DBM FACTS

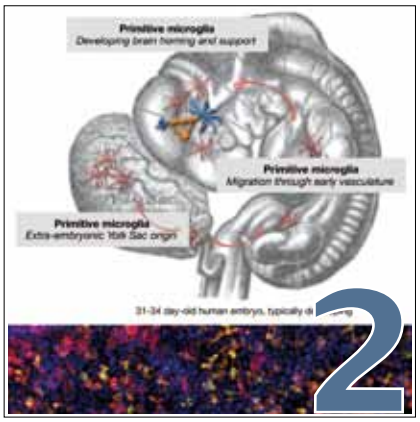
Periodisches Informationsblatt des Departementes Biomedizin
Universität Basel, Universitätsspital Basel und
Universitäts-Kinderspital beider Basel

Neonatal hypoxic ischemia: a model to study neuro-developmental injuries | Life Sciences Training Facility: Focus on genome-wide expression | Christmas in Ireland

4 | 14

INHALT CONTENTS

Departement Biomedizin Basel



Neonatal hypoxic ischemia: a model to study neuro-developmental injuries
from Raphael Guzman and team

Life Sciences Training Facility: Focus on genome-wide expression
from Andreas Papassotiropoulos and Philippe Demougin

Christmas in Ireland
from Paula Cullen

Insights into Ice hockey
from Pascal Rem

Raunächte
von Heidi Hoyermann

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EDITORIAL

Radek Skoda
Leiter DBM

2014 neigt sich seinem Ende zu. In der Medizinischen Fakultät stehen Wechsel an: Thomas Gasser wurde in der Fakultätsversammlung vom 17. November 2014 zum neuen Dekan der Medizinischen Fakultät gewählt. Er wird dieses Amt Ende 2015 antreten. Gleichzeitig wurde Primo Schär zum Vizedekan Forschung gewählt und wird bereits im Januar 2015 seine Tätigkeit aufnehmen. Wir wünschen beiden gutes Gelingen und viel Erfolg bei ihrer neuen Tätigkeit!

Der Architekturwettbewerb für den Neubau des DBM auf dem Schällemätteli-Areal konnte gestartet werden. Fünfzehn Architekturbüros werden am Wettbewerb teilnehmen, dessen Resultate im Spätsommer/Herbst 2015 zu erwarten sein werden. Damit rückt die Zukunft des DBM an einem Standort der Realität einen Schritt näher.

In der letzten Ausgabe des Jahres steht die Forschung von Raphael Guzman und seinem Labor «Brain Ischemia and Regeneration» im Mittelpunkt (ab Seite 2). Andreas Papassotiropoulos und Philippe Demougin lassen uns teilhaben an ihrer Arbeit in der «Life Science Training Facility» (ab Seite 8). Aktuelle Publikationen runden den wissenschaftlichen Teil ab (ab Seite 14). Winterlich geht es weiter: Wir spielen Eishockey mit Pascal Rem (Seite 30), feiern mit Paula Cullen Irische Weihnachten (Seite 26) und gehen auf die Suche nach dem speziellen Zauber der Raunächte (Seite 32).

Schöne Festtage und einen guten Rutsch ins 2015!

2014 is drawing to a close. There are changes on the way for the medical faculty: Thomas Gasser was elected as the new dean of the medical faculty in the faculty meeting of 17th November 2014. He will take up this position at the end of 2015. Primo Schär was elected to the position of vice dean of research at the same time and will take up this position in January 2015. We wish them both every success in their new positions.

The architectural competition for the new DBM building on the Schällemätteli Areal has begun. Fifteen architecture firms will take part in this competition, the results of which are expected in late summer / autumn 2015. With this, the future plans for the DBM take a step closer to becoming reality.

In the final edition of the year the focus is on the research of Raphael Guzman and his Laboratory "Brain Ischemia and Regeneration" (from page 2). Andreas Papassotiropoulos and Philippe Demougin allow us to share in their work in the Life Science Training Facility (from page 8). Current publications round up the scientific section (from page 14). After that we continue in a wintery manner: we play ice hockey with Pascal Rem (page 30), celebrate an Irish Christmas with Paula Cullen (page 26) and go on a quest for the special magic of the "Raunächte" (page 32).

Happy holidays and all the best for 2015!

Neonatal hypoxic ischemia: a model to study neurodevelopmental injuries

Summary

Perinatal brain development is a tightly orchestrated process, involving fine-tuned cellular and molecular crosstalk between different cell lineages. In particular, active myelination reflects the progression of functional brain maturation and connectivity in the first years of life and renders the infant brain particularly vulnerable to injuries. Cerebral palsy (CP) is a broad term used to describe a group of chronic neurodevelopmental disorders where control of movement is impaired following damages to the forming brain. The injury can be caused *in utero* or during early life. While exact etiology often remains unknown, it can associate with sporadic genetic mutations, *in utero* infection, metabolic disorders, severe prematurity with periventricular leukomalacia and hypoxic-ischemic encephalopathy (HI) amongst others. Despite major advances in fetal and neonatal monitoring technology and knowledge of related pathologies, HI remains one of the most common forms of damage to the developing brain, causing significant mortality and precipitating the occurrence of CP.

Currently, only very limited therapy can target the long-term consequences of early brain injury, making regenerative medicine a promising area for treatment exploration. Indeed, several reports suggest that transplanted neural progenitor cells (NPC) may promote CNS tissue repair not merely through cell replacement, but rather by providing immunomodulatory and neurotrophic support for endogenous repair mechanisms.

The Brain Ischemia and Regeneration (BRIR) research group investigates the mechanisms underlying human NPC-mediated brain repair in a rodent model of neonatal HI. Neurogenesis and oligodendrogenesis are key neuroregenerative processes and NPC have been shown to support such repair mechanisms. One hypothesis is that NPC-mediated regeneration occurs through immunomodulation of microglia, the immune cells of the brain with a recently highlighted crucial role in the establishment of brain architecture. Therefore, BRIR studies focus on the neuro-immune interactions in the context of different brain repair processes and neurodevelopmental disorders.

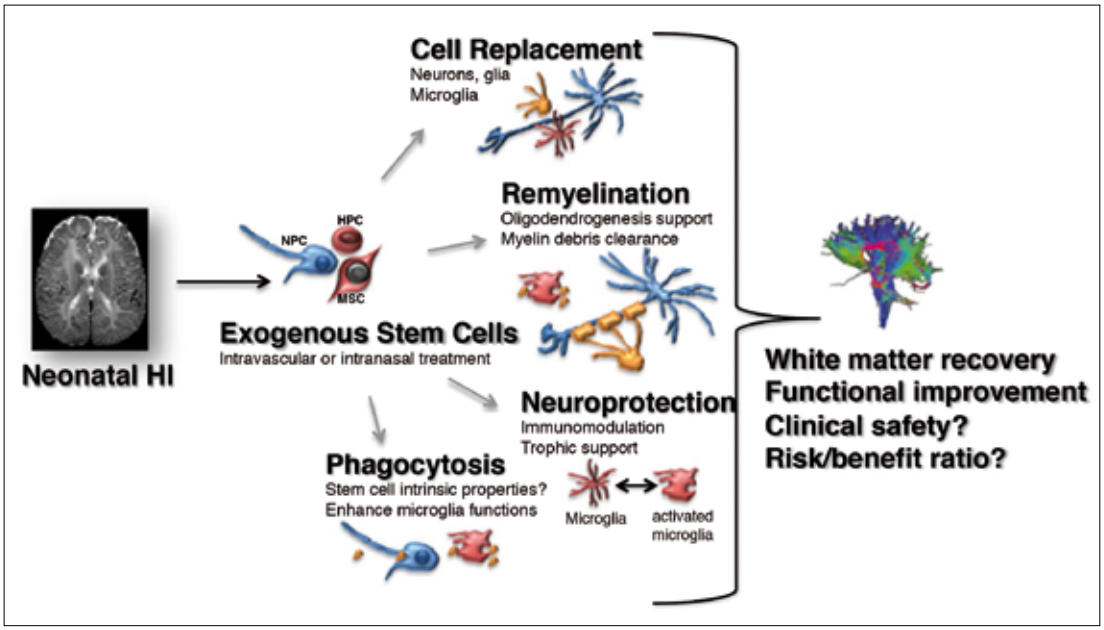

Fig1: Stem cell-induced brain repair mechanisms. Exogenous stem cell administration may promote brain repair not merely through cell replacement, but rather through neurotrophic support of core mechanisms such as remyelination, microglial phagocytosis and immunomodulation.

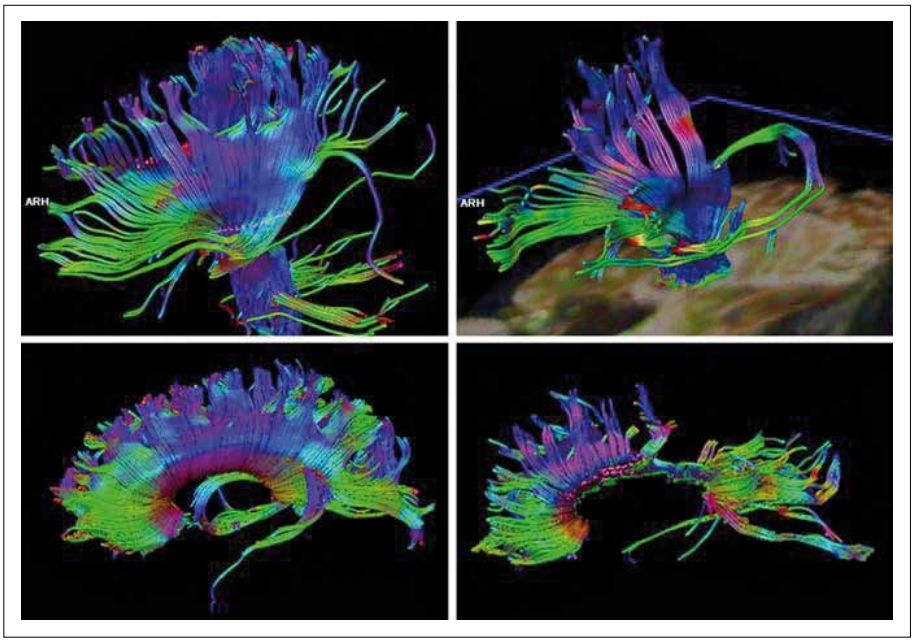

Fig2: Clinical diffusion tensor imaging (DTI). MR tractography of a normal developing child (left column) and a child with severe quadriplegic cerebral palsy (right column). The seed was either placed in the brain stem (top row) or the corpus callosum (bottom row). The images clearly demonstrate a reduction of white matter tracts coming from the central region (Courtesy of Dr. Jacques Schneider, chief pediatric radiology UKBB).

Cerebral palsy

CP is the most common cause of motor disability in children (between 100 and 700 new cases per year in Switzerland) and is often accompanied by impairments in movement, coordination, balance and posture to varying degrees, including spastic and non-spastic forms. Clinical diffusion tensor imaging (DTI) studies have shown that the majority of children suffering from spastic CP present with severe white matter injuries and the importance of the myelin loss appears to correlate with the severity of motor impairments. Rodent models of neonatal HI – first described by Rice and Vanucci in 1981 – have been used for decades now. Specifically, a unilateral carotid ligation is performed at postnatal day 7 (P7) and followed by exposure to hypoxia in specific temperature controlled chambers. In such models, oligodendrocyte progenitor cells (OPC) – the initiators of the myelination processes – have shown to be particularly sensitive to the ischemic environment and rapidly undergo massive apoptosis in response to hypoxic stress. This has been postulated to lead to a depletion of the OPC pool and to subsequently hinder OPC maturation to myelinating oligodendrocytes, therefore impairing the endogenous repair potential[1].

Therapeutic relevance of stem cells for white matter regeneration

As very limited treatment options are available, neonatal HI and CP represent significant medical and socio-economic burden and new therapies that better address the pathophysiology of perinatal white matter damages are needed[2]. Our group demonstrated that endovascular injection of human embryonic stem cell-derived NPC could improve both sensory-motor and cognitive functions in a rat model of neonatal HI (manuscript in preparation). At the cellular level, imaging and histology demonstrated efficient homing of human NPC to the stroked hemisphere up to 30 days after cell injection. We specifically showed that NPC treatment stimulates endogenous OPC for their proliferation, maturation and subsequent myelination in major white matter structures including corpus callosum and striatum as early as 3 days post cell administration. Our data confirm that the increased performances observed on elevated plus maze and novel object recognition in NPC-transplanted animals are likely to rely on a beneficial crosstalk between transplanted human NPC and resident brain cells including OPC. This raises the major question of whether NPC interact directly with OPC or whether the abundant surrounding glia (microglia and astrocytes) act as important bystanders in this cellular crosstalk.

