreting cells and defects in B cell tolerance in Rag-dependent immunodeficiency. J Exp Med *207*, 1541-1554.

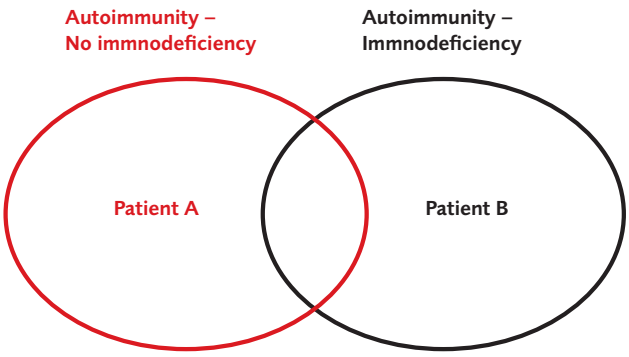

Fig. 1: **PID in a subgroup of patients with autoimmunity.**
Since autoimmune disease may be the initial clinical presentation of a PID, PID should be considered in every patient with autoimmune disease. Our research aims at identifying strategies how to optimize diagnosis and treatment of patient group A (autoimmunity without immunodeficiency) vs. patient group B (autoimmunity with immunodeficiency). The focus of our research is to understand what immunologic mechanisms are involved in PID associated autoimmunity.

Connection to Clinical Practice

Diagnosis and pathogenesis of human primary immunodeficiencies

In order to translate the murine findings to humans, we aim at establishing a cohort of individuals affected with PID. These are recruited from our specialized adult PID clinic at the Medical Outpatient Unit of the University Hospital Basel.
Functional assays of peripheral blood derived immune cells derived from clinically defined groups of PID will help to better characterize and understand the underlying specific immune defect and will point at novel diagnostic and treatment targets.
In addition, our lab provides experimental protocols to diagnose rare PID such as Wiskott-Aldrich syndrome (WAS), x-linked lymphoproliferative disease (XLP), hyper IgE syndrome (Job's syndrome) or veno-occlusive disease with immunodeficiency (VODI).

Selected Publications

- Cattaneo, F., Recher, M., Masneri, S., Baxi, S.N., Fiorini, C., Antonelli, F., Wysocki, C.A., Calderon, J.G., Eibel, H., Smith, A.R., et al. (2013). Hypomorphic Janus kinase 3 mutations result in a spectrum of immune defects, including partial maternal T-cell engraftment. J Allergy Clin Immunol *131*, 1136-1145.
- Recher, M., Fried, A.J., Massaad, M.J., Kim, H.Y., Rizzini, M., Frugoni, F., Walter, J.E., Mathew, D., Eibel, H., Hess, C., et al. (2013). Intronic SH2D1A mutation with impaired SAP expression and agammaglobulinemia. Clin Immunol *146*, 84-89.
- Bloch, D.B., Nobre, R., Steinbicker, A.U., Al-Herz, W., Notarangelo, L.D., and Recher, M. (2012). Decreased IL-10 production by EBV-transformed B cells from patients with VODI: implications for the pathogenesis of Crohn disease. J Allergy Clin Immunol *129*, 1678-1680.
- Recher, M., Burns, S.O., de la Fuente, M.A., Volpi, S., Dahlberg, C., Walter, J.E., Moffi tt, K., Mathew, D., Honke, N., Lang, P.A., et al. (2012). B cell-intrinsic deficiency of the Wiskott-Aldrich syndrome protein (WASp) causes severe abnormalities of the peripheral B-cell compartment in mice. Blood *119*, 2819-2828.
- Recher, M., Berglund, L.J., Avery, D.T., Cowan, M.J., Gennery, A.R., Smart, J., Peake, J., Wong, M., Pai, S.Y., Baxi, S., et al. (2011). IL-21 is the primary common gamma chain-binding cytokine required for human B-cell differentiation in vivo. Blood *118*, 6824-6835.

Innate and Adaptive Immunity

Ectosomes

Inflammasome

Complement

Inflammation

Immuno-nephrology

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Dr. Ceylan Eken (postdoctoral fellow)
Estelle Gerossier (technician)
Dr. Salima Sadallah (postdoctoral fellow)
Dr. Daniel Zecher (postdoctoral fellow)

Inflammation and ectosomes

Our immune system needs to assimilate (satisfy) two seemingly opposing requirements. While on one hand its expected to raise an effective response against infection, on the other hand it should not achieve this at our expense. The stronger the mechanisms in place designed to protect us, the more strictly it should be regulated to remain purposeful. An immune response is therefore a balancing act between pro- and anti- inflammatory mechanisms that ultimately lead to a measured and meaningful response.
Our present investigations are based on our recent observations that different circulating cells (erythrocytes, leukocytes, platelets) release small vesicles by budding from the cell surface. This budding corresponds to a reaction described 20 years ago as "ectocytosis", thus we named these vesicles "ectosomes". Structurally, these ectosomes express surface proteins in a different ratio than found on the originating cell suggesting a specific selection process at the time of budding. In addition, phosphatidylserine (PS) is present on the outer leaflet of the ectosome membrane as found for apoptotic cells. PS serves as receptor for many proteins including C1q, Gas6 and others, which might bridge ectosomes to phagocytes. We have now established that these vesicles have biological functions. Our initial data indicated that ectosomes of PMN, erythrocytes and platelets down-modulate the inflammatory reaction of macrophages and dendritic cells. However, when present in the circulation, PS present on these ectosomes does activate the coagulation cascade.
Our present goals are to define the properties of ectosomes, in particular their capacity to interfere with the function of cells involved in inflammation and immunity, as well as their procoagulant activity *in vivo*.

1) Ectosomes of platelets express TGFbeta, and may regulate T cell differentiation and the function of NK cells. These functions of ectosomes are explored by Salima Sadallah in collaboration with the group of Prof G Iezzi (DBM) and Prof M Stern (DBM) respectively. Initial data indicate that platelet ectosomes shift the differentiation of naïve CD4+ T cells towards functional T regulatory cells (fig. 1).

2) The release of ectosomes is an early phenomenon of neutrophil activation. These ectosomes have anti-inflammatory properties and constitute an early counter-regulatory signal that helps initiate the resolution of inflammation, as shown *in vitro* using uric acid crystals as a model for gout (fig. 2). Arun Cumpelik is currently investigating how these ectosomes affect the course of inflammation in a mouse model of uric acid crystal-induced peritonitis and may be responsible for its self-limiting nature. In addition, he is investigating whether ectosomes aid in maintaining tolerance against neutrophilic self-antigens (myeloperoxidase) in an immunization model of pANCA vasculitis.

3) The role of ectosomes produced or released directly in the blood stream is studied by Daniel Zecher with the help of Arun Cumpelik. He is investigating microvesicle biology in different scenarios: First, their role in transfusion-related complications in mice. He has found in a murine transfusion model that microvesicles derived from aged erythrocytes (E-Ecto) amplify systemic inflammation by thrombin-dependent activation of complement independent of the classical, alternative or lectin pathway (fig. 3). Second, he is studying the release of microvesicles in patients following treatment with depleting antibody preparations in the context of solid organ and stem cell transplantation. The aim is to correlate microvesicle release with treatment efficacy and side effects, most notably hypercoagulability. In another study he is looking at the release of microvesicles

in the context of blood group-incompatible kidney transplantation. In this setting, the shedding of graft endothelial-derived microvesicles might be involved in protecting the graft from antibody-mediated injury, i.e. transplant rejection.

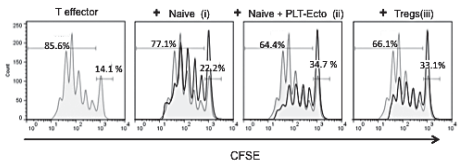

Fig. 1: Suppressive activity of the PLT-Ecto generated Foxp3+ cells. Proliferation of $CD8^+$ T cells(T effector) measured by CFSE staining and analyzed by flow cytometry: (left) cells cultured alone, (i) in co-culture with naïve T cells, (ii) in co-culture with Foxp3 induced naïve T cells, (iii) in co-culture with peripheral blood Tregs.

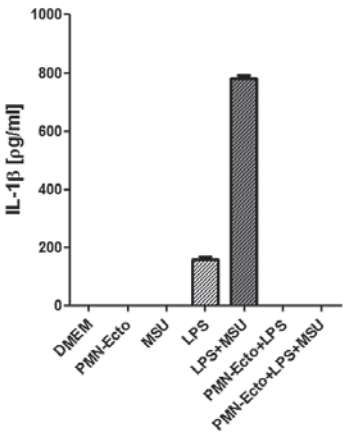

Fig. 2: Human monocyte derived macrophages were pre-incubated with neutrophil ectosomes (PMN-Ecto, $x10^8$) and subsequently primed with LPS (10ng/ml) and stimulated with monosodium urate crystals (MSU, 100µg/ml). The release of IL-1β was determined by ELISA and used as a measure of inflammasome activation. Those cells that did see PMN-Ecto prior to priming or stimulation failed to activate their inflammasomes and release of IL-1β.

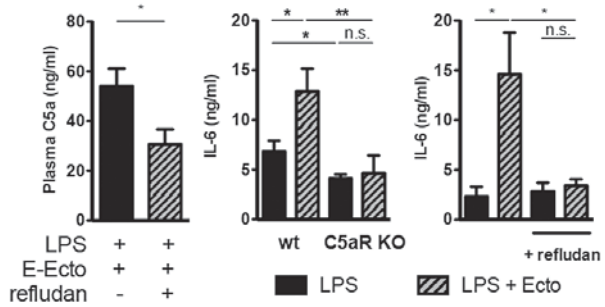

Fig. 3: E-Ecto amplify LPS-induced inflammation by thrombin-dependent activation of complement in mice. Plasma levels of C5a in LPS-primed mice following E-Ecto injection are reduced in mice treated with the specific thrombin-inhibitor refludan (left). Increase of serum IL-6 in LPS-primed mice following injection of E-Ecto depends on C5aR *in vivo* (middle) and can be reversed by refludan (right).

Selected Publications

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- Sadallah et al., 2008, S. Sadallah, C. Eken, JA. Schifferli. Erythrocyte-derived ectosomes have immunosuppressive properties. J Leuk Biol, 84 (2008), PP. 1316-1325
- Eken et al., 2010, C. Eken, PJ. Martin, S. Sadallah, S. Treves, M. Schaller, JA. Schifferli. Ectosomes released by polymorphonuclear neutrophils induce the MerTK-dependent anti-inflammatory pathway in macrophages. J Biol Chem, 285 (2010), PP. 39914-39921
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- Eken et al., 2013, C.Eken, S. Sadallah, PJ. Martin, S. Treves, JA. Schifferli. Ectosomes of polymorphonuclear neutrophils activate multiple signaling pathways in macrophages. Immunobiol, 218 (2013), PP. 382-392
- D. Zecher, A. Cumpelik, J. Schifferli. Erythrocyte-Derived Microvesicles Amplify Systemic Inflammation by Thrombin-Dependent Activation of Complement. Journal of the American Heart Association, Arterioscler Thromb Vasc Biol. 2014;34:00-00.

Transplantation

Tolerance

Regulatory T cells

Lymph Node Stroma Cells

Immunoprivileged Site

Immuno-regulation

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Regulatory T cells and lymph node stroma cells responses during allograft transplantation

The success of solid organ transplantation is determined by the ability to control rejection and establish tolerance. Currently this problem is overcome by treating the patients with immunosuppressive agents, which cause a large number of side effects that compromise the graft survival and patients' life quality. For this reasons tolerance rather than drug-mediated suppression is the ultimate goal in transplantation. Regulatory T cells (Tregs) are a cellular component of the adaptive immune system with the ability to control the activity of graft rejecting effector T cells. Transfer of Tregs was shown to induce tolerance in various murine organ transplantation models. The establishment of tolerance is not an indicator for long term graft acceptance, since Treg-mediated tolerance can be broken. One of the major breaking tolerance causes are strong inflammatory responses.

