DBM FACTS

Periodisches Informationsblatt des Departementes Biomedizin
Universität Basel, Universitätsspital Basel und
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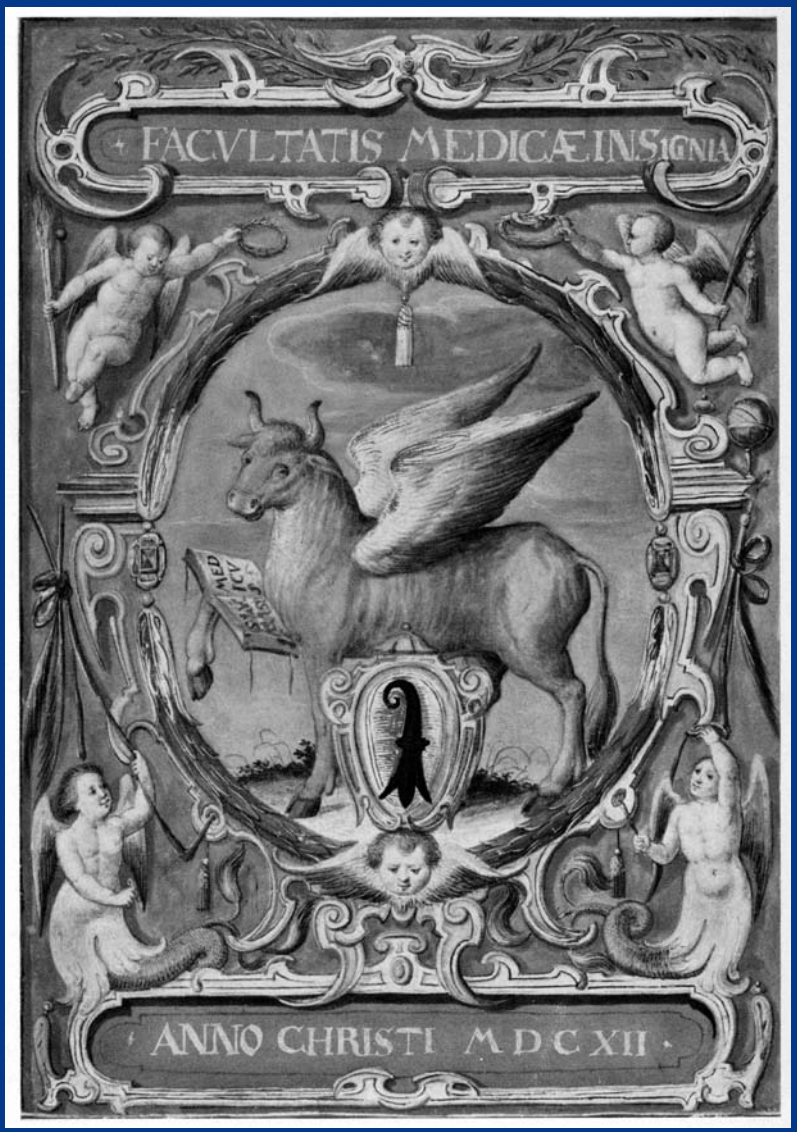

The mouse limb bud: a paradigm model to study the signalling networks that orchestrate organogenesis | Research Group "Gynecological Endocrinology" | My "Fun" days in ICFS-TE

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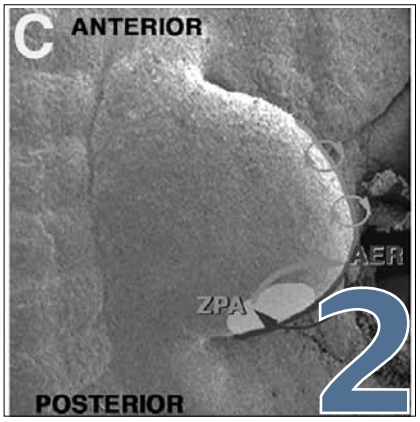
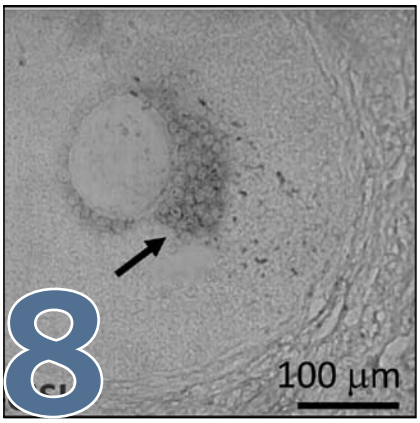


The mouse limb bud: a paradigm model to study the signalling networks that orchestrate organogenesis
from Rolf Zeller



Research Group "Gynecological Endocrinology"
from Christian De Geyter

My «Fun» days in ICFS-TE
from Chitrangada Acharya

Second DBM Summer Event at Sulzkopf in Muttenz

Mit Phyllobates terribilis auf Du und Du
von Peter Zimmermann

IMPRESSUM

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Titelminiatur des 1612 begonnenen zweiten Bandes der medizinischen Matrikel mit dem Wappen der Fakultät, dem geflügelten Stier ihres ursprünglichen Schutzpatrons, des Evangelisten Lukas.
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EDITORIAL

Radek Skoda
Leiter DBM

Liebe Leserinnen und Leser

Endlich ist er da, der Frühling! Und mit ihm die neueste Ausgabe der DBM Facts. Arbeitsreiche Wochen liegen hinter dem DBM, die Research Days mit Besuch des Scientific Advisory Boards haben stattgefunden, mit vielen positiven Anregungen und Rückmeldungen.

Hier einige erfreuliche Meldungen: Ed Palmer durfte als erster Vertreter einer medizinischen Fakultät den renommierten, mit 3 Millionen CHF dotierten "Advanced Investigator Grant" vom European Research Council (ERC) entgegen nehmen. Herzliche Gratulation! Martin Stern hat eine SNF-Förderprofessur erhalten, wozu wir ihm herzlich gratulieren. Er wird über die Erkennung von Tumorzellen durch die "Natural Killer Cells" forschen. Die Universität hat neu eine Tierärztin, Frau Bettina Oswald, eingestellt, die sich um die Tierversuchsstationen der Universität, inklusive des DBM kümmern wird.

Das DBM hat per Ende 2009 Aleksandra Wodnar-Filipowicz verabschiedet, die sich über mehrere Jahrzehnte um das DF bzw. jetzt DBM sehr verdient gemacht hat. Auch in Zukunft wird sie als Koordinatorin für das "Basel Stem Cell Network" der Wissenschaft treu bleiben. Andrej Trampuz hat das DBM im letzten Jahr in Richtung Lausanne verlassen. Wir wünschen ihm einen guten Start und viel Erfolg an seinem neuen Arbeitsort!

In der nun vorliegenden Ausgabe erfahren Sie von Rolf Zeller mehr über die Forschungsaktivitäten des Labors "Developmental Genetics" und Christian De Geyter nimmt uns mit in die wissenschaftliche Welt der "gynäkologischen Endokrinologie". Eine Auswahl der neuesten Publikationen finden Sie ab Seite 18.
Und wie sich mit "phyllobates terribilis" leben lässt, erzählt uns Peter Zimmermann.

Frohe Ostern und einen schönen Frühling!
Radek Skoda

Dear Readers

Springtime has finally arrived! And with it the newest issue of DBM facts. Busy weeks lie behind the DBM – the Research Days took place and there were many positive feedbacks and suggestions from the visiting Scientific Advisory Board.

There are some happy announcements to make. The 3 million CHF "Advanced Investigator Grant" endowed by the European Research Council (ERC) was awarded for the first time to a representative of the medical faculty, Ed Palmer. Congratulations! We would also like to congratulate Martin Stern who obtained an SNF professorship; his research concerns the detection of tumor cells by "Natural Killer Cells". The University has employed a veterinarian, Mrs. Bettina Oswald, who will take care of the University animal facilities, including the ones housed in the DBM.

At the end of 2009, the DBM said goodbye to Aleksandra Wodnar-Filipowicz who was of great service to the DF, renamed DBM, over the past three decades. She will remain faithful to science in her new capacity as coordinator for the "Basel Stem Cell Network". Last year Andrej Trampuz left the DBM for Lausanne. We wish him a good start and much success at his new workplace.

In this issue you will learn more from Rolf Zeller about the research activities of the "Developmental Genetics" laboratory, and Christian De Geyter will take us to the scientific world of "gynaecological endocrinology". A selection of the latest publications can be found from page 18 onwards.

And Peter Zimmerman tells us about one can live with "phyllobates terribilis".

Happy Easter, Happy Spring!
Radek Skoda

The mouse limb bud: a paradigm model to study the signalling networks that orchestrate organogenesis

The Developmental Genetics research group, at the DBM Mattenstrasse, studies what may appear as a rather eclectic collection of research topics at first sight: we aim to understand how mouse limb bud cells send, receive and integrate signals to form the skeletal structures of the upper and lower extremities (arms and legs). To analyse mouse embryonic limb bud development, we employ a hard-core systems biology approach that combines cutting edge mouse molecular genetics with quantitative transcriptome analysis, biochemistry, mathematical simulations and modelling of the relevant interactions (the *in silico* limb – together with Prof. Dagmar Iber and colleagues from D-BSSE). The majority of all humans are born with five fingers and toes (digits), but the formation of additional fingers and/or toes (so-called polydactylies) is rather frequent (about 1 per 500–1000 newborns) and has fascinated humans from prehistoric times onwards (Fig. 1)[1]. One of Marc Chagall's famous self-portraits shows the painter with seven fingers (Fig. 1), but luckily, he only had five fingers, as any polydactyly results in loss of functionality. Pentadactyly (5 digits) of the human hand includes a highly developed thumb that allows us to perform a variety of sophisticated tasks, such as writing novels, painting, carving

Developmental Genetics Group: (from left to right) Simone Probst (PhD student, Basel), Emanuele Pignatti (PhD student, Italy), Javier Lopez-Rios (Postdoctoral fellow, Spain), Jean-Denis Benazet (Postdoctoral fellow, France), Marco Osterwalder (PhD student, Aargau), Alexandra Schauerte (Research assistant, Grisons), Dimitri Robay (Research assistant, France), Alexandre Goncalves (Postdoctoral fellow, Portugal), Rolf Zeller (Group leader, Switzerland), Chris Müller (Personal assistant, United Kingdom), Aimee Zuniga (Group leader, France), Catherine Vaillant (Postdoctoral fellow, France), Susi Boudebaba (Support services, France, not shown)

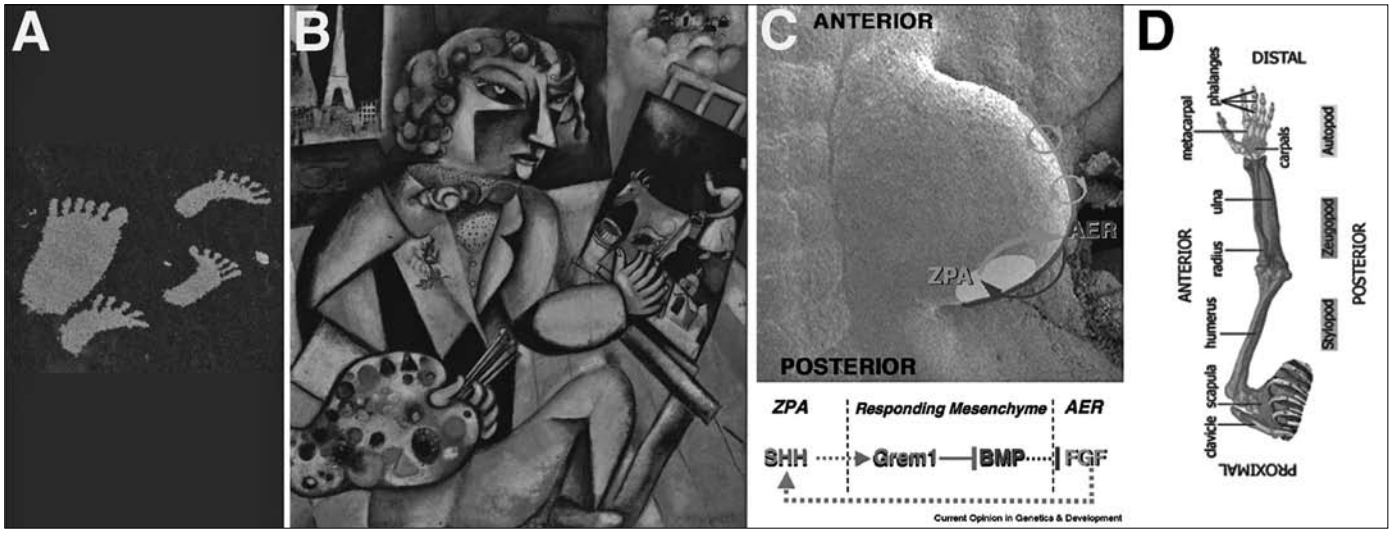

Figure 1. A. Prehistoric rock painting depicting normal and polydactylous feet drawings from Newspaper Rock in Utah (taken from Case et al. 2006 [1]). B. Mark Chagall's self-portrait with seven fingers (Stedelijk Museum, Amsterdam). C. The development of vertebrate limb buds is orchestrated by feedback signalling between two main organizing centres: the SHH expressing ZPA in the posterior mesenchyme and the FGF expressing apical ectodermal ridge (AER). The BMP antagonist Gremlin1 is essential to establish the epithelial-mesenchymal feedback signalling interactions (taken from Zeller and Zuniga, 2007 [20]). D. The skeletal elements of a human arm skeleton (adapted from Vesalius,1543). The stylopod (S; in blue) gives rise to the most proximal limb bud skeletal element, the humerus. The zeugopod forms the radius (anterior) and ulna (posterior) and the distal autopod the wrist (carpals), palm (metacarpals) and digit (phalanges) bones. The scapula and clavicle do not derive from the limb mesenchyme (artwork by Javier Lopez-Rios).

sculptures or playing the piano to create masterpieces like the Goldberg variations. These tools of creativity arise as small limb buds during week 3 of gestation and by 5–6 weeks of human embryonic development the pentadactyly is already apparent. Consequently, any limb malformations arise very early during pregnancy and infants born with additional rudimentary or complete digits are in general surgically treated to correct their digit numbers and normalize the functionality of their hands as much as possible. Therefore, our research interests are not at all focused on finding cures for limb malformations and digit polydactyly, but to understand the molecular signalling mechanisms that control limb bud development and the formation of a normally pentadactylous autopod. This must be quite a robust process as higher mammals and many rodents have pentadactylous limbs. However during evolution, pentadactyly was also a playground for significant variation as not only birds but also bats have modified their upper extremities into wings. Another example is hoofed animals, which make up about six orders of mammals and have given up pentadactyly to build specialised and highly adapted limb structures. In certain species, such as snakes and legless lizards (slow-worms) limbs have been lost altogether as a consequence of surprisingly simple cis-regulatory alterations inactivating *Shh* expression (see below) [2]. This shows that the normally rather robust pentadactylous bauplan can be extensively modified during evolution by altering specific "molecular Achilles heels" – a fascinating topic on its own [3,4].