Exploring the role of microglia in NPC-mediated brain repair mechanisms

The role of microglia in brain homeostasis and CNS disorders is just starting to emerge[3]. Like their systemic

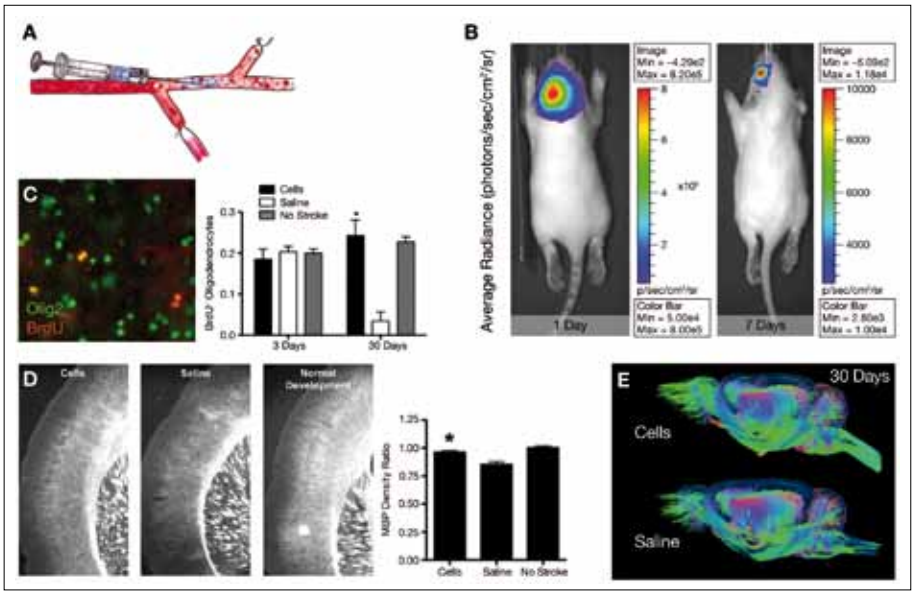

Fig3: Exogenous stem cell-treatment supports remyelination processes in the neonatal rat HI brain. (A) Schematic showing internal carotid artery NPC injection technique. (B) At 1 and 7 days post NPC injection, luciferase positive cells localized to the ischemic brain hemisphere. (C) NPC injection promoted endogenous oligodendrogenesis (Olig2-BrdU immunoreactivity) and (D) improved myelin regeneration at 30 days after injury. (E) This was confirmed using whole brain 3D magnetic resonance diffusion tensor imaging.

counterparts – the macrophages – microglia are professional phagocytes with a pivotal role in innate immunity and subsequent eradication of pathogens and cellular debris. In white matter damages, they might behave as a double-edged sword. On the one hand, they have demonstrated support of cellular regeneration and clearance of inhibitory products but on the other hand, they can also exacerbate inflammation and precipitate cellular damage. We have recently demonstrated that transplanted NPC could modulate endogenous microglial proliferation, activation and phagocytic properties through their specific secretion of vascular-endothelial growth factor (VEGF) in the healthy rodent brain[4]. We are currently investigating these mutual interactions in the context of neonatal HI.

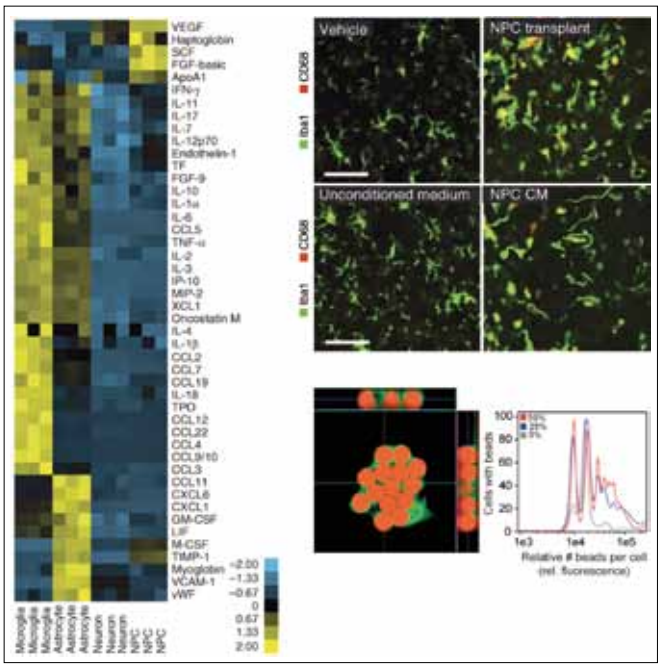

Fig4: NPC regulate microglia functions and activity. (Left panel) Heat map of the secretory profiles of murine NPC, neurons, astrocytes and microglia based on unsupervised clustering of immunoassay measurements of the listed proteins. Yellow shades represent increased expression of proteins relative to other cells types; blue shades, decreased. (Upper right panel) Mice receiving intrastriatal transplants of NPC or NPC-conditioned media (NPC CM) show significant increases in the numbers of Iba-1⁺ CD68⁺ microglia. (Lower right panel) Following treatment with NPC CM, phagocytosis of fluorescent latex beads by BV2, a microglia cell line, is increased. Increase in relative fluorescence within individual cells corresponds to a greater number of engulfed beads per cell as depicted by histograms.

In the course of healthy development and during disease, it remains unclear to which extent microglia support neurogenesis processes. Recent data point to a distinct spatio-temporal activation pattern of microglia in the post-natal rat brain. When such activation patterns are inhibited, significantly reduced neurogenesis could be observed[5]. We believe that this tightly regulated microglial activation is essential for postnatal brain development, and when targeted by neonatal brain injury, can lead to long-lasting neurodevelopmental defects. This is a major research axis of the BRIR group.

Neuro-immune crosstalk in other neurodevelopmental disorders

In order to model mechanisms of neuro-immune interactions in neurodevelopmental disorders, our group

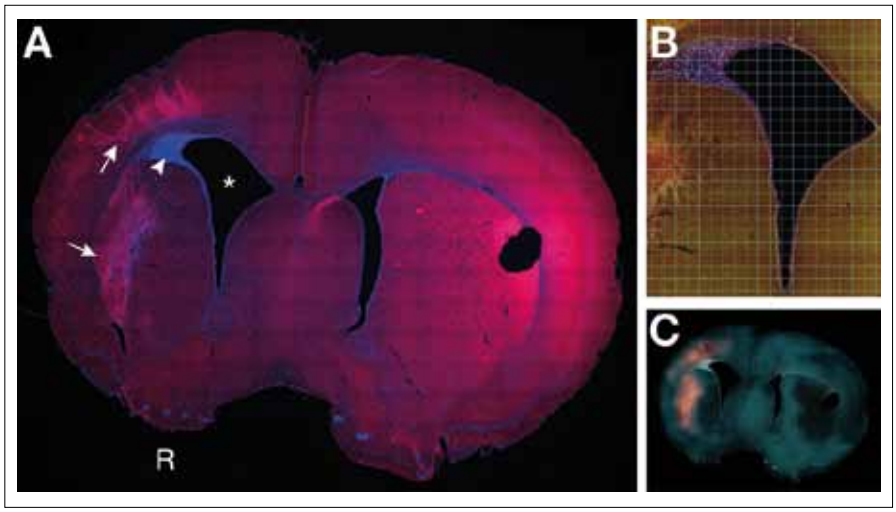

Fig. 5: Microglia accumulation accompanies neurogenic reaction to HI injury in the developing brain. (A) Coronal reconstructed brain section 23 days after injury shows typical features of right-sided (R) injury such as tissue loss with secondary lateral ventricle expansion (asterisk) and accumulation of Iba1⁺ microglia/infiltrating macrophages (arrows). Note that subventricular zone (R) is clearly enlarged at the injured site, likely due to increased neurogenesis (arrowhead). (B) Close-up of subventricular zone (R), showing an increase in activated Iba1⁺/CD68⁺ microglia/macrophages. (C) ImageJ-computed heat map displaying dense population of phagocytic Iba1⁺/CD68⁺ microglia/infiltrating macrophages in the injured R hemisphere.

also has a running project on the use of mouse embryonic stem cells (ESC) and human induced pluripotent stem cells (iPSC) to model the pathogenic contribution of glia in Rett's Syndrome (RTT). RTT is a rare devastating neurodevelopmental disorder, primarily affecting girls with a prevalence of 1 every 10 000 births. This disorder is mainly associated with a loss-of-function mutation in the X-linked *MeCP2* gene encoding methyl-CpG-binding protein 2, a master epigenetic regulator. How *MeCP2* deficiency specifically causes neurological deficits in the growing child is not well understood. However, while RTT has long been viewed as a neuronal lineage-restricted disorder, critical roles for glia including astrocytes, microglia and oligodendrocytes, are just being revealed[6]. In the *MeCP2*-null mouse, experimental evidence suggests that a focal increased release of glutamate in the vicinity of synapses by microglia[7], likely accompanied by an alteration in astrocyte-mediated glutamate clearance, contributes to dendritic spine and synaptic defects. Alterations in the oligodendroglial lineage have also been reported, but specific crosstalk between glial cells in the initiation and progression of the disease has not been systematically investigated.

Taking advantage of *MeCP2* knockout mouse ESC (collaboration with Jacky Guy, University of Edinburgh) and RTT patient-derived human iPSC (collaboration with Alysson Muotri, University of San Diego), we are currently investigating their mesodermal differentiation toward microglia. Generated cells are being thoroughly characterized and compared to their wild-type counterparts, and co-cultures will be performed to model cellular interactions in the context of RTT. Through modeling Rett's Syndrome with pluripotent cells, we should have direct access to the most primitive stages of neuronal and microglial lineages, of particular relevance when studying the impact of immune cells on neurodevelopmental disorders.

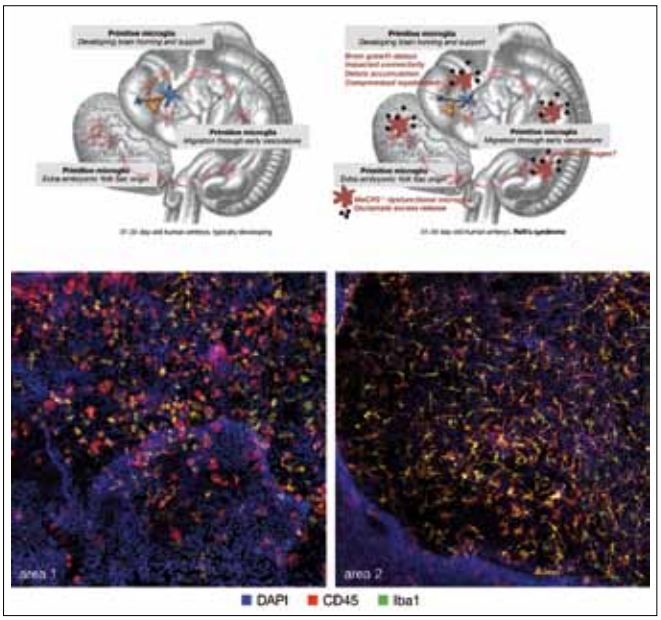

Fig6: Modelling Rett's Syndrome with pluripotent stem cells (Upper panels) Early migration of primitive microglia from the yolk sac to the developing brain might support neurodevelopment in typically developing individuals (upper left). In Rett's syndrome, microglia dysfunction and increased glutamate secretion might affect brain development, including myelination and connectivity, and precipitate the establishment of the disease (upper right). (Lower panel) Proof-of-concept in vitro differentiation of mouse embryonic stem cells (Day 80) toward microglia phenotype with either amoeboid (left) or ramified (right) typical morphology.