In our first project, we aim to examine how Tregs collaborate with the lymph node (Ln) environment to establish and maintain long-term graft tolerance. Therefore, we transplant allogeneic skin grafts on recipient mice and transfer different ratio's of effector CD4 T cells and Tregs. Anti-graft specific effector T cells (ABMs) reject the graft within 12 days (Fig. 1). The co-transfer of polyclonal Tregs delays rejection (2:1) or induces long-term allograft acceptance (10:1). The graft tolerance is sustained despite challenging with high ABM numbers. ABMs do proliferate in all groups, but show impaired proliferation in the presence of Tregs. ABMs are activated as determined by expression of surface markers and display a memory-like phenotype which is independent of Tregs. However, IFN-g-producing effector T cells are less abundant in the presence of Tregs. IFN-g might activate and modulate Ln stromal cells (LnSC) during transplantation tolerance, which in turn support Tregs to maintain long-term tolerance. Indeed, strong IFNg-dependent inflammation reduced the expression of the T cell survival factor IL-7. In contrast, Tregs maintained IL-7 production by LnSC (Fig. 2). IL-7 was mainly used by Tregs and naive ABMs suggesting an additional survival advantage of those after transplantation. High amounts of IL-7 did not lead to survival of all transferred Tregs, but enough to establish a ratio of Tregs to ABMs in the LN. One Treg cell is needed to suppress one ABM cell to achieve long-term tolerance against skin graft rejection. Taken together LnSC respond to Tregs and support their suppression of inflammatory CD4+ T cell responses in the Ln.

Our second project is based on the fact that inflammatory responses influence recruitment and activation of adaptive immune cells in Ln and may even break established graft tolerance. Excessive inflammation mainly occur during infections and autoimmune diseases and often leads to cell death and release of CpG containing motifs of DNA. Thus, we take advantage of the possibility to growth in vitro LnSC (Fig. 3) and study the responses of LnSC upon stimulation with CpG-dependent dendritic cell cytokines. CpG stimulation of DCs induced TLR9 dependent release of various proinflammatory cytokines. Supernatants containing IFN-b induce upregulation of antigen presenting and costimulatory molecules suggesting an active contribution of LnSC in activation of T lymphocytes, which is currently focus of our studies. Taken together LnSC respond to inflammatory cytokines and their producing cells to modify their biological activity to sustain the activation and contraction of T lymphocytes. Understanding the function and responses of LnSC will provide new aspects in the regulation of immune responses by affecting activation and survival of specific T cells.

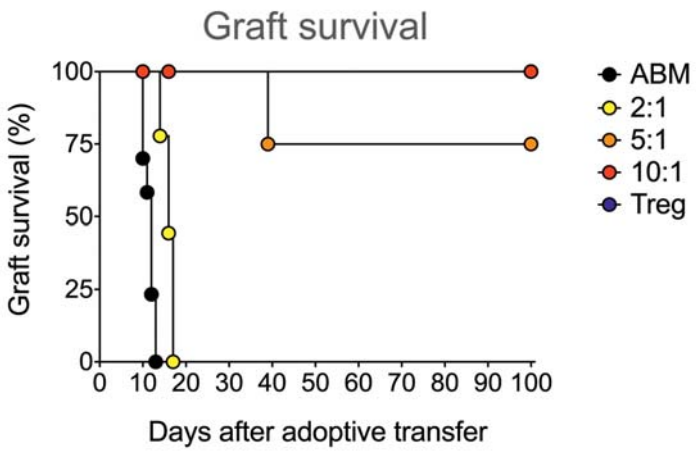

Fig. 1: Skin transplantation graft survival. Rag2-/- mice are adoptively transferred with different ratios of Treg cells and T effector cells (ABM). In this model, graft survival up to 100 days is reached with adoptive transfer of 10 times more Treg cells the effector T cells.

Selected Publications

- Broggi MAS, Schmaler M, Lagarde N, Rossi SW, Isolation of murine lymph node stromal cells, JoVE, In Press
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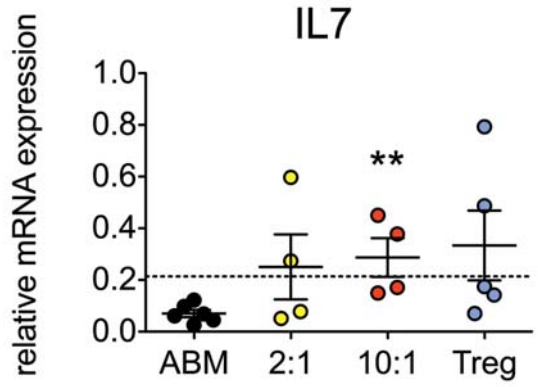

Fig. 2: Isolation and culture of lymph node stroma cells (LnSC) is achieved using magnetic beads selection and consequent expansion in culture. Cultured LnSC are able to maintain the same properties of freshly isolated LnSC.

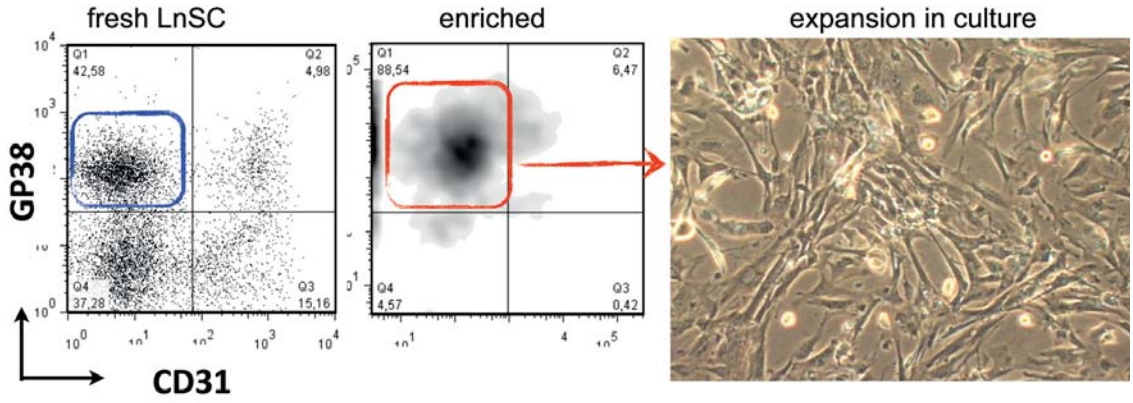

Fig. 3: IL-7 mRNA expression in freshly isolated LnSC from skin transplanted mice adoptively transferred with different rations of Treg cells and effector T cells (ABM). The presence of Treg cells help to maintain normal IL-7 mRNA expression levels in LnSC.

Hematopoietic Stem Cell Transplantation

Multiple Myeloma

Immune Reconstitution

Donor Lymphocyte Infusion

Natural Killer Cells

Cytomegalovirus

Immunotherapy

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Natural killer cells in the control of disease relapse and infection in transplanted patients

Natural killer (NK) cells are a subgroup of lymphocytes that – unlike B- and T-lymphocytes – do not possess rearranged surface receptors but instead are regulated by integration of signals derived from an array of activating and inhibitory receptors. While much progress has been made over the last 10 years in the characterization of NK cell surface receptors and their ligands, the function and ligands of several NK cell receptors are still unknown.

NK cells are of particular importance in patients under pharmacological immunosuppression, e.g. after solid organ or hematopoietic stem cell transplantation. These patients with compromised adaptive immunity are therefore predestined to study the role of natural killer cells in the control of malignant and viral transformation.

Our studies focus on one family of NK cell receptors termed Killer-cell Immunoglobulin-like receptors (KIR). KIR are transmembrane proteins and come in an inhibitory or activating flavor. While the function of inhibitory KIR is clear (providing NK cell tolerance through binding to HLA-class I) both function and ligands of activating KIR are so far undefined. Studies in transplanted patients have hinted that patients carrying activating KIR have a reduced rate of viral infection, pointing to viral proteins as potential activating KIR ligands. Through studies involving solid organ grafts performed in transplant centers in Switzerland reporting to the Swiss Transplant Cohort Study, we have identified activating KIR receptors as having a protective role regarding the occurrence of cytomegalovirus replication after transplantation. These studies are accompanied by in vitro experiments aiming to resolve receptor-ligand interactions relevant for this protective role of NK cells. Another line of research is directed towards the identification of activating KIR receptors involved in the recognition of acute myeloid leukemia cells, where clinical studies have also documented a benefit for patients receiving allografts from a donor carrying such activating receptor genes.

A more recently established line of research analyzes how KIR are involved in the antibody dependent cellular cytotoxicity (ADCC), an important mode of action of therapeutically administered monoclonal antibodies. We could show that the superior efficacy of modern third-generation glycoengineered antibodies is partly due to their ability to overcome inhibitory signaling by KIR receptors.

Finally, in a translational arm of our research and in close collaboration with the Division of Hematology at the University Hospital, we are treating patients with cancers incurable by conventional chemotherapy with highly purified and ex vivo expanded NK cells with the aim to eradicate disease through a combined chemo-/immunotherapy approach.

* left during report period

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- Stern, M., Passweg, J.R., Meyer-Monard, S., Esser, R., Tonn, T., Soerensen, J., Paulussen, M., Gratwohl, A., Klingebiel, T., Bader, P., *et al.* (2013). Pre-emptive immunotherapy with purified natural killer cells after haploidentical SCT: a prospective phase II study in two centers. Bone Marrow Transplant *48*, 433-438.

Immunity to Fungi

T-Cell Therapies

New Adjuvans in Vaccination

Implant Infections with Staphylococcus Aureus and Epidermidis

Infection Biology

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SNSF Ambizione-SCORE

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* left during report period

Host pathogen interaction in fungal infections staphylococcal implant infections

The infection biology research group explores host- and pathogen-specific aspects of infectious diseases in a strong translational setting. One of our main interests is the interaction of innate and adaptive immune response in the context of fungal infections with the goal to generate new immunotherapeutic strategies. A second focus is based on novel anti-infective approaches against foreign-body/implant-associated infections caused by staphylococci.

Host pathogen interaction in fungal infections

Invasive fungal infections belong to the most serious complications in immunocompromised patients and are still associated with an exuberant mortality. Little is known regarding the protective immune response against fungi in humans. In mice, distinct fungi-specific CD4+ T-helper (TH) subsets such as TH1, and probably TH17 cells are important for pathogen control, whereas activation of TH2 cells often exacerbates disease. The T-cell response provides important effector and survival signals towards key players of the innate immunity such as neutrophils or macrophages via secretion of different cytokines. Therefore, understanding this cross-talk in the context of antifungal immunity is important for the development of immunotherapeutic strategies (Figure 1).
We are currently investigating in recipients of hematopoietic stem cell transplantation (HSCT) and in HIV-infected patients receiving antiretroviral therapy the qualitative impairments and the kinetics of recovery of antifungal immunity.