Identification of a self-regulatory and robust signalling network that controls limb organogenesis

Our research aims to gain insight into the molecular nature and functional interactions of the signalling networks that control the formation, outgrowth and patterning of limb buds during embryonic development. In particular, these signalling networks control the embryonic cells that participate in formation of the limb skeleton by specifying their identities and coordinately controlling their proliferation, survival, determination and differentiation programmes. We are interested in understanding how particular embryonic cells are endowed with the power to act as organizers [5,6]. The establishment of such organizing centres is a pre-requisite for the development of all our organs, including kidneys, whose development we also study in the lab [7,8]. These organizing centres in turn orchestrate the signalling networks that pattern the limb bud

along its three axes and determine the identities of the large numbers of chondrocyte progenitors that form the cartilage elements and will give rise to the limb skeleton (Fig. 1). Together with other groups, we already established some time ago that cells responding to such signals activate the expression of specific antagonists, which – if they are secreted – inhibit signalling by either binding to the ligands or receptors or – if they are intracellular – interfere directly with signal transduction. In fact, it has become clear that embryonic cells produce many antagonists to tune down/turn off signals once they have received them. Therefore, a significant part of our current research focuses on understanding how such signalling feedback loops are established and contribute to the generation of robust signalling networks. In particular, we showed that activation of the BMP antagonist Gremlin1 in the limb bud mesenchyme is key to the feedback signalling interactions between the two main organizing centres in limb buds, the mesenchymal ZPA produces the SHH morphogen and the ectodermal AER that produces FGFs (Fig. 1). Our research over the decade has revealed a robust signalling system that regulates limb bud development from initiation to termination in an apparently self-regulatory manner (Fig. 2; described in several key research publications from the group – for details see refs. 5, 7–12). One of the most surprising aspects of this system is the fact that high BMP activity is required to trigger these signalling interactions, but its activity is then rapidly down-regulated by upregulation of the BMP antagonist Gremlin1. Interestingly BMP4 triggers the expression of its own antagonist Gremlin1, which is then reinforced by SHH signalling (Fig. 2). Mathematical simulations reveal the rapid drop of BMP4 activity during onset of limb development. In agreement, genetic analysis shows that early inactivation of BMP4 completely disrupts limb development, while just slightly delayed inactivation causes polydactyly – i.e. the exact opposite phenotype. These amazing results would have been impossible to explain without mathematical simulations and illustrate in a rather radical manner how crucial tight temporal regulation of signalling activities is to normal limb organogenesis [5]. In fact, these dynamic BMP4/*Gremlin1* interactions are equally important for initiation and progression of metanephric kidney organogenesis [8]. Furthermore, our studies reveal that this feedback signalling system interlinks the BMP, SHH and FGF pathways and is able to compensate variations in signalling via its inter-pathway connectivity. Most interestingly, this system has a built-in self-termination mechanism that ends signalling at the right moment during limb bud development (Fig. 2). Our publication on this topic [8] has been recognised as being at the forefront of developmental systems biology [13] and has allowed us to formulate a first integrative model, which provides the conceptual framework for our systems biology approach to limb organogenesis [6]. Using genome-wide and integrative approaches, we have recently been able to gain first insights into how SHH co-ordinately controls development of the two main limb bud axes (Probst, Zuniga and colleagues, submitted)

Polarizing the nascent limb bud field and setting-up the limb bud organiser

Another major aspect of our research aims to uncover the molecular mechanisms that polarize the nascent limb bud mesenchyme and control activation of *Shh* expression at the posterior limb bud margin. The activation of *Shh* expression hallmarks establishment of the ZPA, which yet again appears to be controlled by a mutually antagonistic interaction involving several transcription factors [14,15,16,17]. We have recently shown that the bHLH transcription factor Hand2 is essential for activation of *Shh* expression specifically in limb buds. Hand2 regulates *Shh* activation in concert with HoxD transcriptional regulators and is part of the chromatin complexes bound to the far up-stream cis-regulatory region controlling *Shh* expression in limb buds [14]. Its restriction to the posterior limb bud mesenchyme also depends on the Gli3 repressor protein. Shh is not activated in mouse limb buds lacking *Hand2* and *Gli3* and molecular analysis reveals the complete loss of anteroposterior polarity, which results in formation of up to ten very rudimentary digits [14]. These studies uncover the molecular mechanism that polarizes the nascent limb bud and establishes the *Shh* expressing organizer. In summary, these studies reveal the molecular origin of the signalling asymmetry that controls formation of our evolutionary well adapted and functional hand and feet.

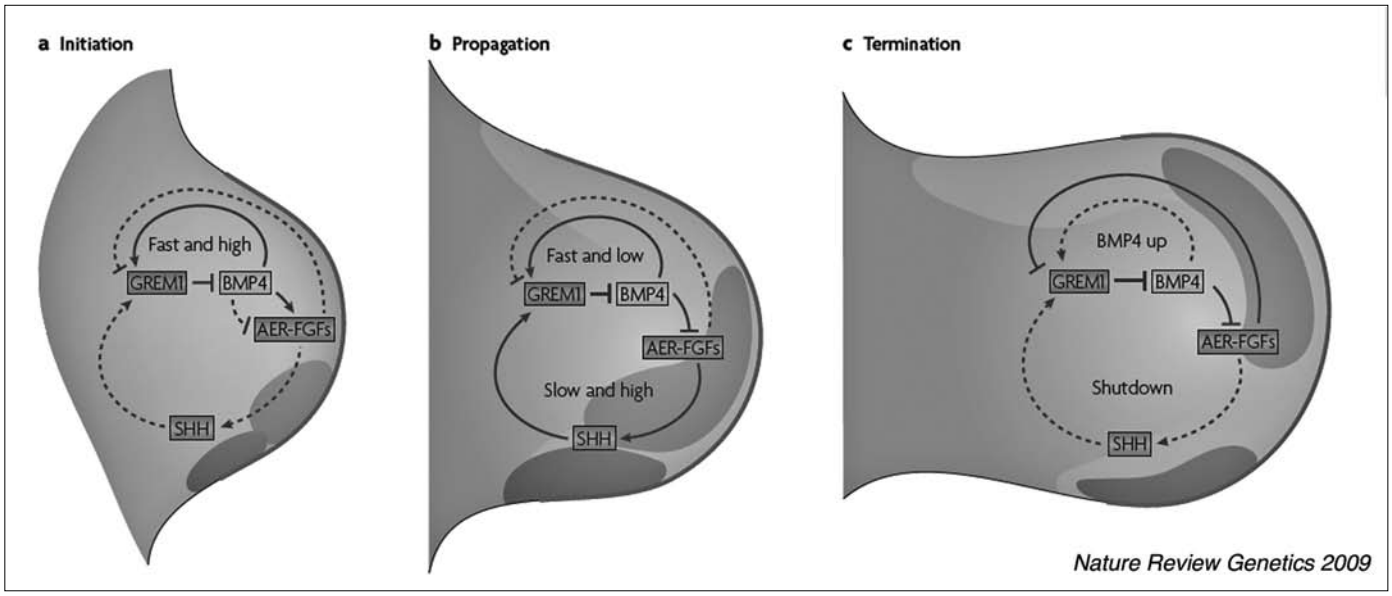

Figure 2. A self-regulatory system of signalling feedback loops controls the orderly progression of limb bud organogenesis from initiation to termination of its initial patterning and outgrowth (taken from Zeller et al., 2009 [6]).

Relevance of our research to regenerative medicine and cancer

Our studies reveal the importance of the strict temporal and spatial control of signalling activities and how changing one signal can affects others by pathway inter-connectivity. These findings are highly relevant to tissue engineering and regenerative medicine. As a first step in this direction, we have initiated a collaboration with the group of Prof. Ivan Martin, who is studying bone development by endochondral ossification of cartilage templates formed by human mesenchymal stem cells (MSCs). In particular, we have begun to analyse the expression and temporal changes of endogenous signals in cartilage and bone templates engineered from MSCs [18]. Building on this successful initial interaction, we plan to study the functions and interactions of the BMP, FGF, IHH and WNT pathways during MSC-mediated cartilage and endochondral bone formation. Particular emphasis will be given to the temporally and spatially controlled local application and induction of morphoregulatory signals, antagonists and effects on endogenous signal/antagonist pathways. Finally, aberrant initiation of such signalling feedback loops and modulation of their activity may underlie initiation and/or progression of tumours. In particular, we have initiated a study dealing with one of the most common and deadly juvenile brain tumours in humans – medullablastomas in collaboration with Stephan Frank (Institute of Pathology). We have previously shown that PN1 is an important extra-cellular modulator of SHH signalling during cerebellar development [19]. Now we have been able to establish that PN1 is highly expressed in the majority of human medullablastoma biopsies and medullablastomas arising in *Ptch1* heterozygous mice as a consequence of elevated SHH signal transduction. Genetic reduction of *PN1* drastically lowers the tumour frequency in *Ptch1* mice. The few neoplasms observed in *Ptch1/PN1* compound mutant mice are much smaller and more differentiated. This analysis indicates that elevation of PN1 during medullablastoma formation is a key event in either tumour initiation or progression. We are now studying this suppression of medullablastomas in *Ptch1* mice to gain insight into the molecular alterations underlying neoplastic cell transformation during postnatal cerebellar development. In the long run, we hope to develop novel therapeutic strategies for medullablastomas and test them in the *Ptch1* mouse model.

Outlook and conclusions

One of the major challenges lying ahead is the systems biology-type analysis of complex signalling networks. We need to quantitate pathway activities over time and space by developing the appropriate real-time sensors (e.g. knock-ins of labile YFP proteins into target genes).

For this purpose, we have developed a novel genetic tool – dRMCE (patent pending) – that allows rapid and highly efficient retargeting of a vast number of genomic loci in mouse ES-cells. Using dRMCE, we can now easily introduce epitope tags into the endogenous key regulator genes to allow their specific and sensitive detection *in vivo*. This will allow us to identify interacting proteins and target genes, which is important for understanding how embryonic cells integrate signalling inputs and activate specific sets of target genes. Another major challenge is the mathematical simulation of signalling interactions over time and space on the basis of quantitative data. This is a truly challenging task, but only systematic genetic and cell-biochemical analysis in combination with the appropriate mathematical simulations will enable us to grasp the complexity by which these signalling networks orchestrate the development of complex and functional organs and tissues.

Acknowledgments

I wish to thank all current and past members of my group, who enable me to enjoy the fun of knowledge-driven basic research albeit the sometimes daunting administrative burdens of my job. I am grateful to SNF, EMBO, EU, Novartis and most recently OncoSuisse for past and current generous support of my research and fellows in the group.

Rolf Zeller

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Research Group "Gynecological Endocrinology"

The research group "Gynecological Endocrinology", situated on the fourth floor of the ZLF building of the Department of Biomedicine, is closely associated to the division of gynecological endocrinology and reproductive medicine at the Women's Hospital of the University of Basel. The latter is mainly specialized in reproductive medicine but is also very involved in the diagnostics, prevention and/or treatment of various female endocrine disorders such as the polycystic ovary syndrome, menopausal climacteric symptoms, estrogen-responsive breast cancer and early pregnancy. Although a considerable number of clinical studies are constantly being performed, among them several multinational, multicentric projects, there is a strong need for experimental research in the field of developmental biology and endocrinology. The research group "gynecological endocrinology", which was installed in 1997 within the frame of the Department of Biomedicine, fulfills that need and their work can be summarized by the following three major topics.

1. Construction of an in vitro model of the ovary

The ovary consists of various functional compartments with the oocyte at its center. Ovarian follicular growth is characterized by a rapid proliferation of granulosa cells, which both nurse the enclosed oocyte

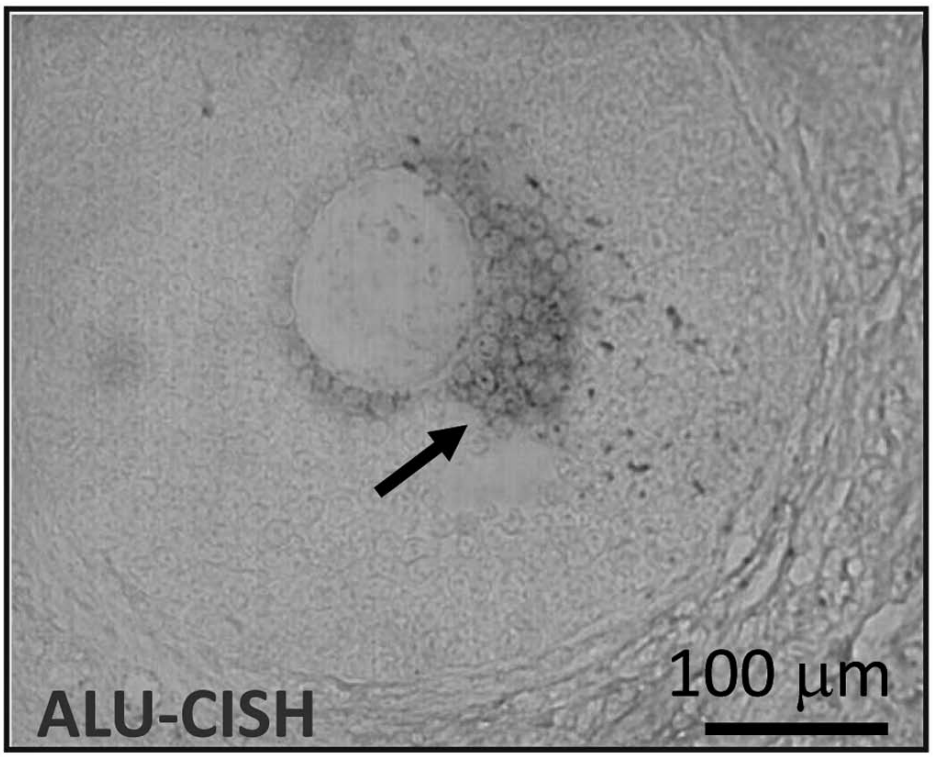

Figure 1:
Human granulosa cells were cultured for three weeks in a three-dimensional pellet made of collagen type I and were then transplanted into the right ovary of an immune-incompetent mouse. Based on primate-specific alu sequences human granulosa cells were visualized (black arrow) integrated within the boundaries of mouse antral follicles closely associated with the enclosed mouse oocyte (Kossowska-Tomaszczuk et al., 2010). This research was carried out in close collaboration with the research group "Tissue Engineering" at the Department of Biomedicine and with the Institute of Laboratory Animals at the University Hospital of Zurich.