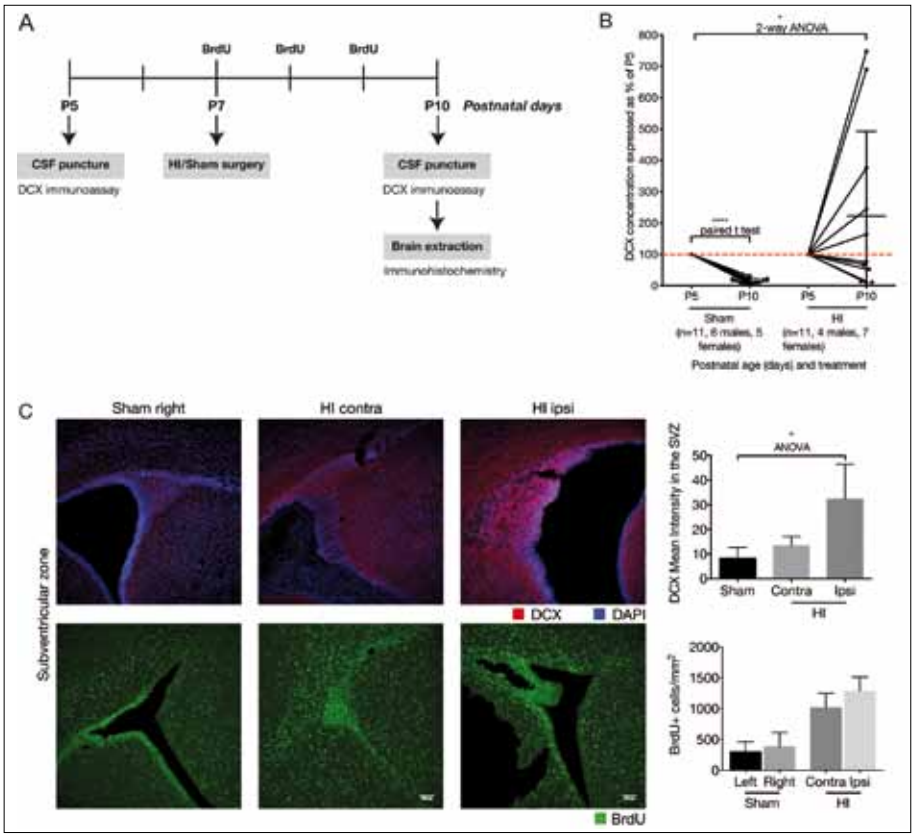

Fig7: The rat model of neonatal hypoxia-ischemia to search for biomarkers of neurogenesis in the CSF-Proof of principle with doublecortin as a candidate biomarker. (A) Scheme showing the experimental timeline. Briefly, neonates underwent sham or HI surgery at P7. CSF from the cisterna magna was punctured before and after surgery, i.e. at P5 and P10. BrdU was injected intraperitoneally at P7, P8 and P9 to label dividing cells. Animals were sacrificed at P10, and brains were collected for immunohistochemistry. (B) Doublecortin (DCX) concentration in the CSF after a sham or HI surgery. Between P5 and P10, the CSF concentration of DCX decreases in sham-exposed animals. In contrast, it increases sharply in HI-injured neonates. (C) Doublecortin/DAPI and BrdU representative immunostainings in the subventricular zone (SVZ) of sham and HI-injured brains 3 days after surgery. The injured side (ipsilateral to the ligation) of the brain from HI-exposed neonates shows enhanced neurogenesis and proliferation in comparison to sham animals, as depicted in the corresponding graphs, i.e. DCX immunointensity and number of BrdU positive cells, respectively.

In search for biomarkers of neurogenesis in neonatal HI injury

There is an unmet clinical need to monitor *in vivo* the endogenous neuroregenerative capacity of the brain including neurogenesis and oligodendrogenesis. For our group, this is of particular relevance in the context of developmental brain disorders. Biomarkers of neurogenesis may prove valuable for diagnostic, prognostic and therapeutic purposes, e.g. to predict or monitor the response to a specific treatment. Neurogenesis is known to persist throughout life in two discrete brain areas – the subventricular zone (SVZ) and the dentate gyrus (DG) of the hippocampus – and to increase in the brain of rodents after neonatal HI injury. Doublecortin (DCX) is a microtubule-associated protein, primarily expressed by immature migrating neuroblasts in a manner closely reflecting neurogenesis. Despite its intracellular localization, DCX can be detected in the cerebrospinal fluid (CSF), likely through release of a C-terminus fragment into the interstitial fluid. Roche has developed a highly specific and sensitive immunoassay to measure such fragment in CSF samples[8]. Using this assay, we quantified DCX release in the CSF of rats before and after neonatal HI injury. While DCX concentration in the CSF (CSF-DCX) showed a clear developmentally regulated decrease between P5 and P10 in sham animals, we found a significant increase in CSF-DCX in animals receiving HI at P7. Moreover, positive correlation between CSF-DCX levels, stroke severity and BrdU/DCX double positivity in SVZ and DG immunohistochemistry could be observed. Our data suggest that levels of DCX in the CSF might reflect the neuronal precursor biosynthesis responses occurring in the rodent brain following an HI insult. Thus DCX in the CSF appears to be a valid *in vivo* biomarker to track neurogenesis (Bregere et al, manuscript in preparation).

Raphael Guzman and team

From left to right: Catherine Brégère, Bernd Schwendele, Tanja Dittmar, Raphael Guzman, Laurie Chicha, Urs Fisch, Stephan Moser (missing on the photo: Pia Bustos)

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Life Sciences Training Facility: Focus on genome-wide expression

The Life Sciences Training Facility (LSTF) of the University of Basel provides access to microarray and deep-sequencing technologies and contributes to the identification of novel molecular pathways in health and disease.

Until recently, researchers were only able to study single or just a few genes related to the biological question they were interested in. Novel genome-wide methods now allow an organism's entire genome to be studied and thereby pave the way towards new discoveries related to the regulation and function of genes. The LSTF provides researchers in Basel and beyond with a dedicated and user-friendly platform to perform their microarray and deep-sequencing experiments. By providing both types of technologies, the LSTF offers a broad panel of tools to get a complete, genome-wide picture of biological systems. The LSTF is well equipped to narrow the gap between genotype and trait.

A Core facility and a research laboratory

The LSTF was established in 2001 as a result of a joint effort led by the DBM and the Biozentrum. It is located on the 5th Floor of the Pharmazentrum (Klingelbergstrasse 50–70, Basel) and is headed by Prof. Andreas Papassotiropoulos, who is also the principal investigator of the Division of Molecular Neurosciences of the University of Basel. His group is dedicated to the identification of novel genetic pathways related to human memory and follows an ambitious integrative approach combining behavioural studies in humans, genetic assays, functional brain imaging and *C. elegans* biology. As a consequence, the Core facility is embedded in a stimulating environment in which experience is shared and intensive cross-talk between users is taking place.

Philippe Demougin is the lead technician and full time coordinator of the LSTF. He is responsible for experimental set up and implementation, for user teaching, and for the testing and optimization of new experimental procedures and tools. Kim-Dung Huynh is specialized on genome-wide level DNA analysis with Affymetrix 6.0 SNP Arrays. Dr. Vanja Vukojevic provides guidance for the design of methylation studies, for the use of the Biotage Pyrosequencer and related downstream statistical analysis.

Kim-Dung Huynh (left) and Dr. Vanja Vukojevic

Access to latest DNA microarray technology

DNA microarrays allow the expression levels of large numbers of genes to be measured simultaneously and for genome-wide genotyping. The LSTF uses the Affymetrix microarray technology to conduct projects in various organisms ranging from worms to humans. These projects have led to the identification of novel genes and molecules, and confirm the notion that there is a lot to be discovered and understood in the context of gene expression and gene regulation.

Affymetrix technology is based on the use of microarrays encapsulated in a protective cartridge (so-called "Genechips"). The arrays are highly reliable due to the way they are manufactured (based on photolithography) and thanks to the automated instrumentation used for all relevant steps: hybridization, washing, staining, scanning, data quality control and basic data analysis. The last generation of expression arrays (Human, Mouse, Rat Transcriptome Arrays 2.0) are the highest

resolution microarrays ever built for gene expression profiling. They offer a comprehensive coverage of more than 245,000 coding and more than 40,000 non-coding transcripts. They are particularly suited for exon-level analysis and experiments related to alternative splicing.

The Affymetrix system at the LSTF
3 Fluidics Stations (left), Scanner 3000 7G with autoloader (right)

Affymetrix microarrays at the LSTF

New directions: Deep-sequencing

The LSTF moved an important step forward and now also provides support for deep sequencing. With this technology it is possible to study the complete sequence of an organism's genome, or selected regions thereof, quickly and at accessible prices. Deep sequencing bears an enormous potential for new discoveries in biological and biomedical research and can also be used for diagnostic purposes.

Within the context of deep sequencing, the LSTF focuses on RNA studies. We provide support for the preparation of libraries starting from total RNA which undergo 2 possible fates:

1. Purification of polyA+ RNA : the procedure starts with a capture of poly-adenylated RNA molecules using Oligo-dT coated magnetic beads. The purified fraction encompasses coding RNA (messenger RNAs typically interrogated in transcriptomic studies) as well as non-coding polyA+ RNA. We use the TruSeq Stranded mRNA kit (Illumina) to ensure the best quality and perfect compatibility with sequencers from Illumina used afterwards.
2. Depletion of ribosomal RNA (rRNA) : The procedure starts by removing both cytoplasmic and mitochondrial ribosomal RNA. The resulting whole transcriptome sequencing (also called Total RNA-Seq) captures a broader range of gene expression changes and enables the detection of novel transcripts in both coding and non-coding RNA species. This process minimizes ribosomal contamination and optimizes the percentage of reads covering RNA species of interest. Cost is higher and data analysis more challenging but this strategy bears a huge potential for the analysis of your samples. We use the TruSeq Stranded Total RNA with Ribo-Zero Gold Kit (Illumina).

In addition to gene expression changes, RNA sequencing can provide information about alternative transcripts, alternative splicing, gene fusions, allele-specific expression and novel transcripts.

RNA-seq versus Microarrays

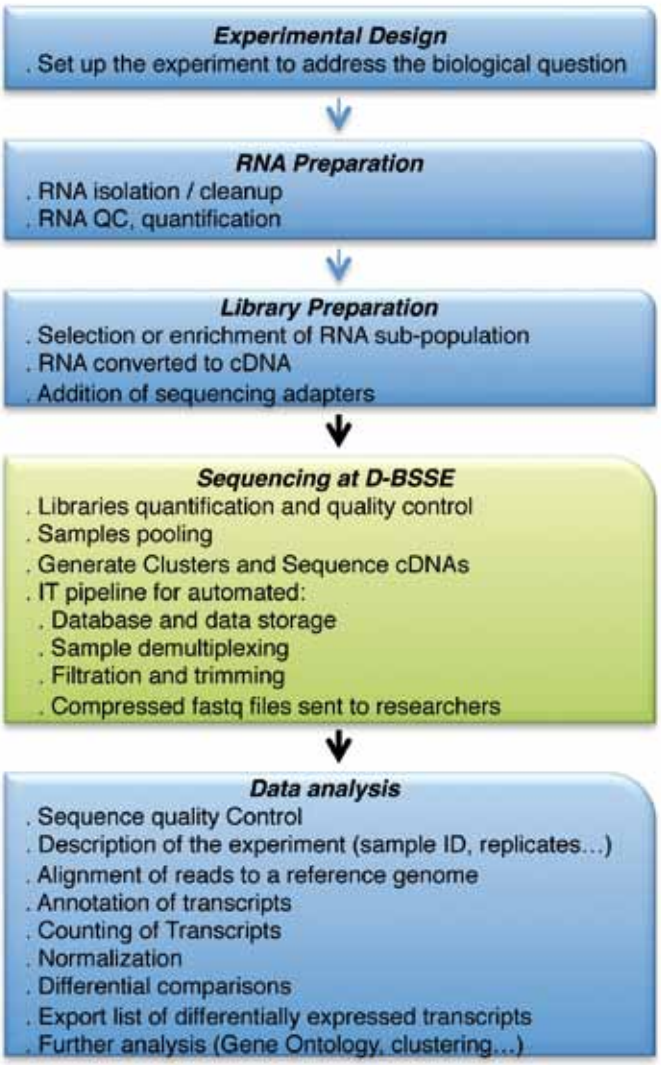
Next-generation sequencing is rapidly becoming the method of choice for transcriptional profiling experiments. In contrast to microarray technology, high throughput sequencing allows the identification of novel transcripts. High throughput sequencing circumvents background noise associated with fluorescence quantification. Furthermore, unlike hybridization-based detection, RNA-seq allows genome-wide analysis of transcription at single nucleotide resolution.

On the other hand microarrays offer the possibility of making gene expression studies more affordable than ever. Signal normalization and data analysis benefit from close to 20 years of development, offering the possibility of obtaining meaningful information related to changes in Gene Expression at the genome-wide level

(coding- as well as non-coding RNA) in a rapid and accessible manner. More complex microarrays, such as the Affymetrix Transcriptome Arrays provide the coverage and accuracy required to accurately detect all known transcript isoforms of a gene at affordable cost. In any case, we strongly encourage researchers to make an appointment and discuss their biological question, the proper experimental design required, all of the technical steps necessary to ensure RNA quality and ultimately to decide on the most suited technology.