Adoptive T-cell therapy for viral and fungal infections

Adoptive T-cell therapy is promising and recommended in patients after HSCT with treatment refractory viral infections caused by adenovirus, cytomegalovirus and Epstein Barr virus. Although fungal infections occur at the same time, there is insufficient experience with adoptive therapy for *Aspergillus* or other fungi mainly due to lack of target T-cell antigens. We have previously identified a T-cell epitope of the cell wall glucanase Crf1 stimulating cross-reactive T cells that recognize *Aspergillus fumigatus* as well as *Candida albicans* (Fig. 2A), which are the two most common fungi causing infections in patients after HSCT. Using a strategy based on activation-dependent expression of CD154, we could simultaneously select and enrich T cells specific for viral and fungal pathogens (Fig. 2B). This approach is promising for future application to prevent post-transplant fungal and viral infections.
To generate efficacious and potent fungus-specific T-cell therapy, T-cells must cover a wide range of different antigens. This is quite challenging as the fungal genome contains up to 10'000 protein-coding genes. We are currently investigating the T-cell responses specific for several *Aspergillus* antigens in patients recovering from fungal infections. This finding will be of great importance to improve the generation of protective fungus-specific T-cells for adoptive transfer.

Staphylococcal implant infections

Bacterial infection of implanted devices is a major health care problem occurring in about 5% of patients. These infections are mainly caused by biofilm-forming *Staphylococcus (S.) aureus* and *S. epidermidis*. Successful treatment requires drugs or coatings, which are active against adhering bacteria which are often less susceptible to antibiotics. We could demonstrate that Serrulatane EN4 that was isolated from an Eremophila plant species seems to be promising to treat these infections (Fig. 3). We are currently investigating different anti-infective coatings and new compounds for their anti-adhesive and anti-microbial activity within different collaborative networks.

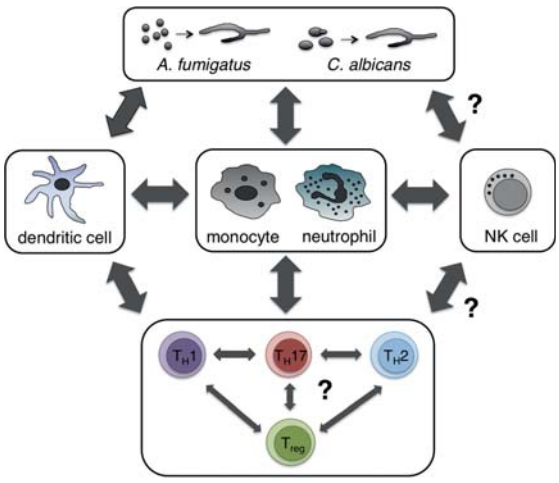

Fig. 1: Crosstalk of immune cells in antifungal immunity.

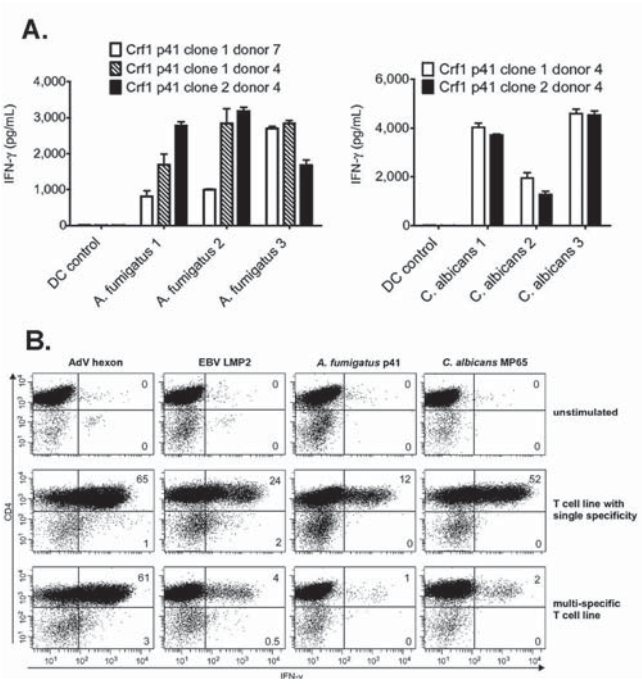

Fig. 2: (A) *Aspergillus fumigatus* Crf1/p41T-cell clones show specific cross-reactivity to clinical isolates of *C. albicans* (B) and can be enriched together with multiple pathogens based on activation-dependent CD154 expression.

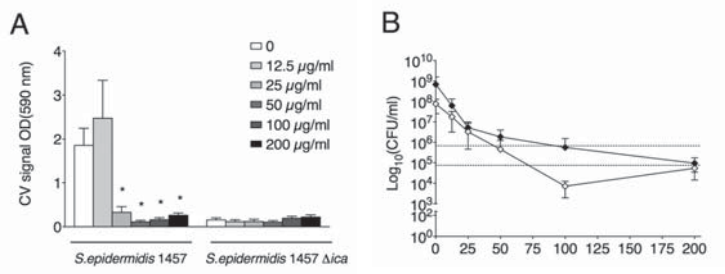

Fig. 3: The influence of EN4 on the biofilm and the viability of *S. epidermidis* 1457 and its isogenic biofilm-deficient Δica mutant was determined by crystal violet (CV) staining (A) and plating of detached adherent bacteria (B), respectively.

Connection to Clinical Practice

Fungal and viral infections have become a leading cause of morbidity and mortality in immunosuppressed patients. Pharmaceutical agents are often less effective in the setting of immunodeficiency, may cause substantial side effects, are expensive and may generate resistance. To overcome these issues, understanding the host-pathogen interaction and exploring strategies such as adoptive T-cell transfer that boost and induce long-term immunity may be promising in these patients.

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Immune regulation

T cell differentiation and identity

Regulatory T cells

Follicular helper T cells

Posttranscriptional gene regulation

microRNAs

Molecular Immune Regulation

New Group starting 2014

Prof. Dr. Lukas Jeker
SNSF Professor
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University Hospital Basel

Group Members
Various positions available:
– Technician
– Students
– Postdocs

The role of microRNAs in immune regulation

Work in our lab is focused on understanding rules underlying regulation of the immune system with a particular focus on various T cells, particularly $CD4^+$ T cell subsets.
Among $CD4^+$ T cells, a subset termed regulatory T cells (Treg) is essential for the establishment and maintenance of immune homeostasis. Due to their proven ability to prevent and even cure autoimmune diseases and their important role in preventing rejection of transplanted organs in preclinical animal models, therapeutic adoptive Treg transfer is a new approach currently being explored in clinical trials to treat various immune related diseases. In contrast, $CD4^+$ follicular helper T cells (T_{FH}) provide classic "help" to B cells and promote autoimmunity. We have previously demonstrated that Treg can lose the hallmark transcription factor FoxP3 and turn into pathogenic cells. Interestingly, other researchers have demonstrated that Treg can, under certain conditions, convert to T_{FH}. Thus, although functionally antagonistic, there is a close molecular interrelationship between Treg and T_{FH}.
On a molecular level, our work is focused on "non-coding RNAs", i.e. RNA molecules whose primary purpose is not to act as an intermediary between DNA and protein. Rather, many classes of non-coding RNAs seem to primarily regulate gene expression but our understanding of these functions is very limited. One of the best studied classes of non-coding RNAs are called microRNAs (miRNA) due to their short length. They act as posttranscriptional negative regulators of other genes. We have demonstrated the dependence of several T cell subsets on miRNAs. Current research projects investigate how individual microRNA loci control T cell differentiation and function. In mice, we have demonstrated that miRNAs are critical for Treg function and stability as miRNA-deficient Treg rapidly lose FoxP3. Furthermore, we found that one particular miRNA locus called miR-17-92 is important for Treg biology and function in vivo. In addition, using T cell-specific genetic ablation of the same miRNA locus in vivo combined with immunization protocols and genome-wide transcript analysis we demonstrated that loss of miR-17-92 leads to impaired differentiation of T_{FH}. Unexpectedly, miR-17-92-deficient cells retained a T_{FH}-like phenotype but acquired features characteristic of other T cell subsets, i.e. cells were displaying a hybrid phenotype. Thus, miR-17-92 is required for the fidelity of T_{FH} gene expression and as such T_{FH} cell identity. In summary, miR-17-92 has pleiotropic effects on diverse T cell subsets for their function and cellular identity.
Future directions include a more in-depth analysis of genetic networks regulated by miR-17-92 in Treg and T_{FH} as well as the analysis of various additional miRNA loci in T cell differentiation, identity and function. We expect that by studying miRNAs and their target genes we will significantly advance our understanding of immune regulation. Furthermore, we are interested in the interplay between miRNAs and other forms of posttranscriptional gene regulation. A better fundamental understanding of molecular control of T cell gene expression and lymphocyte function forms the basis for future therapies. The ultimate goal of our research efforts is to translate our basic research findings to novel concepts and approaches that will benefit patients suffering from a variety of immune related diseases. To this end we are currently exploring approaches to modulate miRNA function as a novel therapeutic modality.

Connection to Clinical Practice

Prof. Dr. Jürg Steiger
Transplantation Immunology
& Nephrology

The role of microRNAs in immune regulation

Our lab is associated with the Transplantation Immunology & Nephrology clinics of the University Hospital Basel. We will collaborate with Prof. Jürg Steiger to translate our basic research findings in clinical settings.

Selected Publications

- de Kouchkovsky, D., Esensten, J.H., Rosenthal, W.L., Morar, M.M., Bluestone, J.A., and Jeker, L.T. (2013). microRNA-17-92 Regulates IL-10 Production by Regulatory T Cells and Control of Experimental Autoimmune Encephalomyelitis. Journal of immunology 191, 1594-1605.
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- Park, C.Y., Jeker, L.T., Carver-Moore, K., Oh, A., Liu, H.J., Cameron, R., Richards, H., Li, Z., Adler, D., Yoshinaga, Y., et al. (2012). A resource for the conditional ablation of microRNAs in the mouse. Cell Reports 1, 385-391.

Diabetic Nephropathy

Podocytes

Free Fatty Acids

Fatty Acid Metabolism

Molecular Nephrology

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Role of free fatty acids and free fatty acid metabolism in the pathogenesis of diabetic nephropathy

Diabetic nephropathy is the most common cause of end-stage renal disease requiring renal replacement therapy, and most patients have type 2 diabetes mellitus. Our major research interest is to understand the pathogenesis of diabetic nephropathy in type 2 diabetic patients. Urinary loss of proteins (= proteinuria) is an early characteristic of diabetic nephropathy. Proteinuria results from an increased passage of proteins through the glomerular filtration barrier as a consequence of raised transcapillary pressure as well as structural alterations. The glomerular filtration barrier consists of capillary endothelial cells, the glomerular basement membrane, and the so called podocytes (highly specialized epithelial cells). Importantly, morphological alterations of podocytes and finally podocyte loss resulting from apoptosis occur at the onset of diabetic nephropathy.