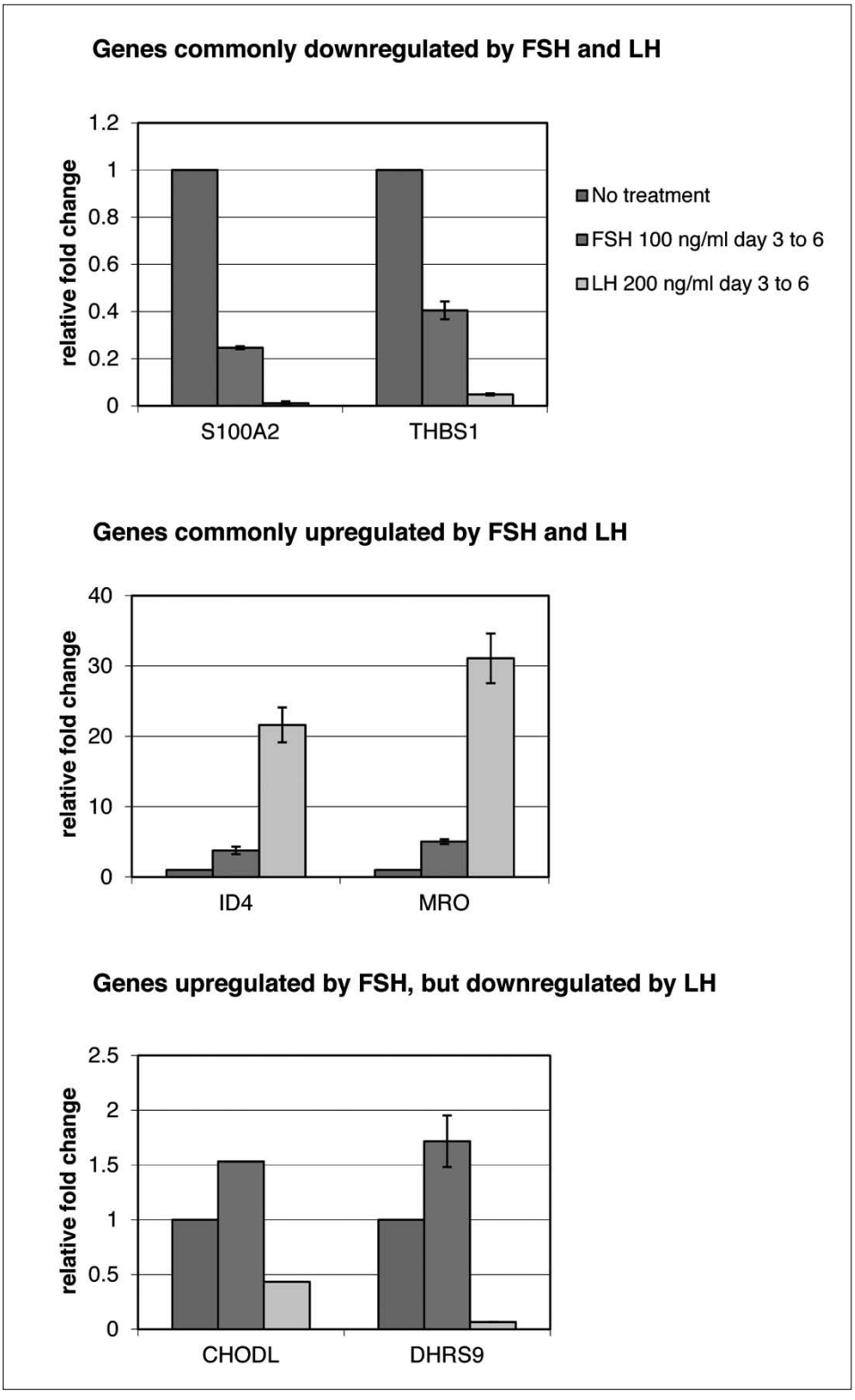

Figure 2:
Genes regulated by FSH or LH in primary human luteinizing granulosa cells collected from infertile women treated for assisted reproduction. This experiment is based on gene expression data provided by Affymetrix microarray and has been carried out with qPCR for various target genes of FSH or LH for confirmation of the microarray results. For each experiment granulosa cells collected from 25 patients were pooled and cultured for 3 days in the absence of LH or FSH and for 3 more days in the presence of either recombinant FSH (100 ng/ml) or recombinant LH (200 ng/ml). Some genes are commonly regulated by FSH or LH, whereas some are differentially regulated by each of both hormones.

but also produce the bulk of the hormones preparing the female organism for reproduction. The granulosa function for its share is regulated by surrounding thecal cells, which not only produce the precursor steroids for the enzymes of the granulosa, but also the blood vessels needed for follicular growth, ovulation and subsequent formation of the luteal body. Although all of these functions can be manipulated either positively or negatively with existing pharmaceuticals, the response of individual patients to medication is highly variable and many aspects of ovarian function still remain unknown. Therefore, through the charac-

Figure 3:
Homozygous of EULIR$^{-/-}$ knockout mouse typically showing exencephaly associated with neural tube defects.

terization of the first immortalized human granulosa cell line (Zhang et al., 2000) we initiated the construction of *in vitro* models for the study of follicular growth. Although through the study of the interaction of these immortalized granulosa cells and immature oocytes a long list of potential signalling molecules could be gathered, it became clear that a more physiological approach was needed. Through a fortunate coincidence, cells with multipotent stem cell-like characteristics were isolated from the antrum of mature human follicles (Kossowska-Tomaszczuk et al., 2008) and through the development of a three-dimensional culture system made of collagen type I these cells can now be cultured over prolonged time periods without loss of their genuine characteristics (Kossowska-Tomaszczuk et al., 2010). These findings provide the basis for the construction of a three-dimensional *in-vitro* follicle-like structures and current research is now being carried out to culture oocyte-enclosed primordial follicles in a three-dimensional environment (Figure 1). Using the opportunity to culture human granulosa cells over prolonged time periods, it is now possible to dissect the differential effects of the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH) on granulosa cell function (Figure 2). This research ultimately aims at setting up an artificial ovary-like system, in which the various compartments can be manipulated in order to identify the regulatory pathways involved in oocyte maturation and follicular growth.

2. Creation of new models of infertility

Based on existing gene expression data but also based on the known pathways involved in apoptosis, which is the counterpart of the endocrine regulation of ovarian follicular development, leading to follicular atresia and luteal demise, various transgenic mouse models were generated for the purpose of identifying novel pathways in infertility.

The first consists of a gene, which encodes a protein (e.g. EULIR) binding to the inhibing binding protein (InhBP/p120), the putative co-receptor of the inhibins. Together with the activins the inhibins (A and B) are structurally related hormones, belonging to the transforming growth factor-β superfamily of proteins, which exert crucial functions in the regulation of the secretion of FSH-synthesis in the pituitary but also in both ovarian and adrenal physiology. EULIR is a E3-ligase protein for InhBP and probably functions in regulating protein turnover. In order to better understand the *in vivo* functions of EULIR, we produced a murine EULIR-knockout model. Deletion of EULIR resulted in embryonic lethality

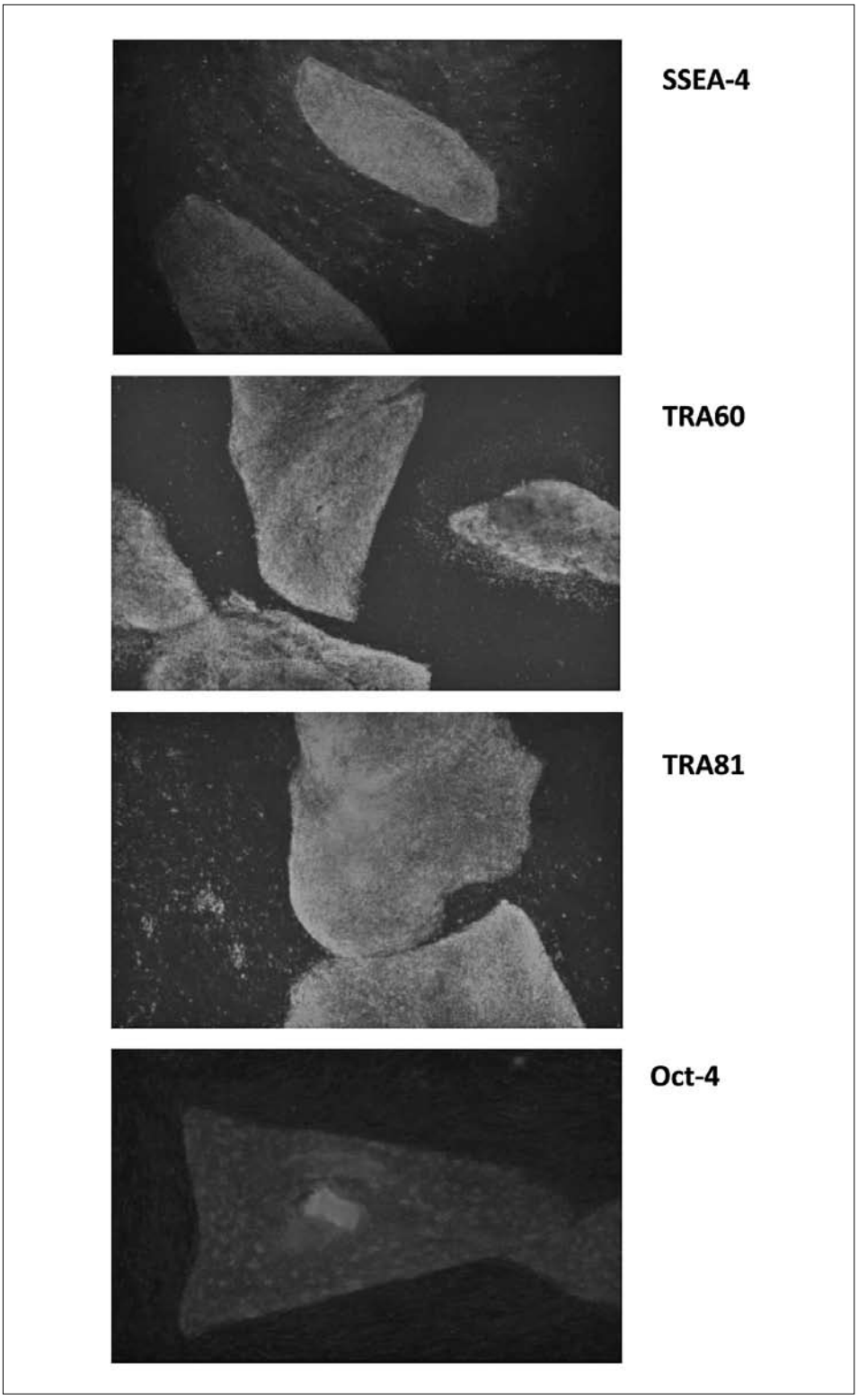

Figure 4:
Demonstration of the presence of four main stem cell markers in the human embryonic stem cell line CHES2, the first produced in Switzerland having a normal karyotype.

since none of the embryos developed beyond the embryonic developmental stage 12.5 d. Homozygous EULIR$^{-/-}$ embryos failed to close the neural tube resulting in exencephaly from the hindbrain to the forebrain (Figure 3) and they also had a very small placenta. As funded by the Repronatal Foundation and the FAG the detailed structure of the placental morphology is currently being studied. In addition, the effect of EULIR on the gonad is now being studied through the creation of a conditional EULIR-knockout model.

Female mammals are endowed at birth with a finite number of primordial follicles, each consisting of an oocyte surrounded by a single layer of somatic cells. These follicles remain in a dormant stage until initiation of their rapid development up to either ovulation or degeneration through atresia. From a quantitative point of view, follicular atresia outnumbers the process of follicular development by far, as only a few hundreds of follicles become ovulated, whereas millions of follicles invariably undergo atresia. Atresia is induced by cellular apoptosis and the various members of the Bcl-2 family serve as central checkpoints in this process. Our interest in apoptosis was initially focused on one particular member of the Bcl-2 family, Bok (Bcl2 ovarian killer), which was originally found to be expressed in the rat ovary only thereby providing us with a potential unique target for medical treatment. The human homologue of Bok (hBok) was first identified in our lab together with the orthologs of Bok in *Drosophila* and chicken (Zhang et al., 2000b). Later studies, however, demonstrated that the expression of hBok is not limited to the gonads but that it plays many roles in various physiological and pathological pathways as well (Gao et al., 2005). Other members of the Bcl-2 family have also been identified in our laboratory, most notably Bcl2l10 (Zhang et al., 2001).

Using transgenic mouse models most phenotypic studies dealing with infertility as caused by members of the Bcl-2 family of proteins have focused on Bak and Bax only. Bax-deficient ovaries contain an unusually large number of follicles with excess granulosa cells reminiscent of the polycystic ovary syndrome in the human. Bax-deficient males, however, were infertile as a result of abnormal seminiferous tubules with an accumulation of atypical premeiotic germ cells but no mature haploid spermatozoa. In contrast, Bak-knockout mice are viable, fertile, normal in size and do not display any gross physical or behavioural abnormalities. However, elimination of both genes dramatically impairs developmental apoptosis in many tissues. In view of these results and taking the existence of other members of the Bax subfamily into account implies that, depending on any particular cell type, the roles of the various members of the Bax subgroup, such as Bak, Bok and Bcl2l13, in the apoptotic pathway are not simply redundant.

To gain insight into the full spectrum of function of the Bcl-2 family of proteins involved in gonadal apoptosis and fertility, we established a homozygous Bcl2l13-deficient mouse line. The $Bcl2l13^{-/-}$ mouse is phenotypically normal and no obvious abnormality was observed in the organs examined as compared to wild-type mice. Both $Bcl2l13^{-/-}$-males and $Bcl2l13^{-/-}$-females were fertile nor did they develop any age-related disorders in over one year of observation. In addition, the interbreeding of $Bax^{+/-}$ with $Bcl2l13^{-/-}$ and $Bax^{-/-}$ with $Bcl2l13^{-/-}$ lead to the production of double knockouts. Preliminary results showed that the $Bak^{-/-}$/$Bcl2L13^{-/-}$-females are infertile, whereas the $Bak^{-/-}$/$Bcl2l13^{-/-}$-males display normal fertility rates. Step by step we are now gaining insight into the redundant processes involved in follicular atresia.