LSTF and Genomics Facility Basel

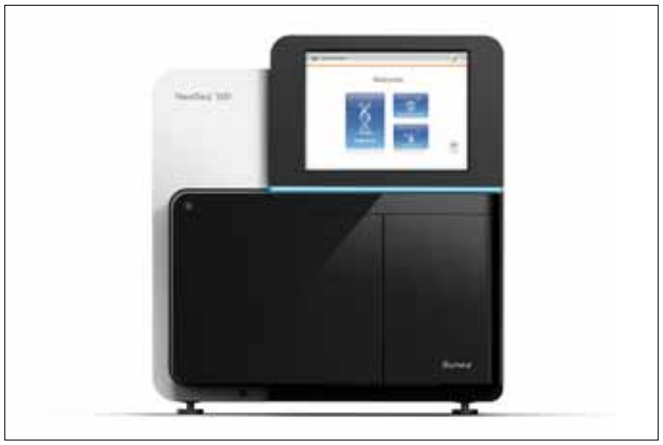
Dr. Christian Beisel is the head of the "Genomics Facility Basel" (located at D-BSSE, Mattenstrasse 26, Basel). This facility results from a joint effort of the D-BSSE of ETH Zurich and the University of Basel, providing guidance and bench work for performing any kind of experiment based on the use of Next Generation Sequencing. The group of Dr. Beisel has expertise in various domains including Chromatin-ImmunoPrecipitation followed by sequencing (ChIP-Seq), Whole-Exome Sequencing and state-of-the-art applications such as single cell gene expression profiling. The LSTF is closely cooperating with the "Genomics Facility Basel". Primarily for RNA-Seq experiments, students and researchers of the University of Basel have the possibility to perform their experiments at the LSTF. Libraries are then passed to the Genomics Facility Basel for further processing. Researchers have then access to 2 workstations available at the LSTF for basic data analysis.



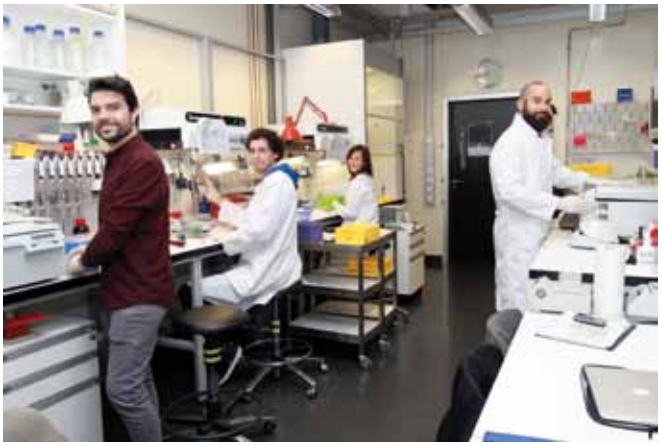
Typical steps of an RNA-Seq experiment. In blue: steps performed in the LSTF, in green: step performed in the Genomics Facility Basel

Workstation : PC with 32 cores, 128 GB RAM

From left to right:
Katja Eschbach, Manuel Kohler, Ina Nissen (Genomics Facility Basel), Philippe Demougin (LSTF), Dr. Christian Beisel (Genomics Facility Basel)

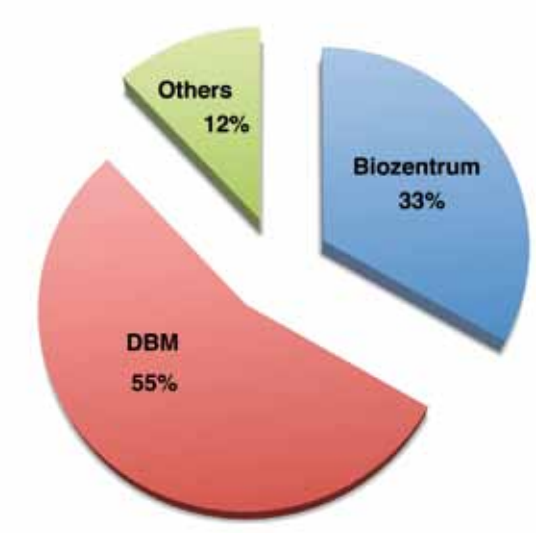


The NextSeq 500 (above) is especially suited for expression studies. The Genomics Facility Basel also owns a HiSeq 2000, a HiSeq 2500, a MiSeq, two cBot instruments as well as a complete Fluidigm system, an amazing technology for performing up to 10,000 simultaneous real-time PCR assays in parallel, even on single-cell level when including the C1 automated single-cell auto prep system.



November 2014, main benches of the LSTF
Dr. Hugo Gante (left) and Nicolas Boileau (right) from the group of Prof. Walter Salzburger, Zoological Insitute
Dr. Reto Kohler (middle left) form the group Gyn. Research, DBM
Lisa Traunmüller (middle) from the group of Prof. Peter Scheiffele, Biozentrum

The LSTF has helped more than 260 different students and researchers over the past 13 years by providing guidance, teaching and regular access to the facility.



LSTF user's affiliations in 2013 and 2014

Current developments

The LSTF runs several projects on the implementation of new procedures related to genome-wide RNA studies. We are currently implementing a safe and standardized way of profiling the level of microRNA expression by deep-sequencing in coordination with Prof. Michaela Zavolan (Biozentrum) and Dr. Christian Beisel (Genomics Facility Basel).

Latest LSTF publications include:

Hadziselimovic et al. Forgetting is regulated via Musashi-mediated translational control of the Arp2/3 complex. ***Cell.*** 2014; 156(6): 1153–66.

Heck et al. Converging genetic and functional brain imaging evidence links neuronal excitability to working memory, psychiatric disease, and brain activity. ***Neuron.*** 2014; 81(5): 1203–13.

Papassotiropoulos et al. Human genome-guided identification of memory-modulating drugs. ***PNAS.*** 2013; 110(46): E4369–74.

Conclusion

The LSTF is dedicated to helping researchers of the University of Basel perform genome-wide gene expression studies (either based on Affymetrix microarrays or Illumina Deep-sequencing). The facility is an ideal training and implementation place, in which researchers learn new methods while performing their own experiments.

We are looking forward to helping you with your project!

Andreas Papassotiropoulos and Philippe Demougin

Dissertationen

Am 17. Oktober 2014 konnte **Lisa Andelfinger** von der Forschungsgruppe Synaptic Plasticity (Departement Biomedizin Klingelbergstrasse) ihre Dissertation mit Erfolg beenden. Sie widmete sich in ihrer Dissertation dem Thema "Modulation of $GABA_B$ receptor signaling by associated proteins and phosphorylation".

Seit dem 14. November 2014 darf sich **Hélène Mereau** von der Forschungsgruppe Childhood Leukemia (Departement Biomedizin Hebelstrasse) Frau Dr. nennen. Sie befasste sich in ihrer Doktorarbeit mit dem Thema: "Targeting the MLL complex in acute leukemia".

Auszeichnungen

Posterpreis an Iris Spörri

Iris Spörri von der Forschungsgruppe Dermatology (Departement Biomedizin Hebelstrasse) hat am Jahrestreffen der ESDR (European Society for Dermatological Research) in Kopenhagen für ihr Poster "Disease Spectrum of Ichthyosis with Confetti" einen Preis gewonnen. Das Preisgeld beträgt 500.– CHF.

Das DBM gratuliert ganz herzlich!

Weihnachtsmarkt am Kölner Dom

The Editorial Team of DBM Facts wishes all its readers a Merry Christmas and a Happy New Year!

Science Translational Medicine | Science Translational Medicine | 2014, Vol 6 Issue 251 251ra119 IF 14,414

Adult human neural crest–derived cells for articular cartilage repair

Karoliina Pelttari[1], Benjamin Pippenger[1], Marcus Mumme[1], Sandra Feliciano[1], Celeste Scotti[2], Pierre Mainil-Varlet[3], Alfredo Procino[4], Brigitte von Rechenberg[5], Thomas Schwamborn[6], Marcel Jakob[1], Clemente Cillo[4], Andrea Barbero[1], Ivan Martin[1]

In embryonic models and stem cell systems, mesenchymal cells derived from the neuroectoderm can be distinguished from mesoderm-derived cells by their Hox-negative profile—a phenotype associated with enhanced capacity of tissue regeneration. We investigated whether developmental origin and Hox negativity correlated with self-renewal and environmental plasticity also in differentiated cells from adults. Using hyaline cartilage as amodel, we showed that adult human neuroectoderm-derived nasal chondrocytes (NCs) can be constitutively distinguished from mesoderm-derived articular chondrocytes (ACs) by lack of expression of specific *HOX* genes, including *HOXC4* and *HOXD8*.In contrast to ACs,seriallycloned NCs could be continuouslyreverted from differentiated to dedifferentiated states, conserving the ability to form cartilage tissue in vitro and in vivo. NCs could also be reprogrammed to stably express Hox genes typical of ACs upon implantation into goat articular cartilage defects, directly contributing to cartilage repair. Our findings identify previously unrecognized regenerative properties of *HOX*-negative differentiated neuroectoderm cells in adults, implying a role for NCs in the unmet clinical challenge of articular cartilage repair. An ongoing phase 1 clinical trial preliminarily indicated the safety and feasibility of autologous NC–based engineered tissues for the treatment oftraumatic articular cartilage lesions.

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The Journal of Experimental Medicine | JEM | 2014 Vol. 211 No. 11 2213–2230 IF 13,912

Myeloproliferative neoplasms can be initiated from a single hematopoietic stem cell expressing *JAK2*–V617F

Pontus Lundberg[1,*], Hitoshi Takizawa[2,*], Lucia Kubovcakova[1,*], Guoji Guo[3], Hui Hao-Shen[1], Stephan Dirnhofer[4], Stuart H. Orkin[3], Markus G. Manz[2], and Radek C. Skoda[1]

The majority of patients with myeloproliferative neoplasms (MPNs) carry a somatic JAK2-V617F mutation. Because additional mutations can precede JAK2-V617F, it is questioned whether JAK2-V617F alone can initiate MPN. Several mouse models have demonstrated that JAK2-V617F can cause MPN; however, in all these models disease was polyclonal. Conversely, cancer initiates at the single cell level, but attempts to recapitulate single-cell disease initiation in mice have thus far failed. We demonstrate by limiting dilution and single-cell transplantations that MPN disease, manifesting either as erythrocytosis or thrombocytosis, can be initiated clonally from a single cell carrying JAK2-V617F. However, only a subset of mice reconstituted from single hematopoietic stem cells (HSCs) displayed MPN phenotype. Expression of JAK2-V617F in HSCs promoted cell division and increased DNA damage. Higher JAK2-V617F expression correlated with a short-term HSC signature and increased myeloid bias in single-cell gene expression analyses. Lower JAK2-V617F expression in progenitor and stem cells was associated with the capacity to stably engraft in secondary recipients. Furthermore, long-term repopulating capacity was also present in a compartment with intermediate expression levels of lineage markers. Our studies demonstrate that MPN can be initiated from a single HSC and illustrate that JAK2-V617F has complex effects on HSC biology.

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[*] P. Lundberg, H. Takizawa, and L. Kubovcakova contributed equally to this paper.

Nature Chemical Biology | nature chemical biology | 2014 | DOI: 10.1038/NCHEMBIO.1650 | IF 13,217

A semisynthetic carbohydrate-lipid vaccine that protects against *S. pneumoniae* in mice

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Severe forms of pneumococcal meningitis, bacteraemia and pneumonia result in more than 1 million deaths each year despite the widespread introduction of carbohydrate-protein conjugate vaccines against *Streptococcus pneumoniae*. Here we describe a new and highly efficient antipneumococcal vaccine design based on synthetic conjugation of S. pneumoniae capsule polysaccharides to the potent lipid antigen α-galactosylceramide, which stimulates invariant natural killer T (iNKT) cells when presented by the nonpolymorphic antigen-presenting molecule CD1d. Mice injected with the new lipid-carbohydrate conjugate vaccine produced high-affinity IgG antibodies specific for pneumococcal polysaccharides. Vaccination stimulated germinal center formation; accumulation of iNKT cells with a T follicular helper cell phenotype; and increased frequency of carbohydrate-specific, longlived memory B cells and plasmablasts. This new lipid-carbohydrate vaccination strategy induced potent antipolysaccharide immunity that protected against pneumococcal disease in mice and may also prove effective for the design of carbohydratebased vaccines against other major bacterial pathogens.