We identified that podocytes are highly susceptible to the saturated free fatty acid (FFA) palmitic acid, which induces podocyte death (Sieber et al., 2010). Mechanistically, palmitic acid-induced podocyte death is linked to endoplasmic reticulum (ER) stress involving the proapoptotic transcription factor C/EBP homologous protein (CHOP) (Sieber et al., 2010). In contrast, we found that monounsaturated FFAs, such as palmitoleic or oleic acid, attenuate palmitic acid-induced lipotoxicity in podocytes (Sieber et al., 2010). In glomeruli of type 2 diabetic patients with diabetic nephropathy we discovered that mRNA expression levels of several key enzymes involved in fatty acid metabolism are altered (Fig. 1, Sieber et al., 2013). The most prominent change is the upregulation of stearoyl-CoA desaturase (SCD)-1, which results mainly from increased expression in podocytes (Figure 2B). SCDs desaturate saturated FFAs to non-toxic monounsaturated FFAs. Pharmacological activation of SCDs or overexpression of SCD-1 was shown to protect podocytes from palmitic acid-induced cell death. Mechanistically, we found that the unsaturated FFA oleic acid or pharmacological stimulation of SCDs promote the incorporation of saturated FFAs, e.g. palmitic acid, into triglycerides, suggesting that the protective effect at least in part results from compartmentalization of palmitic acid in "safe lipid pools" (Sieber et al., 2013). Importantly, our glomerular gene expression analysis in type 2 diabetic patients also suggests disposition for increased fatty acid oxidation as all three isoforms of carnitine palmitoyltransferase (CPT) 1, the rate-limiting enzyme for fatty acid oxidation, are upregulated and acetyl-CoA carboxylase (ACC)-2, which inhibits fatty acid oxidation, is downregulated (Sieber et al., 2013). We hypothesize that this disposition for increased fatty acid oxidation reflects an adaptive, protective mechanism against toxic free fatty acids in diabetic nephropathy. This is supported by our finding that stimulation of fatty acid oxidation in podocytes protects from palmitic acid induced ER stress and cell death whereas inhibition of fatty acid oxidation is deleterious. In addition, we discovered that fatty acid oxidation is critically regulated by acetyl-CoA carboxylases (Kampe, manuscript submitted). This observation potentially explains the results of recent genome wide association studies which discovered that the risk for diabetic nephropathy in type 2 diabetic patients is related to a single nucleotide polymorphism in the ACC-2 gene which results in a higher expression of ACC-2 and likely leads to decreased fatty acid oxidation and accumulation of toxic FFAs.

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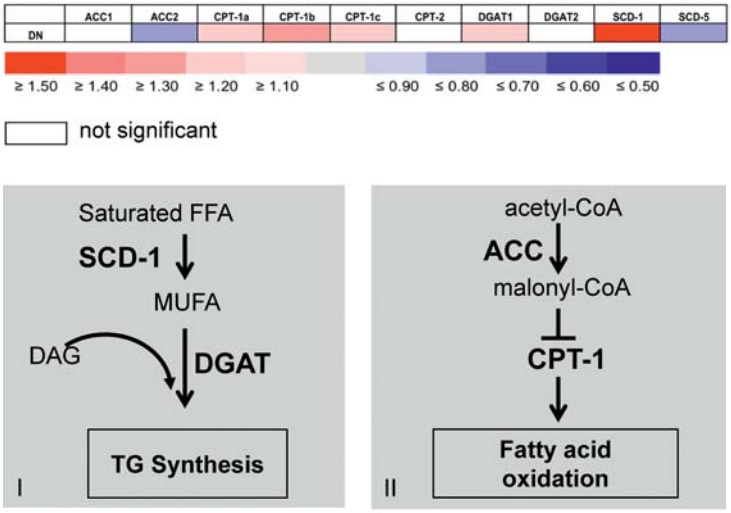

Fig. 1: Expression of fatty acid metabolism associated enzymes in diabetic nephropathy.
Microarray data were obtained from isolated glomeruli of type 2 diabetic patients with diabetic nephropathy and controls (pretransplant allograft biopsies). Gene expressions of ACC2, CPT-1a, CPT-1b, CPT-1c, DGAT1, DGAT2, SCD-1, and SCD-5 were significantly regulated in DN compared to controls with SCD-1 showing the highest upregulation. Upregulated enzymes are indicated in red, downregulated enzymes in blue colors. The related metabolic pathways of enzymes analyzed are depicted in inserts I and II. Abbreviations: ACC-1 = acetyl-CoA carboxylase 1; ACC-2 = acetyl-CoA carboxylase 2; CPT-1 = carnitine palmitoyltransferase 1; DAG = diacylglycerides; DGAT1 = acyl-CoA:diacylglycerolacyltransferases 1; DGAT2 = acyl-CoA:diacylglycerolacyltransferases 2; MUFA = monounsaturated free fatty acid; SCD-1 = stearoyl-CoA desaturase 1; SCD-5 = stearoyl-CoA desaturase 5; TG = triglycerides.

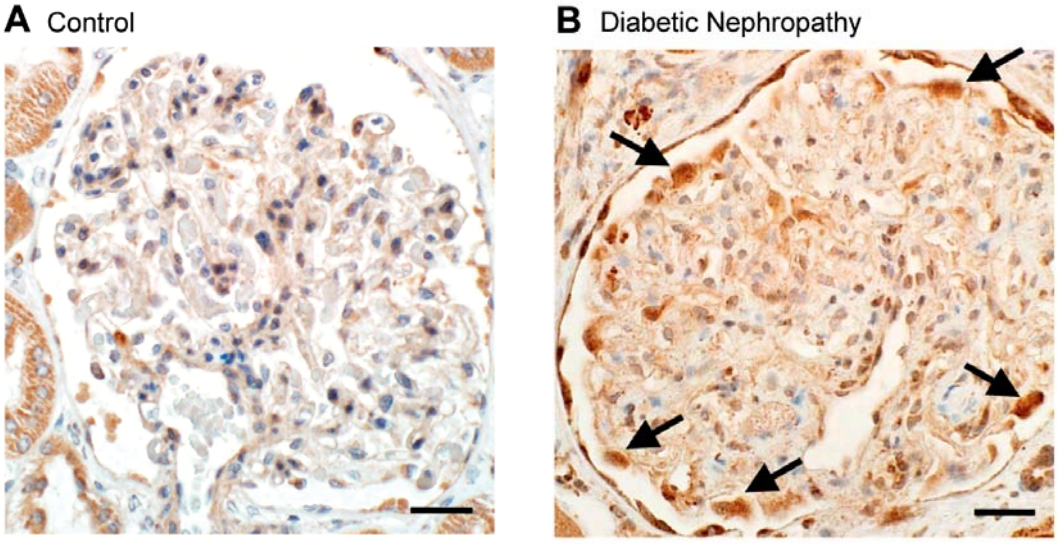

Fig. 2: Glomerular upregulation of stearoyl-CoA desaturase (SCD)-1 in diabetic nephropathy results mainly from increased expression in podocytes.
A: Immunoperoxidase staining against SCD-1 in a tumor nephrectomy specimen from a middle-aged adult without any known history of medical renal disease. No significant expression of SCD-1 is seen in glomeruli. Mild, granular cytoplasmic staining of renal tubules is present. Scale bar = 20 µm. Representative sample of four nephrectomies analyzed is shown. B: Immunoperoxidase staining against SCD-1 in a renal biopsy from a 57 year old male with type 2 diabetes mellitus showing early DN and glomerular hypertrophy. Arrows point to podocytes with intense, granular cytoplasmic staining for SCD-1. There is also some cytoplasmic staining of parietal cells. Scale bar = 20 µm. Representative example of 4 DN samples analyzed.

Selected Publications

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Viral Tropism

HIV Resistance

Persistence

Respiratory Viruses

Diagnostics

Virus Inhibitor

Molecular Virology

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Molecular virology – approaches for new diagnostic tools and towards new targeted drugs

We focus on aspects of the HIV life cycle that are critical for replication and its response to antiretroviral drugs.

1. Reliable tropism testing for the clinics – This major effort bases on the fact that HIV occurs as two distinct forms differing in their receptor dependence. The new HIV inhibitor class targets only CCR5-tropic viruses. This necessitates prior to therapy a tropism determination: Does the virus use CCR5 or the alternative CXCR4 chemokine receptor? We set out to establish a unique, reliable diagnostic test together with our spinoff InPheno AG. The tropism-determining sequence "V3" within the HIV envelope gene is represented in a labeled probe, which is hybridized to a patient-derived blood sample. Our system reads CCR5-/CXCR4-tropism and identifies/dissects mixed virus populations, a main advantage over sequencing systems (Fig1). In 2011 our test passed a European ring trial for quality control; it became accredited and accepted by the Swiss BAG for reimbursement through the Analysenliste.

2. Residual virus despite successful therapy? – In some patients a full suppression of HIV is not achieved although medication appears appropriate. In vitro observations suggest that viral expression from integrated genomes could contribute to this worrisome residual viral RNA, which must not necessarily be a sign of treatment failure. Retroviruses such as HIV require as essential step the genomic integration into the host chromosome. The new integrase inhibitors block this key step possibly leading to the first step towards HIV genome elimination and eradication. However, in a new project we have identified evidence that even unintegrated viral DNA can allow viral gene expression and potentially also the generation of infectious progeny. Our current investigations attempt to verify production of infectious particles despite the presence of therapeutic doses of integrase inhibitors. We will further try to isolate infectious virus from patients with persisting low viremia. This would have important implications for the clinics.

3. Role of HIV Gag in therapy resistance – It is being recognized that even in times of a broad therapy coverage with the highly potent drug class of HIV protease inhibitors (PI) a fair number of patients fail virologically in the absence of signature mutations in the viral protease gene. For addressing this issue we initiated a collaborative project with the Swiss HIV Cohort Study, in which we systematically analyzed virus sequences near the 3' end of the gag gene, where key cleavage sites are encode. The anonymized HIV-1 databases of 2000 sequences stems from a characterized therapy context or from untreated patients. Side-by-side analysis of these two groups enables us to identify alterations emerging only under drug pressure. Mutations can enhance "minor protease mutations" or are by themselves responsible for drug-resistance and clinical therapy failure. Our newly identified mutations associate with clinical PI resistance; they either correspond with earlier publications thus validating our algorithm or they have not been described before as treatment-associated escape mutations (Fig2). Currently we are introducing these mutations into lab strains of HIV-1 in order to phenotypically test if they convey PI resistances. We will further isolate infectious virus from patients with persisting low viremia. This could help understanding "unexplained virological therapy failures" and would be an important addition to the current resistance algorithms.

4. A somewhat "exotic" project aims at widening our views on drug discovery: In a collaboration with the South African CSIR we focus on respiratory infections caused by RSV and influenza. Together with InPheno we have now been able to validate a new phenotypic screening system for in-

hibitors. Various indigenous plants, claimed to possess anecdotal activity, have been extracted and tested in a first pilot study. First promising hits have been identified (Fig3) with selective antiviral activity in the absence of toxicity in cell models and await now further profiling and purification. As long-term goal we aim at a pharmaceutical development.

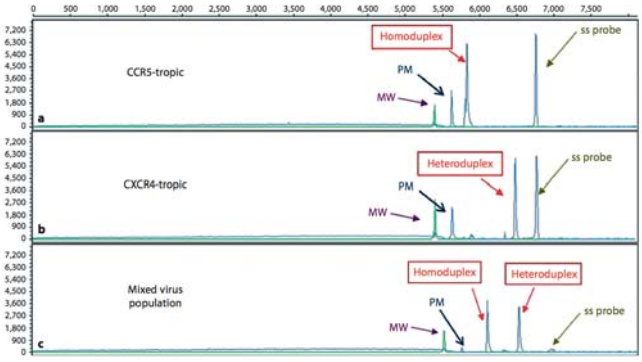

Fig. 1: Principle of our system for HIV tropism. Electrophoregram of typical examples: a) imperfect homoduplex of patient-derived HIV sequence with CCR5 probe; b) CXCR4-tropic heteroduplex; c) mixed virus population with 2 peaks. MW: size marker, PM perfect match of probe-duplex; ss probe singles-stranded labeled probe.