3. Stem cell research

Despite the recent development of induced pluripotent stem cell technology (iPS), embryonic stem cells (ESC) remain the gold standard in stem cell research. The access to supernumerary human embryos, the possibility to trace back the very origin of those supernumerary embryos and the maintenance of quality assurance throughout the entire process justify the development of specialized centres dealing both with assisted human reproduction and the derivation of human ESC lines together with iPS under good manufacturing practice (GMP) conditions. The unit of assisted reproductive medicine located in the University Hospital of Basel has instituted a GMP-grade laboratory for the isolation and characterization of new human ESC lines at the University of Basel. These activities have resulted in the derivation and characterization of three novel human ESC lines (CHES2, CHES3 and CHES5), among them the first in Switzerland with a normal chromosome complement. These were derived from very few embryos, donated for research in accordance with the approval of the local ethics committee (EKBB), of the Federal Ministry of Health (BAG) and through a long-standing collaboration with the Stem Cell Laboratory of the University of Geneva and with the Karolinska Institute in Stockholm, Sweden. In addition, novel protocols for the non-viral-

mediated production of iPS are underway thereby avoiding the integration of vectors into the genome of the reprogrammed somatic cells.

In line with these activities, as funded by the Swiss Centre for Applied Human Toxicology (SCAHT), the stem cell technology and the infrastructure provided by the GMP-setting will be used for the establishment of reproducible differentiation protocols for the purpose of phenotypic screening of both neuronal and germ-line cells in toxicology. Uniform populations of differentiated cells must be achieved reaching an estimated purity of >90 %.

Conclusion and scope

A major motivation for our involvement in research has always been the concomitant improvement of our clinical assisted reproductive medicine programme directly benefiting to our patients. A good example is given by our commitment in hESC-research, which has allowed us to improve and extend the culture conditions in clinical assisted reproductive medicine culminating in improved pregnancy rates and in the avoidance of many multiple pregnancy rates. Furthermore, although our search for genotypes involved in the pathogenesis of human infertility has so far remained unsuccessful, it can be awaited that the generation of the large data sets of genes expressed both in the ovaries and in the breast tissue will lead to the discovery of new signalling pathways, which can be used both in diagnostics and therapeutics.

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My «Fun» days in ICFS-TE

As I write this article, it is close to the end of February and I have already missed one deadline. By the time, it gets printed in DBM facts, most of the events written here will be history. For starters, I am already across the ocean, in a different continent and there is not a day that goes by that I do not reminisce about my "Fun" days at DBM, more particularly at ICFS.

To be honest, I am at a loss. I would like to write something about the time I spent in Basel, but the readers of the magazine are predominantly from Basel, so nothing about Basel would interest them. I am also scared that I will mess-up with locations and directions when I talk about the different spots in Basel....so I guess , I need to be careful !!!

So, maybe it would be better to stick to the microenvironment where I never felt lost and where I got overwhelming love and support. I should write about ICFS-TE, maybe the non-ICFS members of DBM will find something interesting in this article. To the ICFS family, I can only apologize if my article seems mundane and boring.

To start, I go back to the first day I reached the Tissue Engineering Laboratory on the fourth floor. It was two days after Christmas and it took me quite a while to get to lab, as I got confused and lost my way along Hebelstrasse. When I entered the lab I found it, predictably for the season, almost empty. I made my way to the coffee machine and there I found Francine, drinking her espresso and I was immediately taken under her wing. I think she is the person with the biggest network of friends in the building; also her level of energy and stamina are quite contagious, in my mind she is always "Fantastically Fit" Francine. She introduced me to the lab and showed me a place where I could sit *temporarily*. I would realize the significance of the word *temporarily*, only a few days later.

Walking along the Rhine: One of the many things I miss about the city.

To give a little bit of background, I had arrived two days earlier from India, on completion of a doctoral degree from the Indian Institute of Technology, Kharagpur. I was still a bit jet-lagged from the time difference and the long trip. In the next few days, when vacations ended and people started returning to their positions, I gradually started settling into the routine of working at the lab.

For readers who are not acquainted with ICFS, maybe it will help to understand the unique features of this unit. ICFS is a scientific conglomeration of three groups: Tissue Engineering, Oncology and Cell and Gene Therapy, and it brings together approximately 36 people belonging to 11 different countries (this number corresponds to the head count a year ago, and if I am not mistaken, the number is steadily increasing!!!). In most ways, it helped all of us to be in such a cosmopolitan atmosphere, firstly English was the common medium of communication and most of the people could relate to the predicament when a person from a different country lands in Switzerland. We all learnt to play a game of 'international musical chairs', once in ICFS. Given a space crisis, there are always more than two people scouting for a desk space for the day, and it is a matter of joy when a person gets assigned to a desk. We all paid a 'rent' for a newly acquired desk-space; the minimum was a chocolate to all the previous inhabitants of that particular room!!!

I have to return to the beginning. One day, the phone rang at my desk, I answered and since the caller was speaking in German, I promptly passed it to "Super Translator" Sandra (she speaks six languages fluent-

ly, and her list is also increasing!), who spoke into the mouth piece "Institut für Chirurgische Forschung und Spitalmanagement" , smiled and passed it back to me, "Its for you, Chitra!" That began my initiation to the first few baby words in German; and the administrative initiation process had started.

As the Christmas vacations dwindled away, the lab started filling up and my list of friendly faces increased. I met my boss, the "Impressive" Ivan, who completely changed my idea of a leader. Here was a man with a spontaneous smile, intelligent eyes, brilliant mind and exceptionally kind nature. He introduced me to the surgeons and explained briefly the symbiotic relationship shared between scientists and surgeons at ICFS. I met my other boss, "Awesome" Andrea in a few more days' time and gradually we settled into our project. My privileged desk space next to Andrea allowed me to ask questions and explore different ideas whenever they popped into my head. My questions were always answered with a patient look, contemplation, a slow smile and then the solution. Be it a problem of cells not behaving properly, or experiments not making sense, Andrea always made it a priority to address these problems and once the slow smile appears on Andrea's face, solution to even the toughest problem is around the corner!!!

Starting a new project is never without teething troubles, it was quite some time before things started falling into place. The project took me to "Simply Super" Sylvie, together we explored the labeling of cells using different methods. She has the gift of a sharp memory and she can always recommend someone on the floor who had some experience in a particular experiment. Sylvie epitomizes the attitude of the lab, never failing to help others when it is within your capacity to do so.

The desk right next to her was one marked for the rotating surgeons of the lab, at that time it was Arne and a year later it was Franziska. From them, I understood one very important thing about surgeons: given their killing schedules, they have a peculiar in-built sense of humor, probably a result of their training. It is never wise to react to their words immediately, usually all their sentences should be heard out and then you have to wait till their eyes start twinkling. Usually, every sentence from them has a twinkle at the end and till you see that, you would not believe that they were joking!!!

Taken from a cable car: a breath-taking view of this picturesque land.

Adjoining our room, we had more desks occupied by two other bosses: Paul and Arnaud. To me Paul was the "Google of the lab". Be it designing primers, FACS analysis, or troubleshooting about the unpredictability of viruses, he always had some suggestion for everyone. I have never known him to decline helping anyone, most often you will see him handing down laboratory apparatus from the top shelves to diminutive people like me who are spared the effort of scaling ladders!!! Arnaud, with his desk next to Paul, never failed to see the humorous aspect of every situation and we were always in splits when Paul and Arnaud combined their wits together.

Typical of our routine of everyday life, Sylvie would start to assemble people at noon for lunch; this is when I met "Uniquely Unique" Uta and "Really Rosy" Rosaria. I was finding it a little difficult to adjust to the Swiss food habit, and Rosy told me that I was not the only one. With that, I started experiments with Indian cuisine which I started trying on everyone at the lunch table. Uta became my indicator for 'spicy' food: if she turned red, it meant food was still not scaled down to 'European standard', if it was praised by Rosy, I knew it was close to the Indian 'hot' taste!!! With Uta and Rosy by my side, I always had someone to turn to and always found a shoulder to cry on.

Time went really fast, "Shopping Tiger" Nunzia introduced me to the different stores on Freie Strasse and along with Francine, took me to the gym in the neighborhood. "Goldilocks" Karoliina proved to be a saviour when she took me to the public library. We could get up

to 10 books at a time, and I began to complete 2-3 books per week. Sandra was always directing me to some really chic stores for funky jewels and apparel and I was happy to know the correct places to go looking for things!!!

It was not just science in ICFS: we also had ICFS-Social events – Turkish night, Swiss night, the 30's party, Jungle-theme party, football matches and many more. The spirits of the lab were high and anyone going through a bad patch was easily buoyed-up with the easy camaraderie and goodwill shared among all the people. There was always "Charming" Adam to soothe away your worries with his kind words, "Dancing" Daniel, tip-tapping away to glory and never failing to see the positive side of things, "Supportive" Sinan to cheer up your spirits, "Engaging" Elia who always made everyone feel special, and "Bella" Beatrice who always had a ready smile for everyone.

A year passed sooner than I expected, and, as planned, I was supposed to go to The Netherlands on collaboration. Before that, I presented my work in front of the group. I always had trouble speaking in public and suffered from 'stage-fright'. I prepared my slides well in advance, discussed the data with Ivan and rehearsed for the presentation with Andrea. Nothing, however, prepared me for the fright I got when our big boss, Prof. Dr. Michael Heberer walked into the room. I was so scared and conscious that for the first five minutes I fumbled with the computer controls and my whole presentation got a greenish hue as background. Then, Ivan got up and came to help me. He said "even if the projector is not showing the right image, we are here to hear about your work, so don't worry". At that moment, I lost my worries and started talking about my work. At the end of it all, Andrea pumped a fist in the air to say well-done, and Michael and Ivan came over to discuss about the associated work at Holland. Michael asked if I was planning on working on the same project in Holland or would I work on different topics as desired by the company? I was about to react, when I saw the twinkle in his eye!!! After all, he was also a surgeon!!!

The game of musical chairs had restarted; it was my turn to concede my place to "Cherubic" Celeste, a surgeon from Italy who had been waiting for my desk for months. I reached The Netherlands and started working on the same project in the company with whom our lab

Home away from home: Some of the ICFS at the Bollywood night.

was collaborating. This transition involved a lot of effort on the part of my Indian friends as I had to leave behind a lot of luggage with them. Destiny played a peculiar role at this time, my work there got wrapped up in two months and I found myself back-to-work in Basel. In this matter, along with Ivan, I was strongly supported by our Head of Administration, Ms Heidi Hoyermann. Earlier, Heidi had helped me in many other administrative ways, but now her efficiency and support drew admiration from deep inside me. This process was aided by Ms. Caroline Jaussi, Assistant to Professor Michael Heberer, who offered me accommodation and provided a local address for authorities. My Indian friends in Zurich provided me with accommodation for a while, and soon, I was back among the people I loved and who cared for me.

It was my turn to look for a desk in the game of musical chairs and I was overjoyed when I regained my old place, right next to Andrea. We interacted with the Oncology group and based on some discussions with Dr. Giandomenica Iezzi, we were able to complete a few of our objectives.

During my second stint at ICFS-TE, I began to go out and interact with people more than I had done formerly. This time, I was introduced to "Amazing" Anna, a post-doctoral student, who is rightly described by Paul as 'a glass of vitamins'. This bubbly and cheerful girl made the social life inside the group even more vibrant. Francine and Sylvie invited me on many occasions to spend a day with their families; Rosy, Uta, Anna and I met frequently for yoga sessions and meals; and we often

spoke to each other about our lives without hesitation and without fear of being ridiculed.

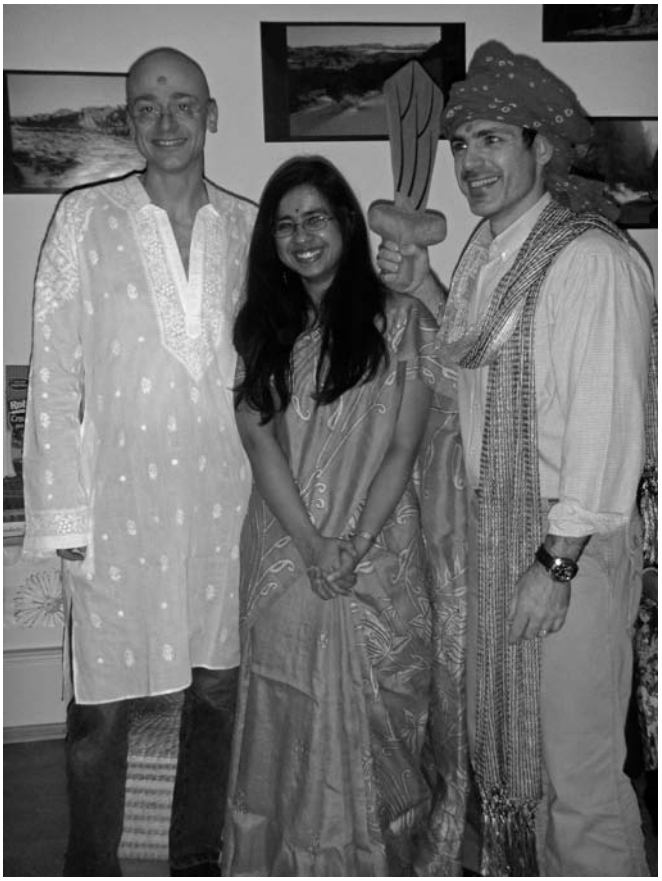
Towards the latter part of the year, I began to apply for a future postdoctoral position within Switzerland first, and later to USA, when Ivan advised that experience in USA might give a greater leverage to my career. We were really amused to receive interview calls, and later appointment offers from more than three big places in the US.

All this while, we kept working and having parties – Wild west, Hippies, Glamour and Glitz and so on. "Efficient and super organized" Anke invited a few of us to her house for a "flaming drink" which I would have never believed possible, had I not witnessed it. Also, I got to know "Elegant" (H)Elena a little better for the caring and affectionate person she really was.