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* These authors contributed equally to this work.

Developmental Cell | Developmental Cell | 31, 345–357, November 10, 2014 | IF 10,366

HAND2 Targets Define a Network of Transcriptional Regulators that Compartmentalize the Early Limb Bud Mesenchyme

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Summary

The genetic networks that govern vertebrate development are well studied, but how the interactions of trans-acting factors with cis-regulatory modules (CRMs) are integrated into spatiotemporal regulation of gene expression is not clear. The transcriptional regulator HAND2 is required during limb, heart, and branchial arch development. Here, we identify the genomic regions enriched in HAND2 chromatin complexes from mouse embryos and limb buds. Then we analyze the HAND2 target CRMs in the genomic landscapes encoding transcriptional regulators required in early limb buds. HAND2 controls the expression of genes functioning in the proximal limb bud and orchestrates the establishment of anterior and posterior polarity of the nascent limb bud mesenchyme by impacting *Gli3* and *Tbx3* expression. TBX3 is required downstream of HAND2 to refine the posterior *Gli3* expression boundary. Our analysis uncovers the transcriptional circuits that function in establishing distinct mesenchymal compartments downstream of HAND2 and upstream of SHH signaling.

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Developmental Cell | Developmental Cell | 30, 701–716, September 29, 2014 | IF 10,366

A Growth Factor-Induced, Spatially Organizing Cytoskeletal Module Enables Rapid and Persistent Fibroblast Migration

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Summary

Directional migration requires robust front/back polarity. We find that fibroblasts treated with plateletderived growth factor (PDGF) and prepolarized by plating on a fibronectin line substrate exhibit persistent migration for hours. This does not occur in the absence of PDGF or on uniformly coated fibronectin substrates. Persistent migration arises from establishment of two functional modules at cell front and back. At the front, formation of a zone containing podosome-like structures (PLS) dynamically correlates with low RhoA and myosin activity and absence of a contractile lamella. At the back, myosin contractility specifically controls tail retraction with minimal crosstalk to the front module. The PLS zone is maintained in a dynamic steady state that preserves size and position relative to the cell front, allowing for long-term coordination of front and back modules. We propose that front/back uncoupling achieved by the PLS zone is crucial for persistent migration in the absence of directional cues.

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Activated group 3 innate lymphoid cells promote T-cell–mediated immune responses

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Group 3 innate lymphoid cells (ILC3s) have emerged as important cellular players in tissue repair and innate immunity. Whether these cells meaningfully regulate adaptive immune responses upon activation has yet to be explored. Here we show that upon IL-1β stimulation, peripheral ILC3s become activated, secrete cytokines, up-regulate surface MHC class II molecules, and express costimulatory molecules. ILC3s can take up latex beads, process protein antigen, and consequently prime $CD4^+$ T-cell responses in vitro. The cognate interaction of ILC3s and $CD4^+$ T cells leads to T-cell proliferation both in vitro and in vivo, whereas its disruption impairs specific T-cell and T-dependent B-cell responses in vivo. In addition, the ILC3–$CD4^+$ T-cell interaction is bidirectional and leads to the activation of ILC3s. Taken together, our data reveal a novel activation-dependent function of peripheral ILC3s in eliciting cognate $CD4^+$ T-cell immune responses.

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Specific Glycosylation of Membrane Proteins in Epithelial Ovarian Cancer Cell Lines: Glycan Structures Reflect Gene Expression and DNA Methylation Status

Merrina Anugraham[1,*], Francis Jacob[2,3,*], Sheri Nixdorf[3], Arun Vijay Everest-Dass[1], Viola Heinzelmann-Schwarz[2,3], and Nicolle H. Packer[1]

Epithelial ovarian cancer is the fifth most common cause of cancer in women worldwide bearing the highest mortality rate among all gynecological cancers. Cell membrane glycans mediate various cellular processes such as cell signaling and become altered during carcinogenesis. The extent to which glycosylation changes are influenced by aberrant regulation of gene expression is nearly unknown for ovarian cancer and remains crucial in understanding the development and progression of this disease. To address this effect, we analyzed the membrane glycosylation of non-cancerous ovarian surface epithelial (HOSE 6.3 and HOSE 17.1) and serous ovarian cancer cell lines (SKOV 3, IGROV1, A2780, and OVCAR 3), the most common histotype among epithelial ovarian cancers. *N*-glycans were released from membrane glycoproteins by PNG*ase* F and analyzed using nano-liquid chromatography on porous graphitized carbon and negative-ion electrospray ionization mass spectrometry (ESI-MS). Glycan structures were characterized based on their molecular masses and tandem MS fragmentation patterns. We identified characteristic glycan features that were unique to the ovarian cancer membrane proteins, namely the "bisecting *N*-acetyl-glucosamine" type *N*-glycans, increased levels of α 2–6 sialylated *N*-glycans and "*N,N'*-diacetyllactosamine" type *N*-glycans. These *N*-glycan changes were verified by examining gene transcript levels of the enzymes specific for their synthesis (*MGAT3, ST6GAL1,* and *B4GALNT3*) using qRT-PCR. We further evaluated the potential epigenetic influence on *MGAT3* expression by treating the cell lines with 5-azacytidine, a DNA methylation inhibitor. For the first time, we provide evidence that *MGAT3* expression may be epigenetically regulated by DNA hypomethylation, leading to the synthesis of the unique "bisecting GlcNAc" type *N*-glycans on the membrane proteins of ovarian cancer cells. Linking the observation of specific *N*-glycan substructures and their complex association with epigenetic programming of their associated synthetic enzymes in ovarian cancer could potentially be used for the development of novel anti-glycan drug targets and clinical diagnostic tools.

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Potent co-operation between the NUP98-NSD1 fusion and the *FLT3*-ITD mutation in acute myeloid leukemia induction

Angeliki Thanasopoulou[1], Alexandar Tzankov[2], and Juerg Schwaller[1]

The NUP98-NSD1 fusion, product of the t(5;11)(q35;p15.5) chromosomal translocation, is one of the most prevalent genetic alterations in cytogenetically normal pediatric acute myeloid leukemias and is associated with poor prognosis. Co-existence of an FLT3-ITD activating mutation has been found in more than 70% of NUP98-NSD1-positive patients. To address functional synergism, we determined the transforming potential of retrovirally expressed NUP98-NSD1 and FLT3-ITD in the mouse. Expression of NUP98-NSD1 provided mouse strain-dependent, aberrant self-renewal potential to bone marrow progenitor cells. Co-expression of FLT3-ITD increased proliferation and maintained self-renewal *in vitro*. Transplantation of immortalized progenitors co-expressing NUP98-NSD1 and FLT3-ITD into mice resulted in acute myeloid leukemia after a short latency. In contrast, neither NUP98-NSD1 nor FLT3-ITD single transduced cells were able to initiate leukemia. Interestingly, as reported for patients carrying NUP98-NSD1, an increased *Flt3-ITD* to wild-type *Flt3* mRNA expression ratio with increased FLT3-signaling was associated with rapidly induced disease. In contrast, there was no difference in the expression levels of the NUP98-NSD1 fusion or its proposed targets *HoxA5, HoxA7, HoxA9* or *HoxA10* between animals with different latencies to develop disease. Finally, leukemic cells co-expressing NUP98-NSD1 and FLT3-ITD were very sensitive to a small molecule FLT3 inhibitor, which underlines the significance of aberrant FLT3 signaling for NUP98-NSD1-positive leukemias and suggests new therapeutic approaches that could potentially improve patient outcome.

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Pharmacological characterization of $GABA_B$ receptor subtypes assembled with auxiliary KCTD subunits

Mathieu Rajalu*, Thorsten Fritzius*, Lisa Adelfinger, Valerie Jacquier, Valerie Besseyrias, Martin Gassmann, Bernhard Bettler

Abstract

$GABA_B$ receptors ($GABA_BRs$) are considered promising drug targets for the treatment of mental health disorders. $GABA_BRs$ are obligate heteromers of principal $GABA_{B1}$ and $GABA_{B2}$ subunits. $GABA_BRs$ can additionally associate with auxiliary KCTD8, 12, 12b and 16 subunits, which also bind the G-protein and differentially regulate G-protein signaling. It is unknown whether the KCTDs allosterically influence pharmacological properties of $GABA_BRs$. Here we show that KCTD8 and KCTD16 slightly but significantly increase GABA affinity at recombinant receptors. However, KCTDs clearly do not account for the 10-fold higher GABA affinity of native compared to recombinant $GABA_BRs$. The positive allosteric modulator (PAM) GS39783, which binds to $GABA_{B2}$, increases both potency and efficacy of GABA-mediated G-protein activation ($[^{35}S]GTP\gamma S$ binding, BRET between G-protein subunits), irrespective of whether KCTDs are present or not. Of note, the increase in efficacy was significantly larger in the presence of KCTD8, which likely is the consequence of a reduced tonic G-protein activation in the combined presence of KCTD8 and $GABA_BRs$. We recorded Kir3 currents to study the effects of GS39783 on receptor-activated G-protein $\beta\gamma$-signaling. In transfected CHO cells and cultured hippocampal neurons GS39783 increased Kir3 current amplitudes activated by 1 μM of baclofen in the absence and presence of KCTDs. Our data show that auxiliary KCTD subunits exert marginal allosteric influences on principal $GABA_BR$ subunits. PAMs at principal subunits will therefore not be selective for receptor subtypes owing to KCTD subunits. However, PAMs can differentially modulate the responses of receptor subtypes because the KCTDs differentially regulate G-protein signaling.

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The glycosphingolipid P_1 is an ovarian cancer-associated carbohydrate antigen involved in migration

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Background: The level of plasma-derived naturally circulating anti-glycan antibodies (AGA) to P1 trisaccharide has previously been shown to significantly discriminate between ovarian cancer patients and healthy women. Here we aim to identify the Ig class that causes this discrimination, to identify on cancer cells the corresponding P_1 antigen recognised by circulating anti-P_1 antibodies and to shed light into the possible function of this glycosphingolipid.

Methods: An independent Australian cohort was assessed for the presence of anti-P1 IgG and IgM class antibodies using suspension array. Monoclonal and human derived anti-glycan antibodies were verified using three independent glycan-based immunoassays and flow cytometry-based inhibition assay. The P_1 antigen was detected by LC-MS/MS and flow cytometry. FACS-sorted cell lines were studied on the cellular migration by colorimetric assay and real-time measurement using xCELLigence system.

Results: Here we show in a second independent cohort ($n = 155$) that the discrimination of cancer patients is mediated by the IgM class of anti-P_1 antibodies ($P = 0.0002$). The presence of corresponding antigen P_1 and structurally related epitopes in fresh tissue specimens and cultured cancer cells is demonstrated. We further link the antibody and antigen (P_1) by showing that human naturally circulating and affinity-purified anti-P_1 IgM isolated from patients ascites can bind to naturally expressed P_1 on the cell surface of ovarian cancer cells. Cell-sorted IGROV1 was used to obtain two study subpopulations (P_1-high, 66.1%; and P_1-low, 33.3%) and observed that cells expressing high P_1-levels migrate significantly faster than those with low P_1-levels.

Conclusions: This is the first report showing that P_1 antigen, known to be expressed on erythrocytes only, is also present on ovarian cancer cells. This suggests that P_1 is a novel tumour-associated carbohydrate antigen recognised by the immune system in patients and may have a role in cell migration. The clinical value of our data may be both diagnostic and prognostic; patients with low anti-P_1 IgM antibodies present with a more aggressive phenotype and earlier relapse.