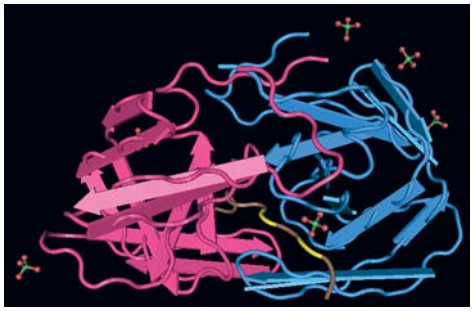

Fig. 2: The dimeric HIV-1 protease is shown in blue and purple. Interacting region of Gag 1/p6 is placed as brown string. Putative resistance mutations at 449 and 451 are highlighted in yellow, indicating an intimate interaction surface.

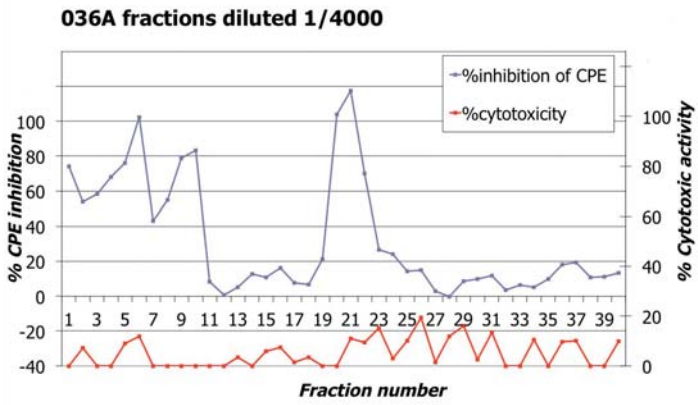

Fig. 3: Cell-based screening of the anti-Influenza activity of a plant extract (036A) in a plaque assay. The y-axis on the left indicates the inhibition of the virus-caused cytopathic effect (CPE) for each fraction (blue line); the axis on the right expresses cytotoxic effects of each fraction on uninfected cells (red line).

Connection to Clinical Practice

Diagnostic tools for improved HIV disease management; new concepts for respiratory diseases

My research group has in the past years developed several key methodologies for analysing HIV therapy. In particular the development of tests for viral drug resistance and for identifying the viruses cell tropism are assisting clinicians for disease management. Emerging from this clinical utility we have focussed research onto newer underlying mechanisms of persisting low viral replication in long-term treated patients or on the predictive value of analyses of viral parameters in cells rather than cell-free blood plasma. Also pilot studies on longer term effects of integrated versus unintegrated viral genomes in the patient may help to understand viral progression or may be useful for concepts towards HIV elimination.

Beyond HIV as human pathogen part of our research can directly be applied to other viruses with clinical importance. In fruitful international collaborations we have invested in the identification of new inhibitory concepts targeting vital virus functions. Herein we concentrate on respiratory pathogenic viruses such as Influenza, parainfluenza, and RSV. With a group in Pretoria we recognize the intriguing source of natural compounds in South African plants, known in traditional medicine to possess disease-fighting properties. Our plan is to identify therein the active principles and make them available for pharmaceutical exploitation.

Selected Publications

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- Vandekerckhove, L.P., Wensing, A.M., Kaiser, R., Brun-Vezinet, F., Clotet, B., De Luca, A., Dressler, S., Garcia, F., Geretti, A.M., Klimkait, T., et al. (2011). European guidelines on the clinical management of HIV-1 tropism testing. The Lancet infectious diseases *11*, 394-407.
- Klimkait, T. (2012). The XTrack system: application and advantage. Intervirology *55*, 118-122.
- Scherrer, A.U., Boni, J., Yerly, S., Klimkait, T., Aubert, V., Furrer, H., Calmy, A., Cavassini, M., Elzi, L., Vernazza, P.L., et al. (2012). Long-lasting protection of activity of nucleoside reverse transcriptase inhibitors and protease inhibitors (PIs) by boosted PI containing regimens. PloS one *7*, e50307.
- Vidal, V., Potterat, O., Louvel, S., Hamy, F., Mojarrab, M., Sanglier, J.J., Klimkait, T., and Hamburger, M. (2012). Library-based discovery and characterization of daphnane diterpenes as potent and selective HIV inhibitors in Daphne gnidium. Journal of Natural Products *75*, 414-419.
- Masimba, P.J., Kituma, E., Klimkait, T., Horvath, E., Stoeckle, M., Hatz, C., Mossdorf, E., Mwaigomole, E., Hamis, S., Jullu, B., *et al.* (2013). Prevalence of Drug-Resistance Mutations and HIV-1 Subtypes in a HIV-1 Infected COHORT in rural Tanzania. AIDS research and human retroviruses.

Immunology

T Cell

Thymus Development

Genetics, Epigenetics

Paediatric Immunology

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The immunobiology of the thymus

T cell mediated responses play a crucial role in providing protective immunity, but at the same time are also responsible for a broad range of autoimmune pathologies when directed against an individual's own tissues. The linage commitment and maturation of T cells is instructed during their thymic development consequent to a close physical and functional interaction with the organs stromal microenvironment. Thymic epithelial cells (TEC) constitute an essential component of this stroma whereby Cortical (c) and medullary (m) TEC have distinct structural, antigenic and functional features. cTECs provide signals that commit hematopoietic precursor cells to a T cell fate and select those immature T cells for further differentiation that express a functionally competent T cell antigen receptor. In contrast, mTEC contribute to the establishment of self tolerance via the expression of peripheral tissue-specific antigens (PTA).

The research of the laboratory of Paediatric Immunology focuses on (i) a detailed understanding of the genetic and epigenetic control of TEC development and (ii) the functional potential of TEC to support the reconstitution of the T cell compartment following hematopoietic stem cell transplantation (HSCT). For this purpose, we have generated specific genetic gain and loss of function mouse models that allow a precise interrogation of particular molecular mechanisms relevant for thymus organogenesis and function.

Our recent research has defined the role of gene dosage for the transcription factor Foxn1 which is essential for the commitment of endodermal epithelial cells to a TEC fate. Moreover, studies have further characterised the importance of the polycomb repressive complex 1 for the maintenance of this cell lineage commitment. Differences in DNA methylation and the generation of micro-RNA constitute additional epigenetic mechanims that we have identified to play an essential role in TEC fate, maintenance and function, including the expression of PTA. The expression of some of these PTA is controlled by the nuclear protein Autoimmune Regulator (Aire). A comprehensive next generation sequencing transcriptome analysis of TEC subpopulations proficient or deficient in Aire expression further revealed that the thymic epithelial cell as a whole express virtually all protein coding genes including all tissue specific antigens and that specific TEC populations display a complex and in part lineage specific gene expression pattern. Importantly for the underlying mechanism of promiscuous gene expression the loci of tissue-specific genes are characterised by unique histone marks, which likely provide a molecular recognition for their transcription either by an Aire dependent or independent mechanism.

In experiments that model allogeneic HSCT, we have furthermore demonstrated that the expression of the molecular mirror representing self-antigens by TEC is severely compromised in the context of acute graft-versus-host disease providing a mechanism for how autoimmunity develops as a consequence of thymus-directed alloimmunity.

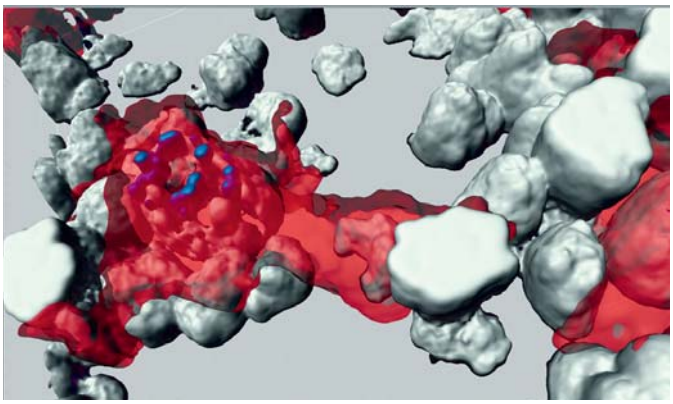

Fig. 1: 3D reconstruction of the mTEC visualized by RFP expression expressing Aire protein (blue). Grey is DAPI staining nucleus.

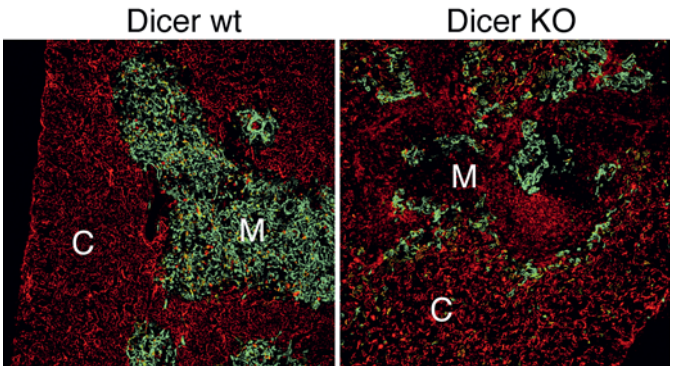

Fig. 2: Adult thymus from 3 week old Dicer wt and Dicer KO mice stained for CK8 (red, cortex C) and CK14 (green, medulla M).

Selected Publications

- Kelly RM, Goren EM, Bouillet P, Strasser A, Contag CH, Scott HS, Gudkov AV, Holländer GA and Blazar RB. (2010). Short term inhibition of p53 combined with Keratinocyte Growth factor improves thymic epithelil cell recovery and enhances T cell reconstitution after murine bone marrow transplantation. Blood;115: 1088-97.
- Papadopoulou AS, Dooley J, Linterman MA, Pierson W, Ucar O, Kyewski B, Zuklys S, Hollander GA, Matthys P, Gray DH, De Strooper B, Liston A. (2011). The thymic epithelial microRNA network elevates the threshold for infection-associated involution via miRNA29a mediated suppression of the IFN-a receptor. Nat Immunol;13:181-7.
- Zuklys S, Mayer CE, Zhanybekova S, Stefanski HE, Nusspaumer G, Gill J, Barthlott T, Chappaz S, Nitta T, Dooley J, Nogales-Cadenas R, Takahama Y, Finke D, Liston A, Blazar BR, Pascual-Montano A, Holländer GA. (2012). MicroRNAs Control the Maintenance of Thymic Epithelia and Their Competence for T Lineage Commitment and Thymocyte Selection. J Immunol;189(8):3894-904
- Ohigashi I, Zuklys S, Sakata M, Mayer, CE, Zhanybekova S, Murata S, Tanaka K, Hollander G* and Takahama Y*. (2013). Aire-expressing thymic medullary epithelial cells originate from β5t-expressing progenitor cells. Proc Natl Acad Sci;110:24, 9885-90 *= shared senior authorship.
- Dertschnig S, Nusspaumer G, Ivanek R, Hauri-Hohl MM, Holländer GA*, Krenger W*. (2013). Epithelial cytoprotection sustains ectopic expression of tissue-restricted antigens in the thymus during murine acute GVHD. Blood;122(5):837-41 *= shared senior authorship.

Autoimmunity

GCA

TCR

Treg

Th17

Translational Immunology

New Group starting 2014

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Giant cell arteritis – towards a better understanding of pathogenesis

Failure of the immune system to discriminate self from non-self can result in autoimmune disease. An integrative model suggests that the balance between auto-reactive T cells and so-called regulatory T cells (Tregs) dictates the likelihood to develop autoimmunity. We aim to test this hypothesis in patients with Giant cell arteritis (GCA), an autoimmune disease of the blood vessels. Both, IL-17 producing Th17 cells – that represent the prototype of auto-reactive effector T cells – and Tregs, have been linked to GCA pathogenesis. We will investigate, whether dysregulated Treg function contributes to disease pathogenesis, e.g. via insufficient suppression of auto-aggressive T-cells or by inducing a Th17 promoting cytokine milieu.