My next progress report was looming around the corner and this time too, I was petrified of talking in front of the group. After it finished, I was glad and relieved that Ivan, Andrea and Michael found my work to be significant. Ivan mentioned that this would be my last presentation for the group and he wished me courage and strength for my next venture. At that time, though I was facing a second departure from the group; I was kept from depression and was busy with different administrative events and proceedings.

My friends were very interested in wearing Indian costumes and one day we planned to meet at Uta's place to try out different Indian clothes. To my utmost surprise, I reached there to find all the labmates assembled for a "Hollywood meets Bollywood" night, intended as a farewell for me. I was flabbergasted, touched and taken unaware. It was so special to see everyone wearing Indian ensemble and I was extremely touched to see the effort everyone had made to wear ethnic apparel and to organize such an event. Dressed as Gandhi, Ivan made a quiz for me to guess the gift the lab had chosen for me. I could only guess it after four hints – it was a traveling bag!!! Later on, the lab also provided me with two other bags, to make my traveling easier.

After the arrival of the US visa, I booked my tickets to leave within a week. Our manuscript was still in the process of being finalized and beside that, I always found some excuse to go back to lab to see my dear friends. Francine and her husband offered to help me with my



With the bosses at the Bollywood night.

bags till Basel airport, but even when I had packed my bags, I was unable to believe that I was really leaving, maybe never to see my friends again. Life was going to change and it was really difficult to accept.

Then, one evening when I was printing-out my manuscript, the phone on my desk rang again. This time I knew enough German to say "Institut für Chirurgische Forschung und Spitalmanagement", but the caller didn't want to speak to me. The call was for Benjamin who would be taking over my desk in a day's time. The game of musical chairs was continuing, finally, it was time for me to leave.

This article was written after I reached Boston, when I was reminiscing about the good times at Basel. Now, in a new environment, I can feel the full impact of the hospitality and warmth extended to me by the entire ICFS family.

Chitrangada Acharya

Selected publications by DBM members

Below 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. The DBM affiliation must be mentioned in the authors list as it appeared in 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 primarily concentrating on original articles. 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

Deadline for the next issue is April 30, 2010.

Hepatology *Hepatology* Epub Nov 13, 2009 IF11,4

Cleavage of mitochondrial antiviral signaling protein in the liver of patients with chronic hepatitis C correlates with a reduced activation of the endogenous interferon system

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Abstract:
Hepatitis C virus (HCV) infection induces the endogenous interferon (IFN) system in the liver in some but not all patients with chronic hepatitis C (CHC). Patients with a pre-activated IFN system are less likely to respond to the current standard therapy with pegylated IFN-. Mitochondrial antiviral signaling protein (MAVS) is an important adaptor molecule in a signal transduction pathway that senses viral infections and transcriptionally activates IFN-. The HCV NS3-4A protease can cleave and thereby inactivate MAVS in vitro, and, therefore, might be crucial in determining the activation status of the IFN system in the liver of infected patients. We analyzed liver biopsies from 129 patients with CHC to investigate whether MAVS is cleaved in vivo and whether cleavage prevents the induction of the endogenous IFN system. Cleavage of MAVS was detected in 62 of the 129 samples (48%) and was more extensive in patients with a high HCV viral load. MAVS was cleaved by all HCV genotypes (GTs), but more efficiently by GTs 2 and 3 than by GTs 1 and 4. The IFN-induced Janus kinase (Jak)-signal transducer and activator of transcription protein (STAT) pathway was less frequently activated in patients with cleaved MAVS, and there was a significant inverse correlation between cleavage of MAVS and the expression level of the IFN-stimulated genes IFI44L, Viperin, IFI27, USP18, and STAT1. We conclude that the pre-activation status of the endogenous IFN system in the liver of patients with CHC is in part regulated by cleavage of MAVS.

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Blood blood 115, 2003–2007, 2010 IF10,4

Clonal analysis of *TET2* and *JAK2* mutations suggests that *TET2* can be a late event in the progression of myeloproliferative neoplasms

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Abstract:
Somatic mutations in *TET2* occur in patients with myeloproliferative neoplasms and other hematologic malignancies. It has been suggested that TET2 is a tumor suppressor gene and mutations in *TET2* precede the acquisition of *JAK2*-V617F. To examine the order of events, we performed colony assays and genotyped *TET2* and *JAK2* in individual colonies. In 4 of 8 myeloproliferative neoplasm patients, we found that some colonies with mutated *TET2* carried wild-type *JAK2*, whereas others were *JAK2*-V617F positive, indicating that *TET2* occurred before *JAK2*-V617F. One of these patients carried a germline *TET2* mutation. However, in 2 other patients, we obtained data compatible with the opposite order of events, with *JAK2* exon 12 mutation preceding *TET2* mutation in one case. Finally, in 2 of 8 patients, the *TET2* and *JAK2*-V617F mutations defined 2 separate clones. The lack of a strict temporal order of occurrence makes it unlikely that mutations in *TET2* represent a predisposing event for acquiring mutations in *JAK2*.

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PNAS PNAS 106, 20081–20086, 2009 IF9,4

The selective antagonist EPPTB reveals TAAR1-mediated regulatory mechanisms in dopaminergic neurons of the mesolimbic system

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Abstract:
Trace amine-associated receptor 1 (TAAR1) is a G protein-coupled receptor (GPCR) that is nonselectively activated by endogenous metabolites of amino acids. TAAR1 is considered a promising drug target for the treatment of psychiatric and neurodegenerative disorders. However, no selective ligand to identify TAAR1-specific signaling mechanisms is available yet. Here we report a selective TAAR1 antagonist, EPPTB, and characterize its physiological effects at dopamine (DA) neurons of the ventral tegmental area (VTA). We show that EPPTB prevents the reduction of the firing frequency of DA neurons induced by *p*-tyramine (*p*-tyr), a nonselective TAAR1 agonist. When applied alone, EPPTB increases the firing frequency of DA neurons, suggesting that TAAR1 either exhibits constitutive activity or is tonically activated by ambient levels of endogenous agonist(s). We further show that EPPTB blocks the TAAR1-mediated activation of an inwardly rectifying K^+ current. When applied alone, EPPTB induces an apparent inward current, suggesting the closure of tonically activated K^+ channels. Importantly, these EPPTB effects were absent in Taar1 knockout mice, ruling out off-target effects. We additionally found that both the acute application of EPPTB and the constitutive genetic lack of *TAAR1* increase the potency of DA at D2 receptors in DA neurons. In summary, our data support that TAAR1 tonically activates inwardly rectifying K^+ channels, which reduces the basal firing frequency of DA neurons in the VTA. We hypothesize that the EPPTB-induced increase in the potency of DA at D2 receptors is part of a homeostatic feedback mechanism compensating for the lack of inhibitory TAAR1 tone.

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Clinical Infectious Diseases 48, 1459–1466, 2009 IF8,3

Incidence and Outcome of Progressive Multifocal Leukoencephalopathy over 20 Years of the Swiss HIV Cohort Study

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Abstract:

Background: We investigated the incidence and outcome of progressive multifocal leukoencephalopathy (PML) in human immunodeficiency virus (HIV)–infected individuals before and after the introduction of combination antiretroviral therapy (cART) in 1996.

Methods: From 1988 through 2007, 226 cases of PML were reported to the Swiss HIV Cohort Study. By chart review, we confirmed 186 cases and recorded all-cause and PML-attributable mortality. For the survival analysis, 25 patients with postmortem diagnosis and 2 without CD4+ T cell counts were excluded, leaving a total of 159 patients (89 before 1996 and 70 during 1996–2007).

Results: The incidence rate of PML decreased from 0.24 cases per 100 patient-years (PY; 95% confidence interval [CI], 0.20–0.29 cases per 100 PY) before 1996 to 0.06 cases per 100 PY (95% CI, 0.04–0.10 cases per 100 PY) from 1996 onward. Patients who received a diagnosis before 1996 had a higher frequency of prior acquired immunodeficiency syndrome–defining conditions ( ) but similar CD4+ T cell counts (60 vs. 71 cells/μL;), compared with patients who received a diagnosis during 1996 or thereafter. The median time to PML-attributable death was 71 days (interquartile range, 44–140 days), compared with 90 days (interquartile range, 54–313 days) for all-cause mortality. The PML-attributable 1-year mortality rate decreased from 82.3 cases per 100 PY (95% CI, 58.8–115.1 cases per 100 PY) during the pre-cART era to 37.6 cases per 100 PY (95% CI, 23.4.–60.5 cases per 100 PY) during the cART era. In multivariate models, cART was the only factor associated with lower PML-attributable mortality (hazard ratio, 0.18; 95% CI, 0.07–0.50;), whereas all-cause mortality was associated with baseline CD4+ T cell count (hazard ratio per increase of 100 cells/μL, 0.52; 95% CI, 0.32–0.85;) and cART use (hazard ratio, 0.37; 95% CI, 0.19–0.75;).

Conclusions: cART reduced the incidence and PML-attributable 1-year mortality, regardless of baseline CD4+ T cell count, whereas overall mortality was dependant on cART use and baseline CD4+ T cell count.

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Clinical Pharmacology & Therapeutics 86, 644–650, 2009 IF7,6

Orally Administered Glucagon-Like Peptide-1 Affects Glucose Homeostasis Following an Oral Glucose Tolerance Test in Healthy Male Subjects

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Abstract:

Glucagon-like peptide-1 (GLP-1) exerts several effects on glucose homeostasis and reduces food intake. After its release from intestinal L cells, GLP-1 is subject to (i) rapid breakdown by dipeptidyl peptidase IV and (ii) high liver extraction. The highest concentrations of GLP-1 are found in the splanchnic blood rather than in the systemic circulation. An oral delivery system would mimic endogenous secretion. Here we investigated the pharmacokinetic/pharmacodynamic (PK/PD) effects of a single dose (2 mg) of oral GLP-1 administered prior to an oral glucose tolerance test (OGTT) in 16 healthy males. GLP-1 was rapidly absorbed from the gut, leading to tenfold higher plasma concentrations compared with controls. The PD profile was consistent with reported pharmacology; GLP-1 significantly stimulated basal insulin release ($P < 0.027$), with marked effects on glucose levels. The postprandial glucose peak was delayed with GLP-1, suggesting an effect on gastric emptying.

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The Sushi Domains of $GABA_B$ Receptors Function as Axonal Targeting Signals

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Abstract:
$GABA_B$ receptors are the G-protein-coupled receptors for GABA, the main inhibitory neurotransmitter in the brain. Two receptor subtypes, $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$, are formed by the assembly of $GABA_{B1a}$ and $GABA_{B1b}$ subunits with $GABA_{B2}$ subunits. The $GABA_{B1b}$ subunit is a shorter isoform of the $GABA_{B1a}$ subunit lacking two N-terminal protein interaction motifs, the sushi domains. Selectively $GABA_{B1a}$ protein traffics into the axons of glutamatergic neurons, whereas both the $GABA_{B1a}$ and $GABA_{B1b}$ proteins traffic into the dendrites. The mechanism(s) and targeting signal(s) responsible for the selective trafficking of $GABA_{B1a}$ protein into axons are unknown. Here, we provide evidence that the sushi domains are axonal targeting signals that redirect $GABA_{B1a}$ protein from its default dendritic localization to axons. Specifically, we show that mutations in the sushi domains preventing protein interactions preclude axonal localization of $GABA_{B1a}$. When fused to CD8α, the sushi domains polarize this uniformly distributed protein to axons. Likewise, when fused to mGluR1a the sushi domains redirect this somatodendritic protein to axons, showing that the sushi domains can override dendritic targeting information in a heterologous protein. Cell surface expression of the sushi domains is not required for axonal localization of $GABA_{B1a}$. Altogether, our findings are consistent with the sushi domains functioning as axonal targeting signals by interacting with axonally bound proteins along intracellular sorting pathways. Our data provide a mechanistic explanation for the selective trafficking of $GABA_{B(1a,2)}$ receptors into axons while at the same time identifying a well defined axonal delivery module that can be used as an experimental tool.

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Biomaterials **Biomaterials** 31, 2252–2259, 2010 IF6,6

Engineering human cell-based, functionally integrated osteochondral grafts by biological bonding of engineered cartilage tissues to bony scaffolds

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Abstract:
In this study, we aimed at developing and validating a technique for the engineering of osteochondral grafts based on the biological bonding of a chondral layer with a bony scaffold by cell-laid extracellular matrix. Osteochondral composites were generated by combining collagen-based matrices (Chondro-Gide®) containing human chondrocytes with devitalized spongiosa cylinders (Tutobone®) using a fibrin gel (Tisseel®). We demonstrate that separate pre-culture of the chondral layer for 3 days prior to the generation of the composite allows for (i) more efficient cartilaginous matrix accumulation than no pre-culture, as assessed histologically and biochemically, and (ii) superior biological bonding to the bony scaffold than 14 days of pre-culture, as assessed using a peel-off mechanical test, developed to measure integration of bilayered materials. The presence of the bony scaffold induced an upregulation in the infiltrated cells of the osteoblast-related gene bone sialoprotein, indicative of the establishment of a gradient of cell phenotypes, but did not affect per se the quality of the cartilaginous matrix in the chondral layer. The described strategy to generate osteochondral plugs is simple to be implemented and – since it is based on clinically compliant cells and materials – is amenable to be readily tested in the clinic.

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Autoantibodies against C1q in Systemic Lupus Erythematosus Are Antigen-Driven

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Abstract:
Autoantibodies against complement C1q (anti-C1q Abs) were shown to strongly correlate with the occurrence of severe nephritis in patients with systemic lupus erythematosus (SLE), suggesting a potential pathogenic role by interfering with the complement cascade. To analyze the humoral immune response against C1q at the molecular level, we screened a bone marrow-derived IgGκ/IgGλ Fab phage display library from a SLE patient with high anti-C1q Ab titer against purified human C1q. Six Fabs that exhibited strong binding to C1q in ELISA were isolated. The anti-C1q Fabs recognized neoepitopes that were only exposed on bound C1q and not present on soluble C1q mapping to different regions of the collagen-like region of C1q. Analysis of the genes encoding the variable H and L chains of the IgG-derived anti-C1q Fab revealed that all the variable H and L chain regions were highly mutated, with nucleotide and amino acid homologies to the closest germline in the range of 71–97% (average 85 ± 4) and 72–92% (average 88 ± 6), respectively. In addition, the variable region of the Fabs exhibited high replacement to silent ratios. The six anti-C1q Fabs were shown to be of high affinity, with a K_d ranging from of 8.4×10^{-8} M to 1.4×10^{-7} M, comparable to an antiviral immune response. Our data underlines the notion that the development of anti-C1q Abs in SLE is the consequence of an Ag-driven, affinity-matured immune response. Those anti-C1q Fabs are unique tools to address how complement C1q is implicated in the pathogenesis of SLE.