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Biochemical Pharmacology 91 (2014) 369–379 IF 4,650

$GABA_B$ receptor phosphorylation regulates KCTD12-induced K^+ current desensitization

Lisa Adelfinger[a,*], Rostislav Turecek[a,b,*], Klara Ivankova[a], Anders A. Jensen[a], Stephen J. Moss[c], Martin Gassmann[a], Bernhard Bettler[a]

Abstract

$GABA_B$ receptors assemble from $GABA_{B1}$ and $GABA_{B2}$ subunits. $GABA_{B2}$ additionally associates with auxiliary KCTD subunits (named after their K^+ channel tetramerization-domain). $GABA_B$ receptors couple to heterotrimeric G-proteins and activate inwardly-rectifying K^+ channels through the $\beta\gamma$ subunits released from the G-protein. Receptor-activated K^+ currents desensitize in the sustained presence of agonist to avoid excessive effects on neuronal activity. Desensitization of K^+ currents integrates distinct mechanistic underpinnings. $GABA_B$ receptor activity reduces protein kinase-A activity, which reduces phosphorylation of serine-892 in $GABA_{B2}$ and promotes receptor degradation. This form of desensitization operates on the time scale of several minutes to hours. A faster form of desensitization is induced by the auxiliary subunit KCTD12, which interferes with channel activation by binding to the G-protein $\beta\gamma$ subunits. Here we show that the two mechanisms of desensitization influence each other. Serine-892 phosphorylation in heterologous cells rearranges KCTD12 at the receptor and slows KCTD12-induced desensitization. Likewise, protein kinase-A activation in hippocampal neurons slows fast desensitization of $GABA_B$ receptor-activated K^+ currents while protein kinase-A inhibition accelerates fast desensitization. Protein kinase-A fails to regulate fast desensitization in KCTD12 knock-out mice or knock-in mice with a serine-892 to alanine mutation, thus demonstrating that serine-892 phosphorylation regulates KCTD12-induced desensitization in vivo. Fast current desensitization is accelerated in hippocampal neurons carrying the serine-892 to alanine mutation, showing that tonic serine-892 phosphorylation normally limits KCTD12-induced desensitization. Tonic serine-892 phosphorylation is in turn promoted by assembly of receptors with KCTD12. This crossregulation of serine-892 phosphorylation and KCTD12 activity sharpens the response during repeated receptor activation.

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Eur J Nutr Springer (2014) 53:1313–1325 IF 3,840

Effect of carnitine, acetyl-, and propionylcarnitine supplementation on the body carnitine pool, skeletal muscle composition, and physical performance in mice

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Abstract

Purpose Pharmacokinetics and effects on skeletal muscle and physical performance of oral acetylcarnitine and propionylcarnitine are not well characterized. We therefore investigated the influence of oral acetylcarnitine, propionylcarnitine, and carnitine on body carnitine homeostasis, energy metabolism, and physical performance in mice and compared the findings to non-supplemented control animals.

Methods Mice were supplemented orally with 2 mmol/ kg/day carnitine, acetylcarnitine, or propionylcarnitine for 4 weeks and studied either at rest or after exhaustive exercise.

Results In the supplemented groups, total plasma and urine carnitine concentrations were significantly higher than in the control group receiving no carnitine, whereas the skeletal muscle carnitine content remained unchanged. The supplemented acylcarnitines were hydrolyzed in intestine and liver and reached the systemic circulation as carnitine. Bioavailability of carnitine and acylcarnitines, determined as the urinary excretion of total carnitine, was in the range of 19 %. Skeletal muscle morphology, including fiber-type composition, was not affected, and oxygen consumption by soleus or gastrocnemius fibers was not different between the groups. Supplementation with carnitine or acylcarnitines had no significant impact on the running capacity, but was associated with lower plasma lactate levels and a higher glycogen content in white skeletal muscle after exhaustive exercise.

Conclusions Oral supplementation of carnitine, acetylcarnitine, or propionylcarnitine in mice is associated with increased plasma and urine total carnitine concentrations, but does not affect the skeletal muscle carnitine content. Despite better preservation of skeletal muscle glycogen and lower plasma lactate levels, physical performance was not improved by carnitine or acylcarnitine supplementation.

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Cytomegalovirus Serology and Replication Remain Associated With Solid Organ Graft Rejection and Graft Loss in the Era of Prophylactic Treatment

Martin Stern[1], Hans Hirsch[2,3], Alexia Cusini[4], Christian van Delden[5], Oriol Manuel[6], Pascal Meylan[7], Katia Boggian[8], Nicolas J. Mueller[9], and Michael Dickenmann[10] and on behalf of all members of the Swiss Transplant Cohort Study

Background. Cytomegalovirus (CMV) replication has been associated with more risk for solid organ graft rejection. We wondered whether this association still holds when patients at risk receive prophylactic treatment for CMV.

Methods. We correlated CMV infection, biopsy-proven graft rejection, and graft loss in 1,414 patients receiving heart (n=97), kidney (n=917), liver (n=237), or lung (n=163) allografts reported to the Swiss Transplant Cohort Study.

Results. Recipients of all organs were at an increased risk for biopsy-proven graft rejection within 4 weeks after detection of CMV replication (hazard ratio [HR] after heart transplantation, 2.60; 95% confidence interval [CI], 1.34Y4.94, PG0.001; HR after kidney transplantation, 1.58; 95% CI, 1.16Y2.16, *P*=0.02; HR after liver transplantation, 2.21; 95% CI, 1.53Y3.17, *P*<0.001; HR after lung transplantation, 5.83; 95% CI, 3.12Y10.9, *P*<0.001. Relative hazards were comparable in patients with asymptomatic or symptomatic CMV infection. The CMV donor or recipient serological constellation also predicted the incidence of graft rejection after liver and lung transplantation, with significantly higher rates of rejection in transplants in which donor or recipient were CMV seropositive (non-D−/R−), compared with D− transplant or R− transplant (HR, 3.05; *P*=0.002 for liver and HR, 2.42; *P*=0.01 for lung transplants). Finally, graft loss occurred more frequently in non-D− or non-R− compared with D− transplant or R− transplant in all organs analyzed. Valganciclovir prophylactic treatment seemed to delay, but not prevent, graft loss in non-D− or non-R− transplants.

Conclusion. Cytomegalovirus replication and donor or recipient seroconstellation remains associated with graft rejection and graft loss in the era of prophylactic CMV treatment.

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Expansion of Human Mesenchymal Stromal Cells from Fresh Bone Marrow in a 3D Scaffold-Based System under Direct Perfusion

Adam Papadimitropoulos[1,*], Elia Piccinini[1,*], Sophie Brachat[2], Alessandra Braccini[1], David Wendt[1], Andrea Barbero[1], Carsten Jacobi[2], Ivan Martin[1]

Abstract

Mesenchymal stromal/stem cell (MSC) expansion in conventional monolayer culture on plastic dishes (2D) leads to progressive loss of functionality and thus challenges fundamental studies on the physiology of skeletal progenitors, as well as translational applications for cellular therapy and molecular medicine. Here we demonstrate that 2D MSC expansion can be entirely bypassed by culturing freshly isolated bone marrow nucleated cells within 3D porous scaffolds in a perfusionbased bioreactor system. The 3D-perfusion system generated a stromal tissue that could be enzymatically treated to yield CD45-MSC. As compared to 2D-expanded MSC (control), those derived from 3D-perfusion culture after the same time (3 weeks) or a similar extent of proliferation (7–8 doublings) better maintained their progenitor properties, as assessed by a 4.3-fold higher clonogenicity and the superior differentiation capacity towards all typical mesenchymal lineages. Transcriptomic analysis of MSC from 5 donors validated the robustness of the process and indicated a reduced inter-donor variability and a significant upregulation of multipotency-related gene clusters following 3D-perfusion-as compared to 2D-expansion. Interestingly, the differences in functionality and transcriptomics between MSC expanded in 2D or under 3D-perfusion were only partially captured by cytofluorimetric analysis using conventional surface markers. The described system offers a multidisciplinary approach to study how factors of a 3D engineered niche regulate MSC function and, by streamlining conventional labor-intensive processes, is prone to automation and scalability within closed bioreactor systems.

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Journal of Psychopharmacology | Journal of Psychopharmacology | DOI: 10.1177/0269881114542454 | IF 3,396

Differential effects of MDMA and methylphenidate on social cognition

Yasmin Schmid[1], Cédric M Hysek[1], Linda D Simmler[1], Molly J Crockett[2], Boris B Quednow[3] and Matthias E Liechti[1]

Abstract

Social cognition is important in everyday-life social interactions. The social cognitive effects of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') and methylphenidate (both used for neuroenhancement and as party drugs) are largely unknown. We investigated the acute effects of MDMA (75 mg), methylphenidate (40 mg) and placebo using the Facial Emotion Recognition Task, Multifaceted Empathy Test, Movie for the Assessment of Social Cognition, Social Value Orientation Test and the Moral Judgment Task in a cross-over study in 30 healthy subjects. Additionally, subjective, autonomic, pharmacokinetic, endocrine and adverse drug effects were measured. MDMA enhanced emotional empathy for positive emotionally charged situations in the MET and tended to reduce the recognition of sad faces in the Facial Emotion Recognition Task. MDMA had no effects on cognitive empathy in the Multifaceted Empathy Test or social cognitive inferences in the Movie for the Assessment of Social Cognition. MDMA produced subjective 'empathogenic' effects, such as drug liking, closeness to others, openness and trust. In contrast, methylphenidate lacked such subjective effects and did not alter emotional processing, empathy or mental perspective-taking. MDMA but not methylphenidate increased the plasma levels of oxytocin and prolactin. None of the drugs influenced moral judgment. Effects on emotion recognition and emotional empathy were evident at a low dose of MDMA and likely contribute to the popularity of the drug.

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Above you can find the abstracts of recent articles published by members of the DBM. The abstracts are grouped according to the impact factor of the journal where the work appeared. To be included, the papers must meet the following criteria:

1. The first author, last author or corresponding author (at least one of them) is a member of the DBM.
2. Department of Biomedicine and University of Basel affiliation must be mentioned in authors list as published by the journal.
3. The final version of the article must be available (online pre-publications will be included when the correct volume, page numbers etc. becomes available).

We are focussing on original publications. Due to page constraints, abstracts of publications that appeared in lower ranked journals may not be able to be included. Review articles are generally not considered, unless they appeared in the very top journals (e.g. Cell, Science, Nature, NEJM, etc.). The final decision concerning inclusion of an abstract will be made by the chair of the Department of Biomedicine.

If you wish that your article will appear in the next issue of DBM Facts please submit a pdf file to the Departmental Assistant, Manuela Bernasconi: manuela.bernasconi@unibas.ch

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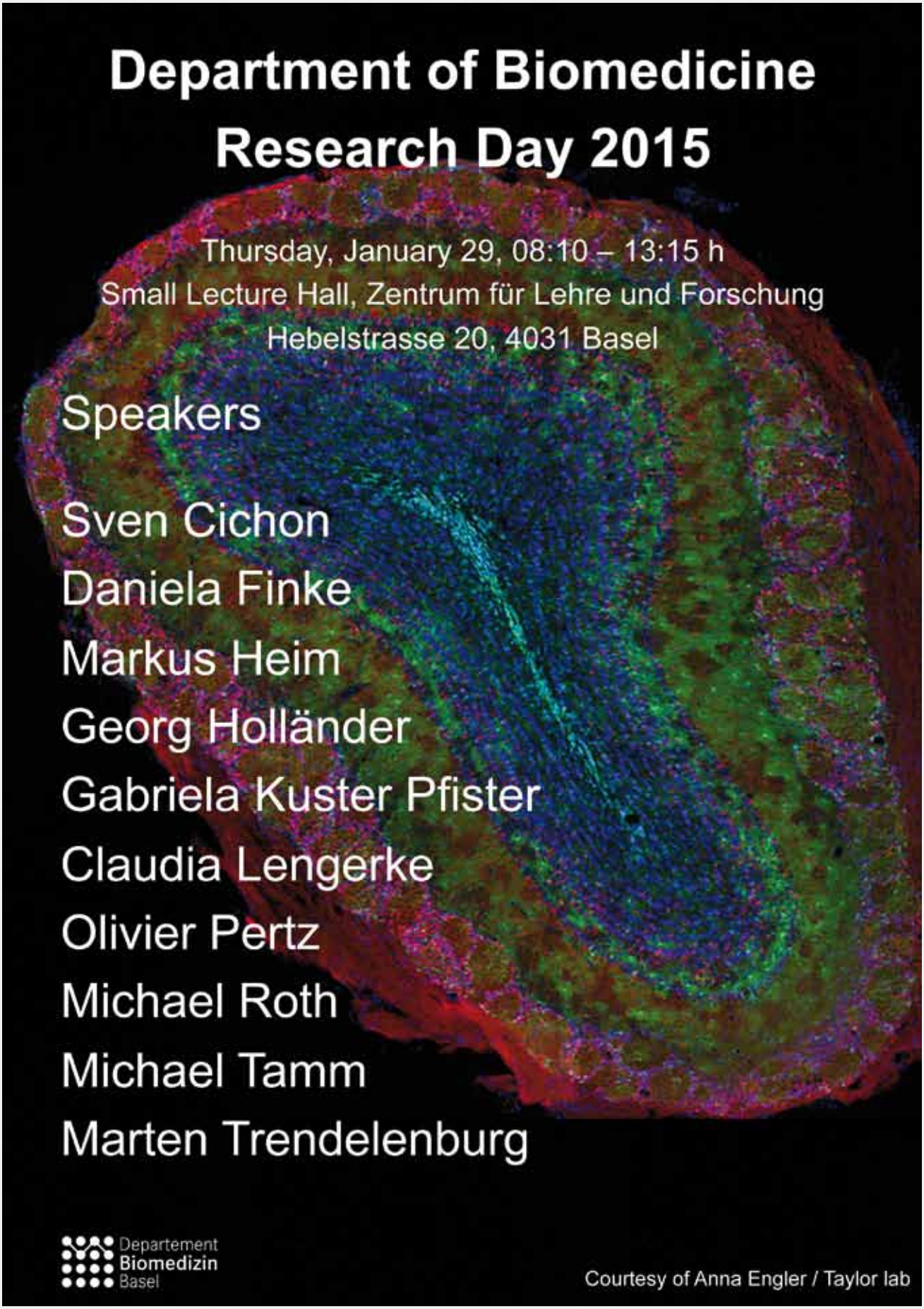
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Hebelstrasse 20, 4031 Basel
Speakers
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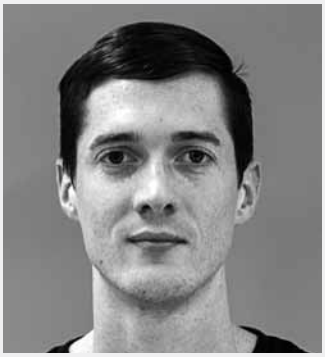