Following antigenic stimulation, T effector cells expand clonally. T cell receptor (TCR) sequencing can be used to assess the clonal T cell repertoire. In chronic infections, certain cancers and autoimmune diseases, T cell clones have been identified that occur frequently and are shared between different patients affected with the same disease ("public T cell clones"). Little is known about the TCR repertoire in GCA. We hypothesize that clonally expanded T cells detect common vascular self-antigens in GCA, which should be reflected in a narrow TCR repertoire and the presence of public TCR. To test this hypothesis, we aim to define the TCR repertoire at the site of autoimmune inflammation in GCA, taking advantage of laser capture microdissection of infiltrating T cells, combined with an unbiased PCR approach. Using computational epitope prediction tools, the target antigen will then be further characterized. As a clinical application, identification of disease-specific TCR clonotypes in inflamed tissue would permit to track and characterize them in the peripheral blood, opening the possibility for highly specific biomarker-research.

Connection to Clinical Practice

PD Dr. Thomas Daikeler, Prof. Dr. Christoph Hess
Division of Rheumatology and Department of Internal Medicine

The Basler Giant Cell Arteritis Cohort

Giant cell arteritis (GCA) is the most prevalent of the primary vasculitis syndromes with an increasing disease incidence. Patients typically present with constitutional symptoms, headache, and a systemic inflammatory syndrome. To date therapy of GCA is based largely on steroids, and guided by parameters reflecting disease activity only partially, as indicated by recent imaging-studies. Furthermore, intensity and duration of steroid therapy remain a matter of debate, and no consensus exists in defining remission. Both GCA itself and the steroid based therapy are associated with significant morbidity. Improving diagnostic accuracy and monitoring of disease activity thus would be of great importance. To study these clinical problems, we established a prospective interdisciplinary cohort of patients with GCA. Relevant clinical data, laboratory parameters, serum and peripheral blood mononuclear cells from all patients are collected at longitudinal time-points. Vascular disease activity is assessed using new technologies such as color-coded duplex ultrasound and positron emission tomography. Thereby we aim at integrating clinical data, imaging studies, and extended immunological and histomorphological assessments for a more detailed understanding of the immunopathogenesis of GCA. This may help to (i) further develop precise, ideally non-invasive, tools to diagnose and monitor disease activity, and (ii) generate strategies towards interfering with specific pathways associated with disease activity and/or complications.

Selected Publications

- Berger, C.T., Carlson, J.M., Brumme, C.J., Hartman, K.L., Brumme, Z.L., Henry, L.M., Rosato, P.C., Piechocka-Trocha, A., Brockman, M.A., Harrigan, P.R., et al. (2010). Viral adaptation to immune selection pressure by HLA class I-restricted CTL responses targeting epitopes in HIV frameshift sequences. J Exp Med 207, 61-75.
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T Cells Tolerance

Autoimmunity

Regulatory T Cells

TCR Signaling

Transplantation Immunology and Nephrology

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Dr. Ondrej Stepanek (postdoctoral fellow)
Lena Wyss (PhD student)

Understanding the principles of naturally occuring T cell tolerance

One of the central mysteries of immunology is self-tolerance. How does the human body select ~10e^{12} T lymphocytes, that are reactive to foreign pathogens but tolerant to normal cellular constituents of the host? The work of our laboratory seeks to understand the general principles by which a healthy individual's immune system achieves a state of self-tolerance. We are particularly interested in how a tolerant T cell repertoire is selected during development and how it's maintained during adult life. The knowledge derived from our research may eventually impact organ transplantation and autoimmune diseases.

Over the last few years, we demonstrated that the affinity threshold for negative selection is a constant for all thymocytes expressing MHC I restricted TCRs. This binding affinity threshold (KD = 6 μM; estimated T1/2 ≈ 2 sec) is a fundamental biophysical parameter used by developing CD8 lineage cells to establish a tolerant T cell repertoire. More recent experiments indicate that thymocytes destined to enter the CD4 lineage use an affinity threshold (KD = 300 μM; estimated T1/2 ≈ 0.1 sec) for negative selection that is 10-50 fold lower than that used for their CD8 lineage counterparts. These differences are explained by the fraction of the corresponding co-receptor (CD8 or CD4) which carries the initiating kinase, lck. 1% of CD8 vs 10% of CD4 molecules are loaded with lck. The emerging picture is that MHC I restricted thymocytes require a high affinity (longer duration) interaction between the TCR and the self-antigen to initiate negative selection because so few (< 1%) of the CD8 co-receptor molecules carry lck. MHC II restricted thymocytes on the other hand undergo negative selection with a much lower affinity (shorter duration) interaction because a higher proportion (10%) of the CD4 molecules carry lck. Imaging studies and molecular modeling have provided evidence that an antigen engaged TCR must undergo hundreds of collisions with the relevant co-receptors to eventually engage a co-receptor molecule which actually carries the initiating kinase, lck. Based on the number of molecules and the biophysical parameters describing their movement, we developed a mathematical model which describes a mechanism where the TCR can actually 'read' antigen affinity and establish an affinity (antigen dwell time) threshold for self-tolerance.

We also examined the affinity threshold required for the induction of experimental autoimmune diabetes. This involves the activation of the integrin LFA-1, formation of long-lasting T cell - antigen presenting cell conjugates, asymmetric cell division and differentiation into short-lived effector cells. Related to this, we are examining the origin of autoimmune T cells; they frequently express threshold affinity TCRs, which are inefficiently removed by clonal deletion. We are also trying to define the minimum number of T cells required to initiate an autoimmune disease.

Another focus of the laboratory is to understand the basic biology of regulatory T cells. Our experiments support the idea that Helios+ FoxP3+ regulatory T cells are survivors of negative selection in the thymus. This implies that the TCR repertoire expressed on Helios+ regulatory T cells is high affinity anti-self. In vitro experiments demonstrated that Tregs require contact with MHC II expressing APCs and IL-2 from conventional T cells to proliferate. We are also using monoclonal Tregs and monoclonal Tconv cells to examine the basis of Treg mediated suppression. An additional project focuses on the role of regulatory T cells in maintaining peripheral tolerance.

Finally, we studied the role if MHC II expression in intestinal epithelial cells. In mice specifically lacking MHC II expression in this cell type, we observed an epithelial lymphocytosis.

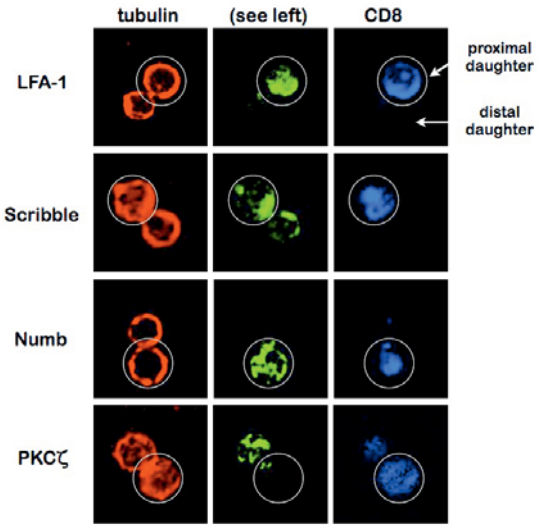

Fig. 1: Asymmetric T cell division following stimulation with high affinity antigen.

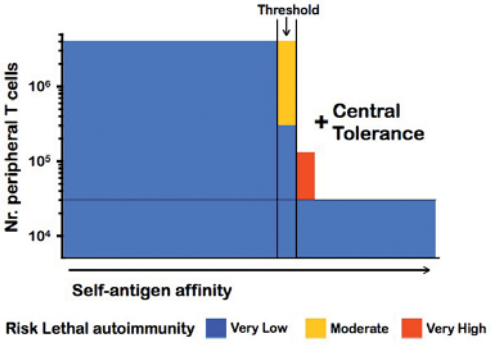

Fig. 2: Relationship between self-antigen affinity, number of antigen specific T cells and risk of developing experimentally induced autoimmune diabetes.

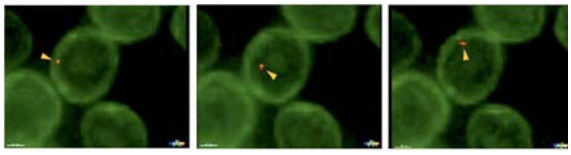

Fig. 3: Dwell times of single pMHC antigen molecules labeled with quantum dots determined using confocal microscopy.

Connection to Clinical Practice

Prof. Dr. med. Jürg Steiger
Clinic of Transplantation and Immunology

Advances in nephrology research

Jürg Steiger heads the Clinic for Transplantation Immunology and Nephrology and leads a team of 7 clinical nephrologists and 6 fellows, which oversees 60 new kidney transplantations, follow-up of 600 transplanted patients, 16'000 dialyses as well as 1300 in- and out-patient consultations each year. The team covers basic, clinical and translational research in different areas (www.unispital-basel.ch/das-universitaetsspital/bereiche/medizin/kliniken-institute-abteilungen/transplantationsimmunologie-nephrologie/lehre-forschung/forschung/).
Min-Jeong Kim's research interest is IgA-nephropathy; she explores signaling in mesangial cells and the clinical impact of differentially glycosylated IgA. Andreas Jehle and his team investigate molecular mechanisms of podocyte function in health and disease. Michael Dickenmann introduced ABO-incompatible living donor kidney transplantation to Basel and investigates clinico-pathological outcomes in these patients. Patrizia Amico, Patricia Hirt-Minkowski, Gideon Hönger and Stefan Schaub study the clinical significance of donor-specific HLA-antibodies and explore novel biomarkers for non-invasive monitoring of renal allograft recipients. Jürg Steiger heads the Swiss Transplant Cohort Study (STCS), a multicenter cohort study of all solid organ recipients in Switzerland. The STCS integrates all information on transplant activities providing a basis for high quality clinical research. The data center of the STCS is led by Michael Koller. Finally, Christa Nolte, Felix Burkhalter and Jürg Steiger analyze short and long-term outcomes of living kidney donors in the Swiss organ living donor health registry (SOL-DHR).

Selected Publications

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- Schrum, A.G., Gil, D.,Turka, L.A. and Palmer, E. (2011) Physical and Functional Bivalency Observed Among TCR/CD3 Complexes Isolated from Primary T Cells. J. Immunol. Jul15;187(2):870-8.
- Currie, J., Castro, M., Lythe, G., Palmer, E., Molina-Paris, C. (2012) A stochastic T cell response criterion. J R Soc Interface. Jun 28, 2012 doi: 10.1098/rsif.2012.0205.
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- Irla, M., Guerri, L., Guenot, J., Sergé, S., Lantz, O., Liston, A., Imhof, B.A., Palmer, E. and Reith, W. (2012) Control of thymic medulla expansion and homeostasis by autoreactive CD4+ thymocytes. PLoS ONE 7(12): e52591. doi:10.1371/journal.pone.0052591

Virus
Immune Response
Immunodeficiency
Immunosuppression
Transplantation
Diagnostics

Transplantation and Clinical Virology

Prof. Dr. Hans H. Hirsch
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Marion Wernli (technician)

Virus, immune response and clinical implication

Virus infection and host response enter a critical virus-host balance, which is influenced by pathogenic factors of the virus and innate and adaptive host responses. Our approach is translational: Clinical observations suggesting a viral complication are corroborated through specific and quantitative virus diagnostics in the Division of Infection Diagnostics, a fully accredited medical microbiology laboratory according to the EN17025 (Swiss Testing Site 217). In the research group Transplantation & Clinical Virology, we aim at characterizing 1) key determinants of virus biology, 2) potential targets of antiviral intervention, 3) relevant immune responses. This combined approach should allow to improve the risk stratification and monitoring of patients, the identification of antiviral targets, and the design of protective vaccines and/or adoptive transfer of T-cells for clinical use.