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Human acute myeloid leukemia $CD34^+CD38^-$ stem cells are susceptible to allorecognition and lysis by single KIR-expressing natural killer cells

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Abstract:
The concept of tumor immunosurveillance has raised prospects for natural killer cell-based immunotherapy of human cancer. The cure of acute myeloid leukemia may depend on eradication of leukemic stem cells, the self-renewing component of leukemia. Whether natural killer cells can recognize and lyse leukemic stem cells is not known. To develop strategies that effectively target acute myeloid leukemia-leukemic stem cells, we investigated anti-leukemic effects of human alloreactive single KIR+ natural killer cells. Natural killer effectors with KIR specificity mismatched with respect to HLA class I allotype of target cells effectively recognized acute myeloid leukemia-leukemic stem cells defined phenotypically as $CD34^+CD38^-$, while healthy bone marrow-derived $CD34^+CD38^-$ hematopoietic stem cells were spared, as demonstrated by cytotoxicity and hematopoietic colony-forming assays. The HDAC inhibitor valproic acid increased the activating NKG2D ligand-dependent lysis of acute myeloid leukemia-$CD34^+CD38^-$ leukemic stem cells. These results show that alloreactive natural killer cells have the potential to detect and target leukemic stem cells, and thus to improve the treatment outcome in acute myeloid leukemia.

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Prevalence of Polyomavirus BK and JC Infection and Replication in 400 Healthy Blood Donors

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Abstract:

Background: The replication of BK virus (BKV) and JC virus (JCV) is linked to polyomavirus-associated nephropathy, hemorrhagic cystitis, and multifocal leukoencephalopathy in immunodeficient patients, but the behavior of these viruses in immunocompetent individuals has hardly been characterized.

Methods: We used EIA to study samples obtained from 400 healthy blood donors aged 20–59 years for BKV- and JCV-specific antibodies against virus-like particles. We also studied BKV and JCV loads in plasma and urine among these individuals by use of real-time polymerase chain reaction.

Results: IgG seroprevalence was 82% (328 of 400 donors) for BKV and 58% (231 of 400) for JCV. As age increased (age groups were divided by decade), the seroprevalence of BKV decreased from 87% (87 of 100) in the youngest group (aged 20–29 years) to 71% (71 of 100) in the oldest group (aged 50–59 years) (p = .006), whereas the seroprevalence of JCV increased from 50% (50 of 100) in the youngest group to 68% (68 of 100) in the oldest group (p = .06). Asymptomatic urinary shedding of BKV and JCV was observed in 28 (7%) and 75 (19%) of 400 subjects, respectively, with median viral loads of 3.51 and 4.64 log copies/mL, respectively (p <.001). Unlike urinary BKV loads, urinary JCV loads were positively correlated with IgG levels. The shedding of JCV was more commonly observed among individuals who were seropositive only for JCV, compared with individuals who were seropositive for both BKV and JCV, suggesting limited cross-protection from BKV immunity. Noncoding control regions were of archetype architecture in all cases, except for 1 rearranged JCV variant. Neither BKV nor JCV DNA was detected in plasma.

Conclusions: Our study provides important data about polyomavirus infection and replication in healthy, immunocompetent individuals. These data indicate significant differences between BKV and JCV with respect to virus-host interaction and epidemiology.

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Simultaneous Isolation of DNA, RNA, and Proteins for Genetic, Epigenetic, Transcriptomic, and Proteomic Analysis

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Abstract:

Analysis of DNA, RNA, and proteins for downstream genetic, epigenetic, transcriptomic, and proteomic analysis holds an important place in the field of medical care and life science. This is often hampered by the limited availability of sample material. For this reason, there exists an increasing interest for simultaneous isolation of DNA, RNA and proteins from a single sample aliquot. Several kit-systems allowing such a procedure have been introduced to the market. We present an approach using the AllPrep method for simultaneous isolation of DNA, RNA and proteins from several human specimens, such as whole blood, buffy coat, serum, plasma and tissue samples. The quantification and qualification of the isolated molecular species were assessed by different downstream methods: NanoDrop for measuring concentration and purity of all molecular species; DNA and RNA LabChip for fractionation analysis of nucleic acids; quantitative PCR for quantification analysis of DNA and RNA; thymidine-specific cleavage mass array on MALDI-TOF silico-chip for epigenetic analysis; Protein LabChip and two-dimensional (2D) gel electrophoresis for proteomic analysis. With our modified method, we can simultaneously isolate DNA, RNA and/or proteins from one single sample aliquot. We could overcome to some method limitations like low quality or DNA fragmentation using reamplification strategy for performing high-throughput downstream assays. Fast and easy performance of the procedure makes this method interesting for all fields of downstream analysis, especially when using limited sample resources. The cost-effectiveness of the procedure when material is abundantly available has not been addressed. This methodological improvement enables to execute such experiments that were not performable with standard procedure, and ensures reproducible outcome.

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Molecular Cancer 8, 105–113, 2009 IF5,4

Levels of plasma circulating cell free nuclear and mitochondrial DNA as potential biomarkers for breast tumors

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Abstract:

Background: With the aim to simplify cancer management, cancer research lately dedicated itself more and more to discover and develop non-invasive biomarkers. In this connection, circulating cell-free DNA (ccf DNA) seems to be a promising candidate. Altered levels of ccf nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) have been found in several cancer types and might have a diagnostic value.

Methods: Using multiplex real-time PCR we investigated the levels of ccf nDNA and mtDNA in plasma samples from patients with malignant and benign breast tumors, and from healthy controls. To evaluate the applicability of plasma ccf nDNA and mtDNA as a biomarker for distinguishing between the three study-groups we performed ROC (Receiver Operating Characteristic) curve analysis. We also compared the levels of both species in the cancer group with clinicopathological parameters.

Results: While the levels of ccf nDNA in the cancer group were significantly higher in comparison with the benign tumor group ($P < 0.001$) and the healthy control group ($P < 0.001$), the level of ccf mtDNA was found to be significantly lower in the two tumor-groups (benign: $P < 0.001$; malignant: $P = 0.022$). The level of ccf nDNA was also associated with tumor-size (<2 cm vs. >2 cm<5 cm; 2250 vs. 6658; Mann-Whitney-U-Test: $P = 0.034$). Using ROC curve analysis, we were able to distinguish between the breast cancer cases and the healthy controls using ccf nDNA as marker (cut-off: 1866 GE/ml; sensitivity: 81%; specificity: 69%; $P < 0.001$) and between the tumor group and the healthy controls using ccf mtDNA as marker (cut-off: 463282 GE/ml; sensitivity: 53%; specificity: 87%; $P < 0.001$).

Conclusion: Our data suggests that nuclear and mitochondrial ccf DNA have potential as biomarkers in breast tumor management. However, ccf nDNA shows greater promise regarding sensitivity and specificity.

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JC Virus-Specific Immune Responses in Human Immunodeficiency Virus Type 1 Patients with Progressive Multifocal Leukoencephalopathy

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Abstract:

Progressive multifocal leukoencephalopathy (PML) is a frequently fatal disease caused by uncontrolled polyomavirus JC (JCV) in severely immunodeficient patients. We investigated the JCV-specific cellular and humoral immunity in the Swiss HIV Cohort Study. We identified PML cases ($n = 29$), as well as three matched controls per case ($n = 87$), with prospectively cryopreserved peripheral blood mononuclear cells and plasma at diagnosis. Nested controls were matched according to age, gender, $CD4^+$ T-cell count, and decline. Survivors ($n = 18$) were defined as being alive for >1 year after diagnosis. Using gamma interferon enzyme-linked immunospot assays, we found that JCV-specific T-cell responses were lower in nonsurvivors than in their matched controls ($P = 0.08$), which was highly significant for laboratory- and histologically confirmed PML cases ($P = 0.004$). No difference was found between PML survivors and controls or for cytomegalovirus-specific T-cell responses. PML survivors showed significant increases in JCV-specific T cells ($P = 0.04$) and immunoglobulin G (IgG) responses ($P = 0.005$). IgG responses in survivors were positively correlated with $CD4^+$ T-cell counts ($P = 0.049$) and negatively with human immunodeficiency virus RNA loads ($P = 0.03$). We conclude that PML nonsurvivors had selectively impaired JCV-specific T-cell responses compared to $CD4^+$ T-cell-matched controls and failed to mount JCV-specific antibody responses. JCV-specific T-cell and IgG responses may serve as prognostic markers for patients at risk.

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Flt3 ligand–receptor interaction is important for maintenance of early thymic progenitor numbers in steady-state thymopoiesis

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Abstract:
T-cell production throughout life depends on efficient colonization and intrathymic expansion of BM-derived hematopoietic precursors. After irradiation-induced thymic damage, thymic recovery is facilitated by Flt3 ligand (FL), expressed by perivascular fibroblasts surrounding the thymic entry site of Flt3 receptor-positive progenitor cells. Whether intrathymic FL-Flt3 interactions play a role in steady-state replenishment of T cells remains unknown. Here, using competitive BM transplantation studies and fetal thymic organ cultures we demonstrated the continued numerical advantage of $Flt3^+$ intrathymic T-cell precursors. Sub-kidney capsule thymic transplantation experiments, in which WT and $FL^{-/-}$ thymic lobes were grafted into $FL^{-/-}$ recipients, revealed that FL expression by the thymic microenvironment plays a role in steady-state thymopoiesis. The deficiency of the most immature thymic T-cell precursors correlated to upregulation of FL by thymic $MTS15^+$ fibroblasts, suggesting that the number of $Flt3^+$ progenitor cells may regulate the thymic expression of this cytokine. Together, these results show that FL expression by thymic stromal fibroblasts interacting with $Flt3^+$ T-cell progenitors is important for the physiological maintenance of early T-cell development.

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Biochemical Journal | 424, 119–127, 2009 | IF4,4

The human urocortin2 gene is regulated by hypoxia: identification of a hypoxia-responsive element in the 3′ -flanking region

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Abstract:
Ucn2 (urocortin 2) has been shown to exert potent beneficial effects in the cardiovascular system, including inhibition of apoptosis, improvement of cardiomyocyte contractility and decrease of oxidative stress. The mechanisms that contribute to the regulation of hUcn2 (human Ucn2) expression in cardiovascular pathologies are not known. In the present study, we analysed the mechanism by which hypoxia, a major stimulus in ischaemic heart disease, regulates Ucn2 gene expression. Hypoxia and CPX (ciclopirox olamine), which prevents proteolytic degradation of HIF (hypoxia-inducible factor), significantly increased hUcn2 mRNA levels in TE-671 cells. Gene silencing of endogenous HIF1α abolishes this increase. Hypoxia and CPX activated a luciferase-linked fragment of the 3′FLR (3′-flanking region) of the *hUcn2* gene containing two putative HREs (hypoxia-response elements), HRE1 and HRE2. Site-directed mutagenesis experiments demonstrated that HRE1 is required for HIF1α-dependent luciferase activation. This activation was conserved in constructs with the 3′FLR fragment placed upstream of the luciferase gene, indicating an enhancer function for HRE1. Competition assays revealed direct binding between HRE1 and HIF1α. Regulation of Ucn2 by hypoxia was confirmed in rat neonatal cardiomyocytes and in cardiac-derived H9c2 cells transfected with constructs of the 3′FLR of the *hUcn2* gene. In conclusion, our study demonstrates that hypoxia induces hUcn2 expression via a specific HRE in the 3′FLR of the *hUcn2* gene, which interacts with the transcription factor HIF1alpha. Hypoxia-mediated stimulation of cardioprotective Ucn2 may help to preserve cardiac function and prevent apoptosis in ischaemic conditions in the heart.

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Journal of Clinical Microbiology | Journal of Clinical Microbiology | 47, 2577–2585, 2009 IF3,9

Antibody Responses to Recombinant Polyomavirus BK Large T and VP1 Proteins in Young Kidney Transplant Patients

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Abstract:
BK virus (BKV)-specific immunity is critical for polyomavirus-associated nephropathy, but antibody responses are incompletely defined. We compared the hemagglutination inhibition assay (HIA) with immunoglobulin G enzyme immunoassays (EIA) to BKV proteins expressed in baculovirus-infected insect cells. N-terminal, internal, and C-terminal domains of the BKV large T antigen (BKLT) were fused to glutathione S-transferase (GST), yielding GST-BKLTD1, GST-BKLTD2, and GST-BKLTD3, respectively. The BKV capsid VP1 was expressed as a GST fusion (BKVP1) or as a native VP1 assembled into viruslike particles (BKVLP). We tested 422 sera from 28 healthy donors (HD), 99 dialysis patients (DP; median age, 15 years; range, 3 to 32 years), and 46 age-matched kidney transplant patients (KTP; median age, 15 years; range, 2 to 33 years). In HD, HIA and BKVLP EIA both yielded a 91.7% seroreactivity, whereas all other EIA responses were lower (BKVP1, 83.3%; BKLTD1, 25%; BKLTD2, 29%; BKLTD3, 40%). HIA titers significantly correlated with EIA levels for BKVLP, BKVP1, and BKLTD1 but not for BKLTD2 or BKLTD3, which were barely above the cut-off. In DP, the seroreactivities of HIA, BKVLP, and BKLTD1 were lower than that in HD (63.6%, 86.9%, and 10.1%, respectively) and they had lower titers ($P < 0.001$). In KTP, seropositivities for BKVLP, BKVP1, and BKLTD1 were 78%, 50%, and 17%, respectively, but anti-BKVLP levels increased significantly in KTP with viruria and viremia, whereas anti-BKLTD1 levels increased after clearing sustained BKV viremia. In conclusion, anti-BKVLP is equivalent to HIA in HD but is more sensitive to determine the BKV serostatus in DP and KTP. In KTP, anti-BKVLP responds to recent BKV viruria and viremia, whereas anti-BKLTD1 may indicate emerging BKV-specific immune control.

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Transplantation | 88, 1161–1168, 2009 IF 3,8

Inhibition of Polyomavirus BK-Specific T-Cell Responses by Immunosuppressive Drugs

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Abstract:
Background: Reducing immunosuppression is the treatment of choice for polyomavirus-associated nephropathy in kidney transplant (KT) patients, but strategies and targets are uncertain.