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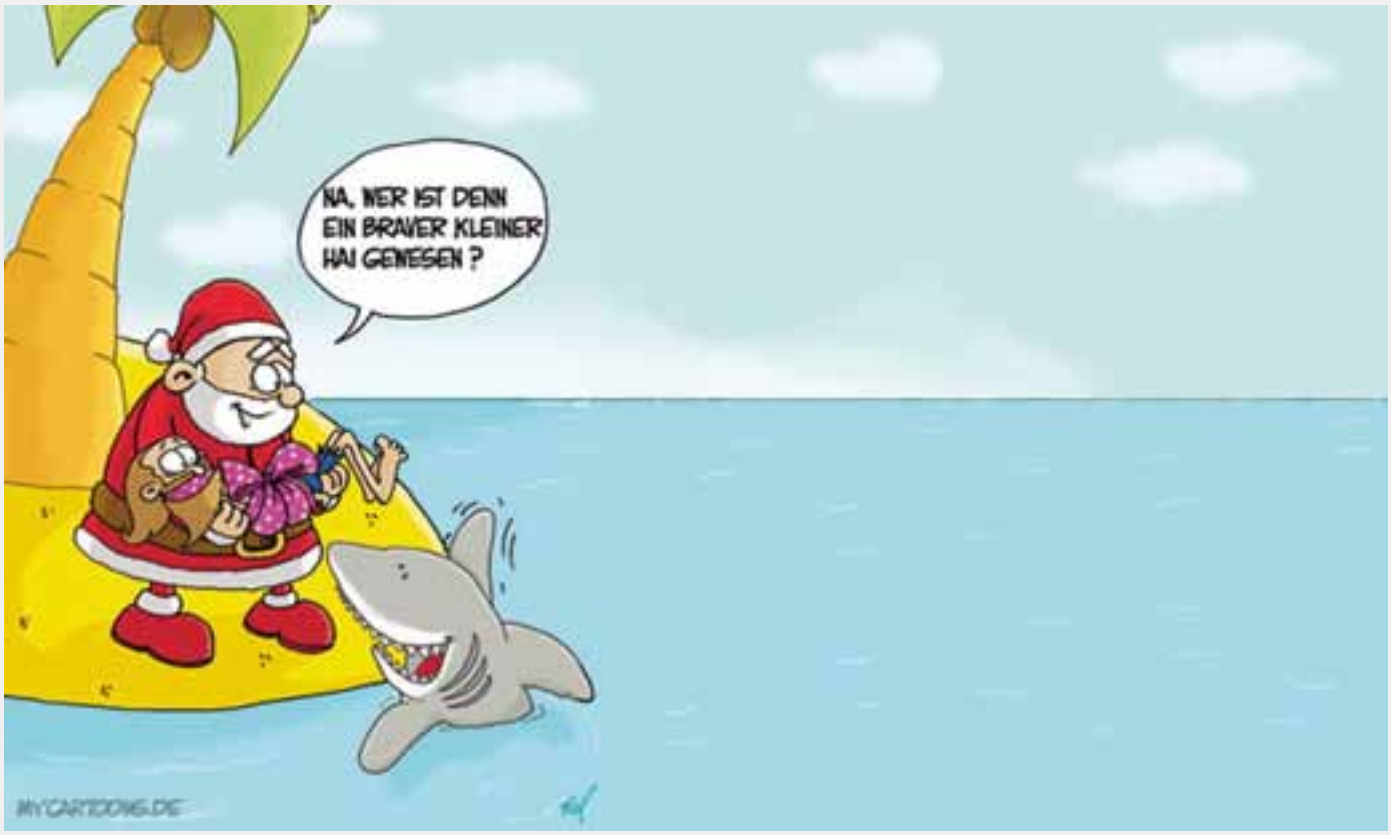


Congratulations

Das DBM gratuliert ganz herzlich!

Ben Patrick Gubser
Geboren am 05.11.2014

Azeglio Campana
Geboren am 30.10.2014

Herzlich willkommen, allerseits!

Vihaan Kampe
Geboren am 25.10.2014

Laura Contador Bustos
Geboren am 23.07.2014

christmas in ireland

Christmas in Ireland is very much a time for tradition and for family. The preparations begin in late November, or even earlier, with the making of the Christmas pudding and the Christmas cake. The former is steamed, the latter baked, but both are laden with dried fruit and require aging and feeding before they are at their best. The making of the pudding is quite a traditional as all members of the family, or at least those present in the house at the time of its making, each stir the pudding three times clockwise with a wooden spoon while making a wish. Once made both the cake and pudding are carefully stored and then usually fed weekly with a few spoonfuls of alcohol, usually whiskey, brandy or even poitín, a traditional Irish distilled spirit, that was essentially Irish moonshine as it was illegal until recent years. Both will keep for at least several months if properly stored and fed, and in our house it is usual for the puddings to be at least a year old before we use them. Both may be eaten for desert after the main Christmas day meal,

although it is more traditional for the pudding to be eaten then whereas the Christmas cake will be served up to guests or as a sweet snack at any time over the Christmas period. A few days before it is needed the cake will be finished with its Christmas decoration. It is first brushed with apricot jam then covered with a layer of marzipan and finally finished with a layer of royal or fondant icing and other decorations.

Christmas cakes

While in the past household decorations were often not put up until the family returned from midnight mass on Christmas Eve, nowadays the 8th of December represents the date on which it is traditional to put up the tree and start decorating. Trees, be they real or artificial, will be decorated with all sorts of Christmas baubles and strings of lights. Holly or Christmas wreaths

will be hung on the front door and a candle or candles will be placed in the front window or windows of the house in preparation for Christmas Eve. In our house it is also traditional for us to supplement the decorations with a sprig of holly placed over each painting in the house on Christmas Eve. The Christmas crib is also an integral part of traditional decoration and it too will be set up with the decorations, although the figures of the baby Jesus and the three kings will not be placed in the crib until the appropriate dates. Decorations come down on Little Christmas, January 6th. Leaving them up beyond that date is considered bad luck.

Christmas Eve sees the start of the Christmas festivities. For us it is a day to come together and start our celebrating. All of us who are home for Christmas get together for a leisurely lunch. Late afternoon sees us preparing for the first of the masses that we will attend. Although most will only attend one Christmas mass we tend to attend several, as my mother sings in the choir at one and my sisters play guitar and sing, either on their own or in a folk group, at two others. For us the first mass will be an early evening special childrens' mass. This is a relatively new idea but a wonderful one as it is aimed at even the smallest of children. It is a noisy and fun filled celebration of all that is important about Christmas and my children and all those that attend really enjoy its simplicity. In the past the first proper Christmas mass would have been at midnight on Christmas Eve, but this has generally moved to the earlier time of 8 or 9pm in most locations. It is one of the few times of the year nowadays that the church will be literally filled to capacity as everyone comes together to celebrate. After this "midnight" mass we will come home and the children will hang up their stockings for Santa, leave him out a drink and a mince pie along with a carrot for Rudolf, before excitedly heading off to be in anticipation of day ahead. I should mention that the mincemeat in the Christmas mince pies is not the ground beef you might think of when first you hear the term but rather a sweet mix of dried fruits, suet, spices and alcohol. We will also light

the candle in the window on this night and on each following night during the holiday season. This tradition actually stems from darker times when religion was suppressed in Ireland. At those times the candle was lit to indicate to any priests in the vicinity that they were welcome and that the house was open to them while those in power were told that the candles is to light the way for Mary and Joseph and let them know they are welcome in the house with open hearts and open doors. This was seen to be "Irish superstition" by those in charge at the time, but it is a "superstition" that has survived to this day.

Christmas day starts early as little children wake early and cannot wait to check what Santa has brought them and finally open the presents that have been waiting under the tree. Even in years when there were no little children in our house the magic of that morning was never diminished and

Christmas dinner

it was the one day of the year you did not mind getting up early. Everyone gathers together to first place the baby Jesus in the crib, in recognition of the true meaning of the day, before moving on to the tree, where gifts are swapped and opened and too many chocolates are undoubtedly eaten by at least one little person. We will eventually sit down to a large traditional Irish breakfast before heading out to mass once more, to join in the celebrations again and enjoy the music. The rest of the day is usually taken over with Christmas dinner. It is a veritable feast that takes hours to prepare and that is usually eaten mid-afternoon. The meal starts with the pulling of Christmas crackers, followed by the donning of paper crowns, telling of jokes and swapping of novelties from inside said crackers. Dinner menus vary but turkey or goose and ham often feature. We start with melon, sometimes followed by smoked salmon and homemade brown bread, and then a turkey broth with vegetable brunoise. After the light start we move on to the main event. There will be roast turkey, baked ham, potato croquettes, brussel sprouts, red cabbage and peas. There will also always be a bread stuffing served with the turkey as well as bread sauce and stewed apple; chutney and mustard go with the ham and there will always be mixed pickles and pickled onions on the side. Dinner is a leisurely affair and we will eventually finish with trifle and the pudding, which is served hot, flambéed with brandy, with whipped or pouring cream on the side.

The 26th of December is St. Stephen's Day and in Ireland this is the day the wren boys will be found, often with blackened faces and wearing old clothes, travelling from house to house playing music and singing. Historically it was thought that the robin, symbolising the New Year, would kill the wren, symbolising the Old Year, at this time of the year. It became tradition for groups of boys to hunt and kill a wren then travel from house to house to entertain the inhabitants while showing off their kill, collecting money for a town celebration, and perhaps gifting the house one of the bird's feathers for good luck. Although nowadays the wren boys are still to be found on their travels they do not carry out the tradition of killing a wren and any money they do collect is for charity or a local school. It is a tradition that is sadly slowly dying out as people become less tolerant of or less willing to let their children take part in such activities or are more interested in maybe attending the horse races that have become tradition at many of the main racecourses on that day or in getting to the first the post Christmas sales in search of the bargains to be had.

The Christmas season finally ends on Little Christmas, January 6th. This is the day on which the decorations must come down, if they haven't already. It is also known as "Nollaig na mBan" or "Women's Christmas" in some areas and is considered a day on which the women of the house get the day off to relax, meet up with their friends and the men must take over the chores and the cooking. Although this tradition was once widespread in the

country it has now become much more localised and the tradition of giving gifts to mothers and grandmothers on that day is similarly dying out.

I hope that you have enjoyed my glimpses into Christmas in Ireland. Before I go I would like to leave you with a tradition Irish greeting for the season ahead and share with you my favourite recipe for Christmas pudding.

Nollaig Shona daoibh go léir agus Athbhliain faoi shéan is faoi mhaise daoibh!

Wishing you all a very merry Christmas and a happy and prosperous New Year!