Community-acquired respiratory virus (CARV) are detected by a multiplex PCR covering >16 different respiratory pathogens including Influenza (FLU) A and B, A/H1N1v ("swine" FLU), bird flu, respiratory syncytial virus (RSV), Metapneumo-, Parainfluenza-, Corona-, Adeno, and Rhinoviruses. Together with colleagues in the clinics for hematology, infectious diseases, and pneumology, we are characterizing virus epidemiology, and trying to link the qualitative and quantitative detection of viruses with clinical presentation and outcomes (Hirsch et al. 2013 Clin Inf Dis 56: 258).

Human Herpes viruses (HHV) now encompass 9 members including cytomegalovirus (CMV) and Epstein-Barr virus (EBV). CMV and EBV are frequent challenges in immunocompromized patients causing viral syndromes, organ-invasive disease and lymphoproliferative disorders such as PTLD. By integrating the replication biology of EBV the contribution of free EBV virion and viral episomal DNA from lysed cells to EBV load, we could develop a first innovative mathematical model allowing to predict the impact of impaired T-cell function, T-cell depletion, and EBV infection recruiting new B-cells (Funk et al. 2007 Lancet Inf Dis 6: 460).

The role of CMV, and specifically the impact of antiviral prophylaxis versus preemptive therapy were studied in the frame of the Swiss Transplant Cohort Study suggesting that graft survival was better in patients receiving antiviral prophylaxis. CMV specific T-cell function appears inactive, but can be restored in vitro using PD-1 receptor blockade. Our recent data support the use of viral loads assays using International Units according to the 1st CMV WHO standard. As a result, International Units for CMV are now recommended in international guidelines (see refs.).

Human Polyomaviruses (HPyV) now consist of 12 different species, 9 of which have only been discovered since 2007. Significant human diseases are recognized for BK virus (BKV), JC virus (JCV), Merkel cell carcinoma and Trichodysplasia spinulosa PyV, all of which arise almost exclusively in immunocompromized patients. BKV causes PyV-associated nephropathy (PyVAN) in kidney transplant patients and late-onset hemorrhagic cystitis in allogeneic bone marrow transplantation. We established that PyVAN is preceded by high-level BKV viruria with decoy cell shedding and BKV viremia. In an international 1:1 randomized-controlled trial comparing tacrolimus with cyclosporine in 682 de novo kidney transplant recipients, we found that BKV viremia was more frequent and higher in the tacrolimus arm posttransplant (see refs.). Based on BKV viremia, reducing immunosuppression becomes an efficient intervention strategy. Accordingly, screening kidney transplant patients for plasma BKV loads is now recommended by international guidelines to identify kidney patients at risk for BKV disease. We speculate that BKV proteins subvert innate and adaptive immune responses (see refs.). Until now, there are no antivirals of proven clinical efficacy, but our recent

in vitro study suggests that the lipid derivative CMX001 may be 400-fold more active compared to the parent cidofovir.

JC virus (JCV) causes progressive multifocal leukoencephalopathy (PML). Together with the US MACS and Swiss HIV Cohort Study, we found that survival of PML patients is associated with early increasing JCV antibody responses. We also showed that CMX001 shows a good JCV inhibitory activity in vitro.

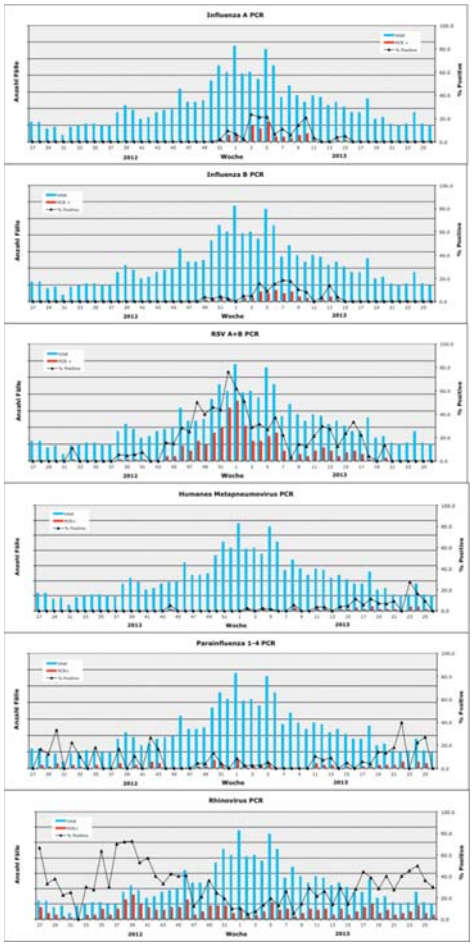

Fig. 1: Community acquired respiratory virus infection in the season 2012–2013 (Dr C. Beckmann, Dr Alexis Dumoulin, Div Infection Diagnostics, DBM Haus Petersplatz)

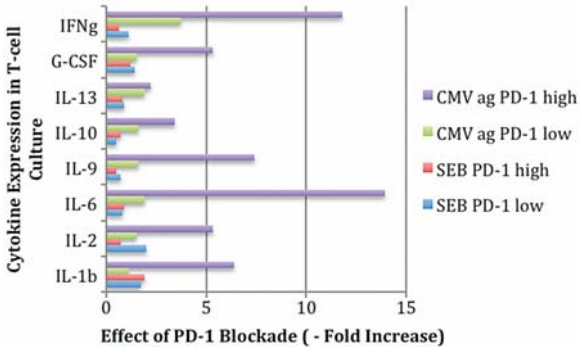

Fig. 2: Restoration of proinflammatory cytokine expression after blockade with anti-PD-L1 and anti-PD-L2 blocking antibodies in kidney transplant patients (Dirks et al. 2013 Transpl Infect Dis 15: 79).

Connection to Clinical Practice

Ongoing collaborations with

Prof. J. Steiger, Prof. J. Passweg, Prof. L. Kappos, Prof. M. Battegay, Prof. U. Heininger

Selected Publications

- Hirsch HH, Lautenschlager I, Pinsky BA, Cardenoso L, Aslam S, Cobb B, Vilchez RA, Valsamakis A. An international multicenter performance analysis of cytomegalovirus load tests. Clin Infect Dis. 2013; 56(3): 367.
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- Cioni M, Mittelholzer C, Wernli M, Hirsch HH (2013) Comparing effects of BK virus agnoprotein and Herpes simplex-1 ICP47 on MHC-I and MHC-II expression. Clin Develop Immunol 2013: 626823.
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- Unterstab G, Gosert R, Leuenberger D, Lorentz P, Rinaldo CH, Hirsch HH. The polyomavirus BK agnoprotein colocalizes with lipid droplets. Virology. 2010; 399(2): 322.

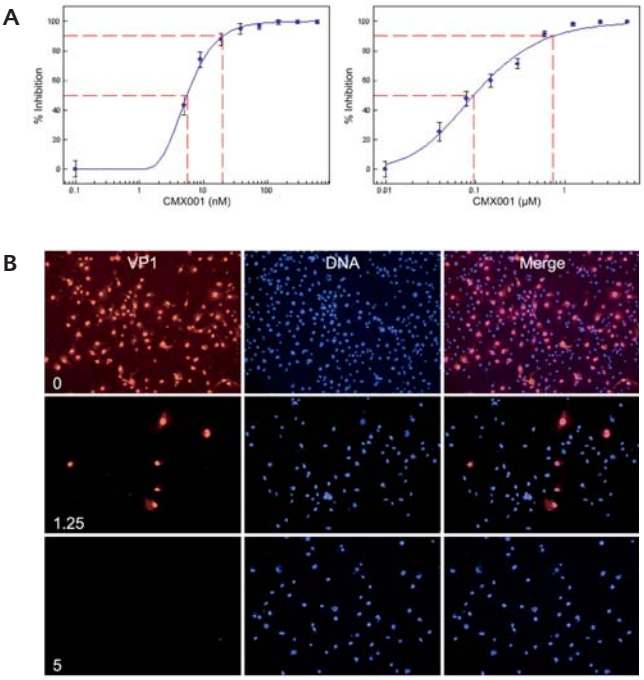

Fig. 3: Inhibition of JC Polyomavirus replication by CMX001 (Brincidofovir). (A) The effect of increasing CMX001 on JCV supernatant loads was analyzed by curve fitting for EC50 and EC90. (B) The effect of increasing CMX001 on JCV infection by staining for the viral capsid protein VP1 (Gosert R et al. 2011 Antimicrob Agents Chemother 55: 2129)

Feral Pigeon *Columba livia*

Epidemiology

Pigeon Control

Wild Boar *Sus scrofa*

Deterrent Systems

Integrative Biology

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*left during report period

Feral pigeons and wild boars

Management of feral pigeon populations
A high reproduction rate and a large food base allow large feral pigeon populations in almost every city worldwide. In order to find the properly designed control strategy that aims at lowering the number of an avian pest, the thorough understanding of the population processes of the considered species is needed. Estimates of demographic parameters as natality, mortality, immigration and emigration and of their variability are crucial when selecting an appropriate control strategy. These evaluations provide sensible hints regarding the feasibility of the intended control method itself. In the city of Basel we run nine feral pigeon lofts for more than 20 years providing broad scientific data. Recent results prove that only a small part of the breeding pairs, less than 10 percent, is able to compensate for losses due to mortality. Control strategies that encompass less than 90% of the breeding pairs therefore are unable to reduce a population. The only way to solve the pigeon problem is the reduction of the food supply by collaborating with general public.

Ectoparasites of feral pigeons and their reactions towards host-related stimuli
The close coexistence of large feral pigeon populations and humans in our cities implicates serious health risks. Several ectoparasites known to infest humans can migrate into human living space. Especially after their natural hosts are excluded from roosting and nesting areas ectoparasites search for new alternative hosts. We study the host seeking behavior of significant human pathogenic feral pigeon ectoparasites. One of these important ectoparasites is the pigeon tick *Argas reflexus*. Being able to starve for years, *A. reflexus* can hide unnoticeably in the cracks and crevices of buildings. When biting a human host, the severity of the reaction to the bite can vary from mild symptoms to life-threatening conditions. With our study we hope to provide information about the main stimuli, which lead the parasites to their hosts. Furthermore we wish to contribute to a risk assessment for humans living close to feral pigeon nests or adjacent to buildings infested with feral pigeons.

Preventing wild boar *(Sus scrofa)* damage in agriculture – investigation of deterrent systems
To prevent economic problems by high wild boar populations, an effective wild boar management has to be established. Besides the regulation of the populations by means of hunting, vulnerable crop fields have to be protected adequately. Crop protection is usually achieved by the use of electric fences, which is costly and time-consuming since fences need regular maintenance. Alternatively, various methods are available that claim effective deterrence of wild boars, however most of them lack scientific proof. In our study conducted in the Canton Basel-Land we investigated the effectiveness of solar-powered blinkers, an odour repellent, and a gustatory repellent in field experiments with free-ranging wild boars.
Solar blinkers and the odour repellent, which were investigated at baited luring sites, reduced the probability of wild boar visits by 8.1% and by 0.4% respectively. The gustatory repellent, which was investigated in experimental fields, did not have a significant effect on the frequency of damage events.
Our study revealed, that none of the deterrents investigated was able to prevent wild boars from entering the experimental sites. To date, the only recommendable means for damage prevention is the electric fence. Our results contribute to an assessment of legal foundations and common practice of field protection, compensation payments, and hunting by cantonal veterinary- and game authorities.