Method: Using interferon-γ ELISpot assays, we investigated immunosuppressive drug levels and polyomavirus BK (BKV) large T-antigen-specific T-cell responses in KT patients in vivo and in healthy donors after titrating immunosuppression in vitro.

Result: In KT patients, BKV-specific T-cell responses were inversely correlated with tacrolimus trough levels (R^2=0.28, P<0.002), but not with mycophenolate levels, prednisone, or overall immunosuppressive dosing. In vitro tacrolimus concentrations above 6 ng/mL inhibited BKV- and cytomegalovirus-specific T-cells more than 50%, whereas less than 30% inhibition was observed below 3 ng/mL. Inhibition by cyclosporine A was more than 50% at concentrations of 1920 ng/mL and less than 30% below 960 ng/mL, corresponding to clinical C_0 trough levels of 200 and 100 ng/mL, respectively. However, mycophenolate up to 8 µg/mL, leflunomide 50 µg/mL, or sirolimus concentrations 64 ng/mL did not inhibit BKV-specific interferon-γ production, but antigen-dependent T-cell expansion.

Conclusions: Calcineurin-inhibitor concentrations are critical for BKV-specific T-cell activation. Reducing calcineurin inhibitors should be considered as first step, whereas conversion to mTOR inhibitors may be an attractive alternative or second step that should be validated in clinical BKV intervention trials.

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European Journal of Pharmaceutical Sciences 38, 384–388, 2009 IF 3,7

Comparison of the dissolution and pharmacokinetic profiles of two galenical formulations of the endothelin receptor antagonist macitentan

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Abstract:
NMacitentan (ACT-064992) is an orally active endothelin receptor antagonist. We first compared the in vitro dissolution characteristics of uncoated and film-coated tablets with hard gelatin capsules containing 10 mg ACT-064992. Subsequently, we compared the oral pharmacokinetics of ACT-064992 and its active metabolite ACT-132577 of the coated tablet and the gelatin capsule formulation in 11 male volunteers.
The dissolution profile showed a rapid disintegration of all formulations with >90% dissolution of ACT-064992 within 45 min. The pharmacokinetics of ACT-064992 and its metabolite ACT-132577 were comparable for the two formulations. ACT-064992 revealed a slow absorption (median t_{max} 8 h) and a terminal half-life of approximately 13 h. Bioequivalence criteria were met for AUC_{0-t} and $AUC_{0-\infty}$. Mean C_{max} was 19% lower after ingestion of the tablet compared to capsules with its lower 90% confidence limit below the accepted bioequivalence range. The pharmacokinetics of the metabolite ACT-132577, characterized by a t_{max} of approximately 48 h and a terminal half-life of approximately 45 h, was not different between the two formulations.
We conclude that the absorption profile of the tablet differs from the capsule in peak but not in total exposure, which is not expected to be of clinical significance.

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Nephrology Dialysis Transplantation 25, 426–433, 2010 IF 3,6

Urinary excretion of carnitine as a marker of proximal tubular damage associated with platin-based antineoplastic drugs

M. Haschke[1], T. Vitins[1], S. Lüde[1], L. Todesco[1], K. Novakova[1], R. Herrmann[2] and S. Krähenbühl[1]

Abstract:
Background: Patients treated with cisplatin or carboplatin show increased renal excretion of carnitine. It is currently unclear whether this is also the case for oxaliplatin and which are the responsible mechanisms.

Methods. We investigated 22 patients treated either with a single dose of cisplatin, carboplatin or oxaliplatin. Carnitine and kidney function parameters were determined in plasma and urine. Inhibition and mRNA expression of OCTN2, the principle carnitine transporter, were assessed in L6 cells overexpressing OCTN2 and in 293-EBNA cells, respectively.

Results: Renal excretion of free and short-chain acylcarnitine increased already at the day of administration was maximal the day after and had normalized 1 week after administration of cisplatin, carboplatin or oxaliplatin. The renal excretion fractions for free carnitine and acylcarnitines increased 4–10 times during treatment with platin derivatives. Renal excretions of 1-microglobulin and other proximal tubular markers were also increased, compatible with a proximal tubular defect. Direct inhibition of OCTN2 expressed in L6 cells by cisplatin, oxaliplatin or platinum2+ could not be demonstrated, and experiments using urine from patients treated with cisplatin inhibited OCTN2 activity no more than expected from the carnitine content in the respective urine sample. Cisplatin was associated with a time- and concentration-dependent decrease of OCTN2 mRNA and protein expression in 293-EBNA cells.

Conclusions: All platin derivatives investigated are associated with renal tubular damage in humans without significantly affecting glomerular function. The rapid onset and complete reversibility of this effect favour a functional mechanism such as impaired expression of OCTN2 in proximal tubular cells.

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Current Neurovascular Research 6, 279–287, 2009 IF 3,6

Cortical and Putamen Age-Related Changes in the Microvessel Density and Astrocyte Deficiency in Spontaneously Hypertensive and Stroke-Prone Spontaneously Hypertensive Rats

M.-F. Ritz, F. Fluri, S. T. Engelter, N. Schaeren-Wiemers and P. A. Lyrer

Abstract:
Cerebral small vessel disease (SVD) is a major contributor to dementia in the elderly, and hypertension represents a major cause for developing the disease. However, little is known about its development and progression. Modifications of large cerebral arteries due hypertension are thought to participate to the development of small ischemic infarcts, but the status of the small vessels before the establishment of hypertension is not well defined. Using spontaneously hypertensive rats (SHR) and stroke-prone SHR (SP-SHR) as a models for SVD, we analysed the effect of hypertension on the microvasculature in the cortex and putamen, and on its relationship with astrocytes in animals aged 2 to 9 months. Compared with the normotensive Wistar-Kyoto rats (WKY), the densities of the collagen type IV-positive capillaries were significantly higher in both brain areas of young SHR and SP-SHR. In contrast, the expression of the astrocytic marker GFAP was significantly lower in these animals, whereas astrogliosis was observed after 6 months in their cortex only. To investigate if chronic hypoxia occurs due to the lower number of astrocytes in young SHR and SP-SHR, we evaluated the levels of HIF-1α in both brain regions. The accumulation of HIF-1α was not observed at the youngest ages, but was apparent in neurons of 9-month-old SHR and SP-SHR. Our results indicate that the brains of young SHR and SP-SHR rats show evidence of cellular imbalance between microvessels and astrocytes at the neurovascular unit that may lead to their higher vulnerability to hypoxic events at older ages.

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BMC Medicine 8, 1–18, 2010 IF 3,3

IGF-I induced genes in stromal fibroblasts predict the clinical outcome of breast and lung cancer patients

M. Rajski[1], R. Zanetti-Dällenbach[4], B. Vogel[1], R. Herrmann[1,3], C. Rochlitz[1,3] and M. Buess[1,2]

Abstract:

Background: Insulin-like growth factor-1 (IGF-I) signalling is important for cancer initiation and progression. Given the emerging evidence for the role of the stroma in these processes, we aimed to characterize the effects of IGF-I on cancer cells and stromal cells separately.

Methods: We used an *ex vivo* culture model and measured gene expression changes after IGF-I stimulation with cDNA microarrays. *In vitro* data were correlated with *in vivo* findings by comparing the results with published expression datasets on human cancer biopsies.

Results: Upon stimulation with IGF-I, breast cancer cells and stromal fibroblasts show some common and other distinct response patterns. Among the up-regulated genes in the stromal fibroblasts we observed a significant enrichment in proliferation associated genes. The expression of the IGF-I induced genes was coherent and it provided a basis for the segregation of the patients into two groups. Patients with tumours with highly expressed IGF-I induced genes had a significantly lower survival rate than patients whose tumours showed lower levels of IGF-I induced gene expression ($P = 0.029$ - Norway/Stanford and $P = 7.96e-09$ - NKI dataset). Furthermore, based on an IGF-I induced gene expression signature derived from primary lung fibroblasts, a separation of prognostically different lung cancers was possible ($P = 0.007$ - Bhattacharjee and $P = 0.008$ - Garber dataset).

Conclusion: Expression patterns of genes induced by IGF-I in primary breast and lung fibroblasts accurately predict outcomes in breast and lung cancer patients. Furthermore, these IGF-I induced gene signatures derived from stromal fibroblasts might be promising predictors for the response to IGF-I targeted therapies.

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BMC Cancer 9, 71–80, 2009 IF3,1

Loss of heterozygosity of TRIM3 in malignant gliomas

J. L. Boulay[1], U. Stiefel[2], E. Taylor[1] , B. Dolder[1] , A. Merlo[1] and F. Hirth[2,3]

Abstract:

Background: Malignant gliomas are frequent primary brain tumors associated with poor prognosis and very limited response to conventional chemo- and radio-therapies. Besides sharing common growth features with other types of solid tumors, gliomas are highly invasive into adjacent brain tissue, which renders them particularly aggressive and their surgical resection inefficient. Therefore, insights into glioma formation are of fundamental interest in order to provide novel molecular targets for diagnostic purposes and potential anti-cancer drugs. Human *Tripartite motif protein* 3 (TRIM3) encodes a structural homolog of *Drosophila brain tumor* (*brat*) implicated in progenitor cell proliferation control and cancer stem cell suppression. *TRIM3* is located within the loss of allelic heterozygosity (LOH) hotspot of chromosome segment 11p15.5, indicating a potential role in tumor suppression. ...

Methods: Here we analyze 70 primary human gliomas of all types and grades and report somatic deletion mapping as well as single nucleotide polymorphism analysis together with quantitative real-time PCR of chromosome segment 11p15.5.

Results: Our analysis identifies LOH in 17 cases (24%) of primary human glioma which defines a common 130 kb-wide interval within the *TRIM3* locus as a minimal area of loss. We further detect altered genomic dosage of *TRIM3* in two glioma cases with LOH at 11p15.5, indicating homozygous deletions of *TRIM3*.

Conclusion: Loss of heterozygosity of chromosome segment 11p15.5 in malignant gliomas suggests *TRIM3* as a candidate brain tumor suppressor gene.

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Journal of Neuroscience Research 88, 774–783, 2010 IF3,1

Reduced size of the dendritic tree does not protect Purkinje cells from excitotoxic death

O. S. Gugger, J. P. Kapfhammer

Abstract:
Purkinje cell loss by excitotoxic damage is a typical finding in many cerebellar diseases. One important aspect of this high sensitivity of Purkinje cells to excitotoxic death might be the enormous size of their dendritic tree, with a high load of excitatory glutamate receptors. We have studied whether reduction in the size of the dendritic tree might confer resistance against excitotoxic death to Purkinje cells. We have grown Purkinje cells in organotypic cerebellar slice cultures under chronic activation of metabotropic glutamate receptors or of protein kinase C. Both treatments strongly reduced dendritic tree size. After this treatment, cells were exposed to the glutamate receptor agonist AMPA, which has a strong excitotoxic effect on Purkinje cells. We found that Purkinje cells with small dendritic trees were as sensitive to AMPA exposure as untreated control cells with large dendritic trees. Immunostaining against vesicular glutamate transporter 1 revealed that the small dendritic trees were densely covered by glutamatergic terminals. Our results indicate that the expansion of the dendritic tree and the total number of AMPA receptors per neuron do not play a major role in determining the susceptibility of Purkinje cells to excitotoxic death.

Anatomical Institute, Department of Biomedicine, University of Basel, Basel, Switzerland

Transplant Infectious Disease 11, 100–105, 2009 IF 2,5

Outcome of influenza infections in outpatients after allogeneic hematopoietic stem cell transplantation

N. Khanna[1,2], I. Steffen[2], J.-D. Studt[3], A. Schreiber[3], T. Lehmann[3], M. Weisser[1], U. Flückiger[1], A. Gratwohl[3], J. Halter[3] and H.H. Hirsch[1,2]

Abstract:

Background: Influenza can cause significant morbidity and mortality in patients after hematopoietic stem cell transplantation (HSCT). The diagnostic methods and antiviral treatment have scarcely been investigated.

Methods: We retrospectively identified influenza-infected patients with upper or lower respiratory tract infection (RTI) diagnosed by culture and polymerase chain reaction (PCR) testing between November 2007 and April 2008. Treatment with oseltamivir 75 mg twice daily and serial nasal swabs were performed at the discretion of the treating physician.

Results: We identified 21 influenza infections in 19 patients: 19 with upper RTI and 2 with lower RTI. At diagnosis, all 21 samples were positive for PCR with a median influenza load of 5.9 log10 copies/mL. Culture was positive in 14 (67%) patients. Influenza A virus was diagnosed in 8 (38%) episodes and influenza B virus in 13 (62%) episodes. Two patients were sequentially infected by influenza A, followed by B after 38 and 47 days, respectively. Eighteen (86%) patients were treated with oseltamivir for 11 days (median, interquartile range [IQR]: 8–14). No progression to lower RTI or mortality occurred. Shedding persisted for 12 days (median, IQR: 8–13). Absolute lymphocyte count at diagnosis correlated inversely with shedding of the virus (P<0.001).

Conclusions: Oseltamivir is well tolerated and may reduce mortality of influenza virus-infected patients after HSCT. PCR may help to optimize diagnosis and to monitor treatment strategies.

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International Journal of Oncology 35, 1123–1131, 2009 IF 2,2

The 10q25.3-26.1 G protein-coupled receptor gene *GPR26* is epigenetically silenced in human gliomas

J.-L. Boulay, M.-C. S. Ionescu, B. Sivasankaran, M. Labuhn, B. Dolder-Schlienger, E. Taylor, P. Morin, B. A. Hemmings, M. M. Lino, G. Jones, D. Maier and A. Merlo

Abstract:
Loss of heterozygosity (LOH) of the entire chromosome 10 is the most frequent genetic alteration in human glioblastoma (GBM). In addition to *PTEN/MMAC1* on 10q23.3, clustering of partial deletion break-points on 10q25.3-26.1 points to a second suppressor locus. The proposed target gene DMBT1 was not confirmed. By somatic deletion mapping of this region, we identified the complementary DNA encoding the human homologue of rat orphan G protein-coupled receptor GPR26. *GPR26* is highly expressed in fetal and adult brain, but frequently reduced or absent in glioma cells and biopsies, due to *de novo* methylation of its 5' CpG island. Silencing of *GPR26* was reversed with 5-aza-deoxycytidine and the histone deacetylase inhibitor trichostatin A. Furthermore, overexpression of *GPR26* in HEK and in U87 glioma cells increased intracellular cAMP concentration which is considered to induce astrocytic differentiation. Interestingly, we observed concomitant silencing of *GPR26* with *O^6-methylguanine-DNA methyl transferase* (*MGMT*), a DNA repair gene co-localized on 10q25.3-26.1 (p=0.0001). We conclude that epigenetic silencing is a common mechanism in malignant gliomas that simultaneously inactivates *MGMT* and *GPR26*. The 10q25.3-26.1 region may contain an important epigenetic pathway in brain tumorigenesis.