Paula Cullen

Christmas pudding (serves 8–10)

225g (8oz) each of raisins, sultanas and currants
1 carton (100g) of both chopped mixed peel and glacé cherries, halved
110g (4oz) each of dried figs, dried dates and blanched almonds chopped
½ medium cooking apple, peeled cored and coarsely grated
Finely grated rind of 1/2 lemon
110g (4oz) plain flour
110g (4oz) suet or butter
110g (4oz) brown sugar, light or dark
110g (4oz) breadcrumbs
About 1 rounded teaspoon each of cinnamon, nutmeg and ground cloves
3 large eggs
150ml (¼pt) stout
2–3 tablespoons whiskey
Juice of half lemon

Method

- Mix together all of the fruits.
- Rub the fat into the flour and mix in the breadcrumbs, sugar and spices. Add the fruit mix and stir well to combine.
- Whisk all of the wet ingredients together (eggs, stout, whiskey and lemon juice) then add the liquid mix to the dry ingredients and mix using a wooden spoon. At this point all of those present in the house at the time should stir the pudding, each person stirring the mix three times clockwise and making one wish as they do so.
- Pour the mixture into a 1.75l/3pt pyrex or other heatproof pudding bowl and top with a double layer of well greased baking parchment. Fold one pleat into the center of the baking parchment which will allow steam to escape as the pudding cooks. Tie the parchment in place with cooking twine.
- To cook, place the prepared pudding into a steamer, over a saucepan of gently boiling water. Cover the steamer with a lid. Or, if you prefer, the pudding can be cooked in a bowl which is standing on a trivet in a saucepan of gently boiling water. The water should come two-thirds of the way up the sides of the bowl. Cover the saucepan with a loose-fitting lid placed at a slight angle. Constantly check the level of water and keep topping it up when necessary with more boiling water. The pudding should be cooked for 5–6 hours.
- Once cool remove the parchment and replace with clingfilm or an airtight lid. The pudding should keep in a cool dark place for several months, or longer. From time to time pour a spoonful of whiskey over the pudding to keep it moist. To reheat the pudding can be steamed for an hour before being turned out and flambéed with some brandy to serve, or alternatively slices of the pudding can be lightly fried in a little butter to heat through.

Insights into Ice hockey

The summer ends and the days are getting shorter. The leaves begin to fall, the temperature decreases and the first snowflakes lay a white carpet on the ground. Finally, the season of one of the most amazing sports starts: Ice hockey! A sport that contains an action-loaded packet of patience, speed, precision, tactics, filigree technique, power, and roughness covered in emotions. Many people may think that ice hockey is brutal. Of course it is a sport, where full contact is a part of it and many players have lost more than one teeth, experienced concussions, and other injuries, but ice hockey is much more. So why to play ice hockey, when 100 kg heavy players are going to hit you at a speed of over 30 km/h and hard rubber pucks whoosh by at an average speed of 150km/h? It is not easy to formulate this answer in words. Hence, let me try to describe you very briefly how it is as a player.

The day of a game is always more special. Hours before the game, you are a bit nervous and every minute, ice hockey determines your thoughts, while everything else is turning more and more into minor matter. The moment you enter the rink and you see the freshly prepared and reflecting ice, you just want to get out there and play. However, first you need to prepare. You enter the locker room, which has its own smell that is mixed of old sweat and different creams. Ice hockey players do not recognise the smell anymore, but if you never tasted it before, it may be a bit harsh. Next to this “smell of hockey” you meet your teammates. This is the first aspect of ice hockey that makes it such an amazing sport, the friendship. Probably, all of you that play a team sport know what I am talking about. You make jokes, tease each other and unsurprisingly talk about hockey. As we are exclusively men, we tend to exaggerate slightly and boast how many hits we will make, how often we will score and what amazing moves we will show. Of course, we also think that the astonishing moves of the players from the NHL (National hockey league, which is the North American hockey league) are easy and that we will show them how to do it properly. The atmosphere in the locker room is very relaxed and you know what ever happens out on the glacier, these guys are there and will help you with all their power. Generally, you feel acceptance and respect.

Since ice hockey is a rough sport, you wear protectors and if you ever saw a filled hockey bag, you know that this is quite large. However, this misleads as you can have changed within 10 minutes. Each player has its own changing ritual, as we all tend to be superstitious, at least for ice hockey. The changing mostly starts with the cup, followed by the socks holder, pant, skates, shine guards, shoulder and elbow pads. Then the shirt, gloves, and helmet are put on. Finally, you grab the gumshield and the stick.

Then the team goes out on the ice for a 15 minutes warm up. The warm up starts with a few rounds around the field and active muscle warm up. You feel the fresh air in the face, hear the cracking of the ice and sense how the blade claw the ice. Next to the contact of the skates with the ice, the first contact with the puck shows you the behaviour of the ice, which is generally important to get a good handling for away games and your personal feeling. Motivated and ready to enter the battle, you return to the locker room, while the ice is cleaned. During this time in higher leagues, there is absolute silence, so

each player can concentrate on his job and prepare mentally for the game. In addition, the coach gives the last important inputs for the game. The tension, which is a mixture of jumpiness, aggression, relaxations, and concentration increases and you are completely ready for the game, willing to give everything for a success of the team.

Finally, the first five field players of each team position for the start. This is the most magnifying moment, where the personal tension is the highest. As defensemen, as I am, you look at "your" attack of the opponent team, ready to stop him. Then the puck drops, you hear the sound of slashing sticks of the centres (the players in the middle, where the puck is dropped). The opponent has the puck and is speeding up to you. You back up with speed as well, focus on him and when the moment comes you stand up, leaning all your weight forward and stretch your muscles. You hit the attack, depending on power, you can actually feel how his chest backs down a bit. The adrenalin shoots through your body, accompanied by the satisfactory feeling that nobody will just pass you. You are the boss! You speed back to get the puck. Within a few seconds, you need to see where your teammate is located to play a pass, because there is someone speeding up to prevent this. You feel each contact of the hit, the compression and slight backing down of the body. Yes it hurts a bit, but the adrenalin gives you this indescribable feeling and when you recognised that you are not made up of glass, the feeling of self-consciousness and being alive increases dramatically. You are an unbreakable mountain! However, there is not much time and the next pass is played. You feel the resistance of the puck in each finger. You speed as fast as you can while you read the game. An opponent focused on you but you are ready for the one on one. At a maximum speed, you move your body fast to provoke a mistake of the defensemen. By the precise control of the puck, you move the puck in one direction while moving your body in the other. Then you play the puck around him and you hunker down and pass the opponent, while he is still trying to push you aside or nail you to the wall. However, your speed and technique prevented you from the danger of a hit and allowed you to overtake him and pass to your teammate. Nobody can stop you! Another nice pass to the next player and a shot. All this within a few seconds.

From time to time, there are some "disagreements" of two players, mainly after an unfair hit or when the goalkeeper is attacked. You can imagine that when two men on ice that are willing to give everything and are loaded with self-consciousness and adrenalin, this ends in some handful arguments. In biology, this is comparable to a fight that is defining who is going to be the alpha male. Something, mostly the mothers do not understand (and will let you know after the game). After the game, you feel completely exhausted and all stress is gone. The tension decreases and relaxation takes over. Afterwards you have the satisfactory feeling of exhaustion and that you had just a lot of fun out there with great blokes.

This is ice hockey – this is fascination! You skate at maximum speed, while your upper body moves independently and your stick precisely controls the puck, all without losing balance and keeping up reading the game. All this makes it a dynamic sport that is not only amazing to watch, but also to play. Just a little hint, for those who have sometimes problems to catch up with the puck when watching: Do not only look at the player with the puck, but also at the other ones and with time, you will see that you can anticipate the next pass. Best would be to go to the rink, grab some skates, a stick and puck and just get the hands on, to get really the feeling how it is to stand on ice sensing the fresh air, ice and puck. Let's go!

Pascal Rem

Raunächte

Zwischen den Jahren … Weihnachten ist vorbei, Neujahr steht bald vor der Tür. Für viele von uns eine geschenkte Zeit, in der wir Freunde einladen, das Jahr Revue passieren lassen und uns auf das nächste vorbereiten. Die Nächte, die dahinter stehen, sind den wenigsten bekannt, die Raunächte. Meist handelt es sich um die zwölf Nächte vom 21. auf den 22. Dezember, die sogenannte Thomasnacht, die längste Nacht des Jahres, die Wintersonnenwende, die Thomas, dem Ungläubigen zugedacht wird, bis zum 5. auf den 6. Januar, die Vigil von Epiphanie, der Erscheinung des Herrn. In unserer Zeit sind sie christlich überlagert. Der Brauch geht jedoch auf die Zeit zurück, als man noch in Mondjahren rechnete. Da ein Mondjahr bei zwölf Monaten nur 354 Tage umfasst, wurden die für ein Sonnenjahr fehlenden 11 Tage oder 12 Nächte als tote Tage, als Tage ausserhalb der Zeit, eingeschoben.

In der Mythologie werden an solchen Tagen die Gesetze der Natur ausser Kraft gesetzt und die Grenzen zu anderen Welten fallen. Mythische und magische Rituale haben sich in vorchristlicher Zeit entwickelt und sind bis heute erhalten: Die Seelen der Verstorbenen haben Ausgang, Geister ziehen mit der wilden Jagd über das Land. So wird an manchen Orten im Alpenraum nicht nur an Silvester, sondern in allen Nächten geböllert, um die Unholde fernzuhalten.

Auch das Befragen von Orakeln war ein häufiger Brauch, den wir heute noch an Silvester mit dem Bleigiessen aufleben lassen. Tiere im Stall sollen um Mitternacht mancher Raunächte die menschliche Sprache sprechen und über die Zukunft erzählen oder sich bei einem Hausgeist über ihren Herrn beschweren können. Hat er sie schlecht behandelt, so wird er bestraft.
Mancherorts galten die Nächte als so gefährlich, dass man sie mit Fasten und Beten beging. Das Haus musste ordentlich sein, es durfte keine weisse Wäsche auf der Leine hängen, damit sie nicht von den Reitern der wilden Jagd im nächsten Jahr als Leichentuch für den Besitzer verwendet werden konnte. Wäscheleinen durften erst gar nicht gespannt werden, damit sich die wilde Jagd nicht in ihnen verfangen konnte. Frauen und Kinder durften in der Dunkelheit das Haus nicht mehr verlassen.

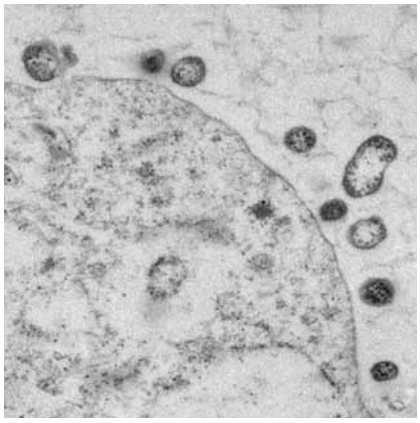
Nicht viele von uns erleben heute noch die nächtliche Einsamkeit im Winter, die mühseligen Lebensbedingungen und das geheimnisvolle Treiben der Naturkräfte, die sich in den düsteren Geschichten widerspiegeln. Doch damals wie heute ist diese Zeit etwas ganz Besonderes.

Heidi Hoyermann

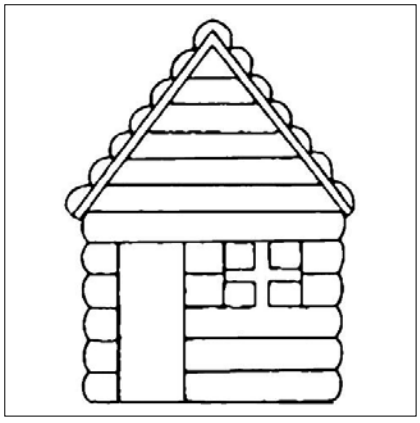
Josef Fruth: Haussegen der Raunacht

VORSCHAU PREVIEW

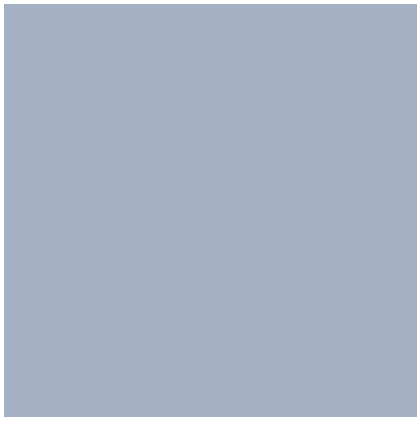
In der nächsten Ausgabe . . .



. . . erfahren wir von Daniel Pinschewer, womit sich seine Forschungsgruppe Experimental Virology beschäftigt



. . . lernen wir mit Felicity Wollseifen die internationale Wohnvermittlung des USB kennen



. . . erleben wir ein Sportereignis der besonderen Art

. . . entdecken wir mit Melanie Neutzner, wie viel Freude ein eigener Gemüsegarten bereitet

. . . heissen wir den Frühling willkommen

Weihnachten – Nächte, die Tage sind.

Erhard Horst Bellermann