Fig. 1: The pigeon tick *Argas reflexus* represents the most significant health hazard posed by feral pigeons. This bloodsucking ectoparasite is widely present at breeding sites and is able to starve for up to 9 years. In predisposed persons the bites release severe allergic reactions. With our study we try to identify the stimuli, which lead the ticks to its hosts.

Fig. 2: Foto of a family group of wild boars at an experimental luring site taken by a camera trap. In the front three piglets are visible. At the age of 4 months they lose their typical stripe pattern and develop a reddish fur, which changes to dark brown at about 12 months. Behind the piglets an adult female feeds from the bait. The luring site was surrounded by posts, to which the odor repellent was affixed. One of the posts is visible in the background (red arrow).

Selected Publications

- Haag-Wackernagel D, 2011. Die Taube – eine Erfolgsgeschichte. Biologie in unserer Zeit 1(41): 44–52.
- Geigenfeind I, Vanrompay D, Haag-Wackernagel D. 2012. Prevalence of Chlamydia psittaci in the feral pigeon population of Basel, Switzerland. J Med Microbiol, 61(Pt2): 261–265.
- Schlageter A, Haag-Wackernagel D. 2011. Effectiveness of solar blinkers as a means of crop protection from wild boar damage. Crop prot 30: 1216–1222.
- Schlageter A, Haag-Wackernagel D. 2012. Evaluation of an odor repellent for protecting crops from wild boar damage. J Pest Sci 85: 209–215.
- Schlageter A, Haag-Wackernagel D. 2012. A Gustatory Repellent for Protection of Agricultural Land from Wild Boar Damage: An Investigation on Effectiveness. J Agr Sci 4(5): 61–68.

Neuromuscular Diseases

Skeletal Muscle Sarcoplasmic Reticulum

Ryanodine Receptor

Calcium Homeostasis

Pharmacogenetics

Novel Proteins

Perioperative Patient Safety

PD Dr. Susan Treves **Prof. Dr. Thierry Girard**

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Esther Schmidt (technician)
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Martine Singer (technician)
Antonio Teixeira (technician)
Prof. Albert Urwyler
Prof. Francesco Zorzato (project leader)

Skeletal muscle calcium dysregulation under normal and pathological conditions

Calcium is a universal second messenger regulating different biological functions from muscle contraction and neuronal excitability, to gene transcription and cell death. Physiologically, Ca^{2+} signals result both from the release of Ca^{2+} from intracellular stores as well as influx from the extracellular environment, via the opening of channels on the plasma membrane. In skeletal muscle, Ca^{2+} regulates contraction and relaxation and alterations in its intracellular concentration can lead to several neuromuscular disorders. Investigations carried out during the past decade have shown that in more than 50% of the cases, Central Core Disease, Multi-minicore disease and Malignant Hyperthermia are linked to point mutations in the gene encoding the skeletal muscle sarcoplasmic reticulum calcium release channel ryanodine receptor (RyR1), which is a key protein involved in releasing the calcium from the sarcoplasmic reticulum after plasma membrane depolarization. Indeed to date more than 200 missense mutations in the RYR1 gene have been identified in patients and associated to the Central core disease, Multiminicore disease, Congenital Fiber type disproportion, Centronuclear myopathy, rhabdomyolyisis and the Malignant Hyperthermia phenotype.
There are three isoforms of the ryanodine receptor that are expressed in different tissues; type 1 is preferentially expressed in skeletal muscles but recent data has shown that it is also expressed in some areas of the central nervous system, in some immune cells and in smooth muscle cells. These results imply that mutations in RYR1 (the gene encoding RyR1) may lead to alterations of Ca^{2+} homeostasis not only in skeletal muscle, but also in other tissues expressing this intracellular calcium release channel. Indeed ryanodinopathies have recently been implicated in other clinical conditions such as sepsis and intensive care polyneuropathy, broadening the clinical spectrum of disorders linked to altered RyR1functions.
The aims of our research are to broaden our knowledge of ryanodinopathies. We plan to investigate the mechanism by which recessive mutations in RYR1 linked to some forms of Multiminicore Disease, Centronuclear myopathy and Congenital Fiber Type disproportion lead to a drastic decrease in RyR1 content in muscle biopsies. In addition, we will investigate the expression profile of major proteins involved in calcium homeostasis, in human ocular muscles. This is important in order to understand why some patients affected by neuromuscular disorders linked to recessive RYR1 mutations exhibit eye muscle involvement (ptosis, ophthalmoplegia) but other patients, particularly those with dominant RYR1 mutations which alter channel function, do not. Finally we will investigate the role of RyR1 in smooth muscle cells and determine if mutations in RYR1 can cause alterations in bleeding times. This is important because prolonged bleeding times have been reported in some patients with dominant RYR1 mutations linked to Malignant Hyperthermia.

Finally by pursuing a proteomic approach of the endo(sarco)plasmic reticulum, the organelle responsible for calcium homeostasis in muscle and non-muscle cells we have characterized at the molecular and functional level a number of proteins including junctate, JP-45, SRP-35. SRP-27. For JP-45 we have made a knock-out animal model which has yielded important information on calcium influx occurring in skeletal muscle during excitation-contraction coupling. In the future we plan on screening the gene encoding human JP45 (JSRP1) for polymorphic variants in order to study their role in neuromuscular disorders. For SRP-35, a 35 kDa a retinol dehydrogenase, that we hypothesize links muscle contraction to the activation of metabolism we have recently made a transgenic animal. We think that this animal model will offer important insight

into the identification of molecular components coupling muscle activity to metabolism and may help identify potential molecular targets for the treatment of age-associated dismetabolic disorders such as type 2 diabetes.

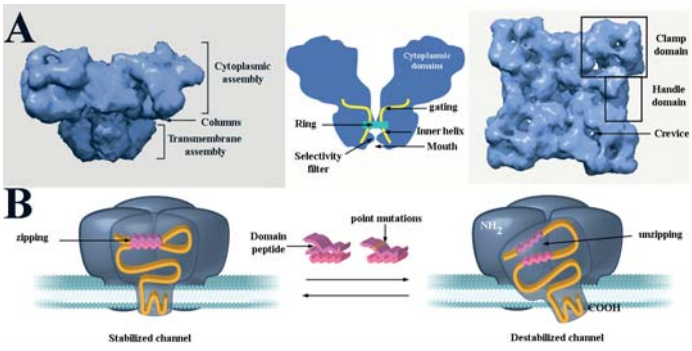

Fig. 1: Structure of the ryanodine receptor 1 (RyR1). **(a)** Proposed architecture of RyR1 based on cryo-electron microscopy (cryo-EM) at 10Å resolution, **(b)** Inter-domain interactions within RyR1. The N-terminal and central domains interact with one another to stabilize the closed configuration of the channel. When this interaction is disrupted and 'unzipped', by means of a competing synthetic domain peptide, pathogenic mutation, or physiological activation, the channel becomes destabilized and allows the passage of calcium ions through the pore.

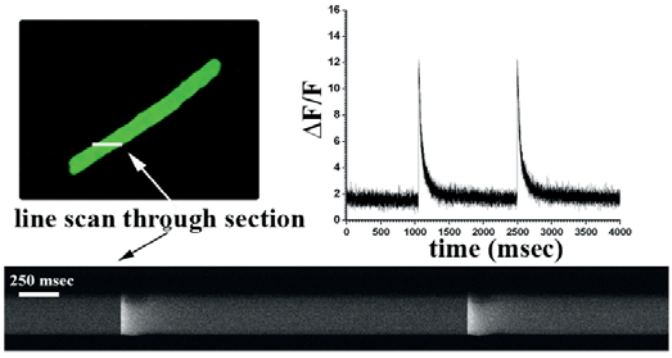

Fig. 2: Line scan image through an isolated FDB fiber loaded with the calcium indicator Fluo-4 and stimulated electrically at 1 Hz. The fiber was observed with a Nikon A1R confocal microscope in resonance mode through a 40x Plan Fluo (1.3 N.A.) objective.

Connection to Clinical Practice

Personalized medicine in anaesthesia and intensive care

Pharmacogenetics deals with influences of individual genetic profiles on the mode of action or duration of action of pharmaceutical agents. Potent drugs with fast onset and short duration of action are typical for anaesthesia and intensive care treatment. At the same time physiological parameters, such as cardiac function, oxygen consumption, CO2 production, core body temperature, renal function and brain activity as well as neuromuscular transmission are measured and controlled.
Skeletal muscle is the largest human organ, with a high metabolic activity. Mutations in skeletal muscle type 1 ryanodine receptor gene (RYR1) predispose for malignant hyperthermia (MH), a hypermetabolic disease with an autosomal dominant mode of inheritance. However, in about 25% of families with MH mutation screening RYR1 supports the view that MH is a heterogenetic disorder. If not immediately treated, MH might be lethal. The search for further genetic loci causing MH will facilitate presymptomatic diagnosis.
Calcium homeostasis and excitation contraction coupling play an important role in a patient's reaction to anesthetic agents and /or in the outcome of general anaesthesia. Thus, one aim of our research is the identification and characterization of polymorphic variants of genes encoding proteins involved in excitation contraction coupling. Further fields of interest are the effect of anaesthetic agents on ion channels, such as chloride and sodium channels of normal and myopathic muscles and molecular mechanisms of muscle atrophy and force reduction, because intact skeletal muscle function is crucial for a successful rehabilitation of patients treated on intensive care units.

Selected Publications

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- Vukcevic, M., Zorzato, F., Keck, S., Tsakiris, D.A., Keiser, J., Maizels, R.M. and Treves, S. (2013). Gain of function of the immune system caused by a ryanodine receptor 1 mutation. J. Cell Sci. 126, 3485-3492.
- Yasuda, T., Delbono, O., Wang, Z.M., Messi, M.L., Girard, T., Urwyler, A., Treves, S. and Zorzato, F. (2013). JSPR1 variants affect skeletal muscle excitation contraction coupling by decreasing the sensitivity of the dihydropyridine receptor. Hum. Mut. 34, 184-190.
- Mosca, B., Delbono, O., Messi, M.L., Bergamelli, L., Wang, Z.M., Vukcevic, M., Lopez, R., Treves, S., Nishi, M., Takeshima, H., Paolini, C., Martini, M., Rispoli, G., Protasi, F. and Zorzato, F. (2013) Enhanced dihydropyridine receptor calcium channel activity restores muscle strength in JP45/CASQ1 double knock-out mice. Nature Communications 4:1541 doi: 10.1038/ncomms2496.

DBM Publications 2011–2013

(Peer reviewed papers only)

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Albrecht, I., R. Bieri, A. Leu, P. Granacher, J. Hagmann, M.W. Kilimann, and G. Christofori. 2013. Paralemmin-1 is expressed in lymphatic endothelial cells and modulates cell migration, cell maturation and tumor lymphangiogenesis. *Angiogenesis* 16:795-807.

Albrecht, I., and G. Christofori. 2011. Molecular mechanisms of lymphangiogenesis in development and cancer. *The International journal of developmental biology* 55:483-494.

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Anetzberger, H., A. Mayer, C.U. Schulz, and M. Muller-Gerbl. 2012b. Computed tomography osteoabsorptiometry is reliable for the determination of the subchondral bone mineralization distribution in the rabbit knee. *European surgical research. Europaische chirurgische Forschung. Recherches chirurgicales europeennes* 48:208-214.

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