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Dermatology 220, 82–92, 2010 IF 2,2

Ectoparasites from Feral Pigeons Affecting Humans

D. Haag-Wackernagel[1] and A. J. Bircher[2]

Abstract:
Feral pigeons pose a considerable health risk to the human population. They are vectors of infectious diseases and source of antigens causing allergic diseases. Breeding and roosting sites of pigeons harbor parasites that may infest humans. In the present article, a concomitant parasitization of a young female with 3 different ectoparasites, the bedbug Cimex lectularius, the pigeon tick Argas reflexus and the red mite Dermanyssus gallinae, is reported. The parasites invaded the apartment from a balcony used as roost by feral pigeons and infested the patient continuously over a period of more than 2 months. To our knowledge this case presents the first record of a coincidental infestation of a single patient with several ectoparasite species deriving from feral pigeons. Additionally we report general symptoms in the patient probably caused by the high number of stings. Dermatologists should be aware of the possibility of an infestation with ectoparasites deriving from feral pigeons. In a review we give an overview on the most important ectoparasites transmitted from feral pigeons to humans and their importance for the dermatologist.

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PLOS one PLOS one 4, e7443, 1–12, 2009 IFN,A

GSK3β Regulates Differentiation and Growth Arrest in Glioblastoma

S. Korur[1], R. M. Huber[1], B. Sivasankaran[1], M. Petrich[1], P. Morin, Jr[2], B. A. Hemmings[2], A. Merlo[1] and M. M. Lino[1]

Abstract:
Cancers are driven by a population of cells with the stem cell properties of self-renewal and unlimited growth. As a subpopulation within the tumor mass, these cells are believed to constitute a tumor cell reservoir. Pathways controlling the renewal of normal stem cells are deregulated in cancer. The polycomb group gene Bmi1, which is required for neural stem cell self-renewal and also controls anti-oxidant defense in neurons, is upregulated in several cancers, including medulloblastoma. We have found that Bmi1 is consistently and highly expressed in GBM. Downregulation of Bmi1 by shRNAs induced a differentiation phenotype and reduced expression of the stem cell markers Sox2 and Nestin. Interestingly, expression of glycogen synthase kinase 3 beta (GSK3β), which was found to be consistently expressed in primary GBM, also declined. This suggests a functional link between Bmi1 and GSK3β. Interference with GSK3β activity by siRNA, the specific inhibitor SB216763, or lithium chloride (LiCl) induced tumor cell differentiation. In addition, tumor cell apoptosis was enhanced, the formation of neurospheres was impaired, and clonogenicity reduced in a dose-dependent manner. GBM cell lines consist mainly of CD133-negative (CD133-) cells. Interestingly, ex vivo cells from primary tumor biopsies allowed the identification of a CD133- subpopulation of cells that express stem cell markers and are depleted by inactivation of GSK3β. Drugs that inhibit GSK3, including the psychiatric drug LiCl, may deplete the GBM stem cell reservoir independently of CD133 status.

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[2] Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Hundedenkmal «Mali»

Auf dem Schulhof der Orientierungsschule
Thomas Platter/Wettstein/Claragraben 50

Im Jahr 1992 wurde die Fassade des Wettsteinschulhauses von einer Maurerfirma saniert. Steinmetz Julijan Bor und sein Hund Mali waren mit von der Partie. Eines Tages kam Herrn Bor, der gerade erst das kriegsgebeutelte Ex-Jugoslawien verlassen hatte, die Idee, nachdem er schon die Felspyramide auf dem Pausenhof mit Masken und Fratzen verziert hatte, die altägyptischen Hundeskulpturen vor Augen seinem treuen Gefährten auf vier Pfoten ein Denkmal in Sandstein zu setzen. Das Hochbauamt willigte ein. So nahm Malis Abbild Platz auf dem Affenfelsen, wie die begeh- und besitzbaren Felspyramiden auf den Schulhöfen genannt werden. Die Schüler waren begeistert und die Identifikation gross, kannten sie doch den Vierbeiner nur zu gut, der immer mit ihnen herum tollte. In einem feierlichen Akt mit Kinderfest wurde der «Hundefelsen» eingeweiht. In der Zwischenzeit kamen andere Schülergenerationen nach, die Mali nicht mehr kannten. Einmal wurde ihm ein Ohr abgebrochen und auch die Schnauze bekam das eine oder andere ab. Nun soll Mali in Bronze gegossen werden, als Andenken an einen liebenswürdigen Hund, der bis zu seinem Tod im Jahr 2005 seinen Herrn so treu begleitete, stellvertretend für alle Hunde dieser Welt, die so unerschütterlich mit uns Menschen durch dick und dünn gehen.

Dissertationen

Seit dem 9. Dez. 2009 darf sich **Franz Schaub** von der FG Exp. Hematology (Dept. Biomedizin USB) Herr Dr. nennen. Er befasste sich in seiner Dissertation mit dem Thema: „Clonal Evolution of Mutation Events in Myeloproliferative Neoplasms".

Am 11. Dez. 2009 hat **Federica Facciotti** von der FG Exp. Immunology (Dept. Biomedizin USB) ihre Dissertation erfolgreich abgeschlossen. Der Titel ihrer Doktorarbeit hiess: „Lipid antigen presentation and thymic selection of iNKT cells ".

Mit der Doktorprüfung am 17. Dez. 2009 schloss **Silvia Francioli** von der FG Tissue Engineering (Dept. Biomedizin USB) erfolgreich ihre Dissertationszeit ab. Das Thema ihrer Doktorarbeit lautete: „Influence of chondrocytes differentiation stage on the capacity to generate cartilaginous tissue in vitro".

Am 21. Dez. 2009 war es für **Valentina Mele** von der FG Oncology Surgery (Dept. Biomedizin USB) soweit, sie beendete erfolgreich ihre Doktorandenzeit, in der sie sich mit „Effects of mesenchymal stromal cells on proliferation and invasive potential of tumor cells of colorectal cancer" auseinandergesetzt hatte.

Ebenfalls im Dez. 2009 stellte sich **Michaela Metz** von der FG Synaptic Plasticity (Inst. für Physiologie) dem Dissertationskomitee. Der Titel ihrer Doktorarbeit lautete: „Characterization of a Novel Family of $GABA_B$ Receptor Interacting Proteins".

Said Abdel Aziz ebenfalls von der FG Synaptic Plasticity (Inst. für Physiologie) ging dem Thema „Identification of serine 867 as new phosphorylation site on the $GABA_B$ receptor: Characterization of physiological effects" nach und darf seit Dez. 2009 den Doktortitel tragen.

Am 27. Jan. 2010 stellte sich **Mirko Vukcevic** von der FG Perioperative Patient Safety (Dept. Biomedizin USB) erfolgreich den Fragen des Dissertationskomitees. Er beschäftigte sich in seiner Doktorarbeit mit dem Thema „Calcium homeostasis and role of ryanodine receptor type 1 (RyR1) in immune cells".

Auszeichnungen

Advanced Investigator Grant from the European Research Council to Ed Palmer

Ed Palmer received an Advanced Grant from the European Research Council, for a project entitled, Terra Incognita: T cell receptor $\alpha\beta$ - in control of signal initiation and T cell fate. The grant provides 3,000,000 SFr to study the development of self-tolerance in the immune system and the failure of self-tolerance in individuals suffering from autoimmune diseases. The grant is one of 89 given in the Life Sciences in Europe, the first Advanced Grant given to a member of the Medical Faculty and the fourth Advanced Grant received by a faculty member at the University of Basel.

Fakultäts- und DBM-Preis an Jean-Denis Benazet

Jean-Denis Benazet hat für seine Dissertation zum Thema „Interlinked signaling feedback loops and self-regulation during vertebrate limb development" (verteidigt am 17. Feb. 2009) am 27. Nov. 2009 den Fakultätspreis der Philosophisch-Naturwissenschaftlichen Fakultät der Universität Basel und am 17. Dez. 2009 den DBM-Preis für die beste Publikation des Jahres 2009 am DBM erhalten (Science:323, 1050-1053, 2009).

Herzliche Gratulation!

DEPARTEMENT BIOMEDIZIN USB

Anne-Sophie Benischke
Gyn. Oncology

Paul Bourgine
Tissue Engineering

Charles Hemion
Ocular Pharmac. & Physiology

Kapil Dev Kampe
Mol. Nephrology

Hélène Mereau
Childhood Leukemia

Philipp Müller
Cancer Immunology

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Cellular Neurophysiology

Michael Barz
Cellular Neurophysiology

Selma Becherer
Cellular Neurophysiology

Stefanie Heigele
Cellular Neurophysiology

Jörg Pohle
Cellular Neurophysiology

Ausserdem haben angefangen:

DEPARTEMENT BIOMEDIZIN USB

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Dominik Eichin, Immunobiology
Sucharita Geiger, Metabolism
Nicole Getzmann, Dermatology
Corina Metaxas, Immunnephrology
Maria Antonietta Sabatino,
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Julia Manzetti ,
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Simone Neu, Transplantation Virology
Edyta Swierad, Serology/Virology

Interne Wechsel:

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Brigitte Schneider,
Gyn. Endocrinology

Austritte:

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Ivana Majic, Infectious Diseases
Andrej Trampuz, Infectious Diseases
Anastasia Badmann, Mol. Nephrology
Martin Clauss, Infectious Diseases
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Daniela Baldoni, Infectious Diseases
Katrin Bühler, Cardiobiology
Anthony Collmann, Exp. Immunology
Silvia Francioli, Tissue Engineering
Naja Jann, Infection Biology
Regine Landmann, Infection Biology
Aleksandra Filipowicz,
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Monika Hermle, Stab
Dejing Pan, Exp. Hematology
Michal Rajski, Medical Oncology
Hui Wan, Clinical Immunology

INSTITUT FÜR ANATOMIE

Hélène Mereau,
Developmental Genetics

INSTITUT FÜR PHYSIOLOGIE

Said Abdel Aziz, Synaptic Plasticity

INSTITUT FÜR MEDIZINISCHE MIKRO-BIOLOGIE

Nesrin Saridas, Technical Support
Melanie Hug, Molecular Diagnostics
Karin Klaus Mühlhauser,
Administration

Congratulations

Das DBM gratuliert ganz herzlich!

Magnus Siegfried Neutzner
Geboren am 17.12.2009

Lisa Brüngger Brecht
Geboren am 1.2.2010

Herzlich willkommen, allerseits!

Aeneas Gianni' Barrera
Geboren am 6.1.2010

Ariadni-Zoe Kyriakakis-Buron
Geboren am 13.1.2010

Bienvenue Eric Spaety, neuer Layouter im DBM Facts Team

Hier stellt er sich vor:

Ich heisse Eric Spaety. Ich bin Franzose. 1958 wurde ich in Munster im Elsass geboren. Von 1974 bis 1977 machte ich eine Lehre als Schriftsetzer Typograph an der «Ecole des Arts Graphiques» in Mulhouse. Nach einer Weiterbildung an der Handwerkskammer in Colmar erhielt ich 1986 den Meisterbrief. Während dieser Zeit arbeitete ich in einer Druckerei in Colmar.

1989 ging ich zur Druckerei «Morf Bimo Print» in Basel, wo ich nun seit 20 Jahren in der «Druckvorstufe» arbeite. Der Buchdruck und der Kommunikationsbereich haben weitgehende Entwicklungen durchgemacht, und in den vergangenen 30 Jahren habe ich einschneidende Veränderungen miterlebt. Ich begann meinen Beruf in einer alten Werkstatt mit dem Drucken von Hand und Bleiwerkstücken in der Gutenbergschen Manier. Heute befinden wir uns im Universum der Informatik und ich drucke mit dem Computer, meinem Handwerkszeug.

In meiner Freizeit habe ich mich sehr im gesellschaftlichen Bereich engagiert. 18 Jahre lang war ich Mitglied des Stadtrates von Colmar. Heute nehme ich im «Marathon Club» von Colmar regelmässig an Läufen teil und bin Präsident des über 150 Mitglieder zählenden Vereins.

Second DBM Summer Event will take place on Thursday, August 12, 2010 on the «Sulzkopf in Muttenz»

Hiking-Tour, Barbecue, Several attractions

Anekdote zur Senkung der Arbeitsmoral

von Heinrich Böll

In einem Hafen an einer westlichen Küste Europas liegt ein ärmlich gekleideter Mann in seinem Fischerboot und döst. Ein schick angezogener Tourist legt eben einen neuen Farbfilm in seinen Fotoapparat, um das idyllische Bild zu fotografieren: blauer Himmel, grüne See mit friedlichen schneeweissen Wellenkämmen, schwarzes Boot, rote Fischermütze. Klick. Noch einmal: klick. Und da aller guten Dinge drei sind und sicher sicher ist, ein drittes Mal: klick.

Das spröde, fast feindselige Geräusch weckt den dösenden Fischer, der sich schläfrig aufrichtet, schläfrig nach einer Zigarettenschachtel angelt; aber bevor er das Gesuchte gefunden, hat ihm der eifrige Tourist schon eine Schachtel vor die Nase gehalten, ihm die Zigarette nicht gerade in den Mund gesteckt, aber in die Hand gelegt, und ein viertes Klick, das des Feuerzeuges, schließt die eilfertige Höflichkeit ab. Durch jenes kaum messbare, nie nachweisbare Zuviel an flinker Höflichkeit ist eine gereizte Verlegenheit entstanden, die der Tourist – der Landessprache mächtig – durch ein Gespräch zu überbrücken versucht.

«Sie werden heute einen guten Fang machen.»
Kopfschütteln des Fischers.

«Aber man hat mir gesagt, dass das Wetter günstig ist.»
Kopfnicken des Fischers.

«Sie werden also nicht ausfahren?»
Kopfschütteln des Fischers, steigende Nervosität des Touristen. Gewiss liegt ihm das Wohl des ärmlich gekleideten Menschen am Herzen, nagt an ihm die Trauer über die verpasste Gelegenheit.

«Oh, Sie fühlen sich nicht wohl?»
Endlich geht der Fischer von der Zeichensprache zum wahrhaft gesprochenen Wort über. «Ich fühle mich grossartig», sagt er. «Ich habe mich nie besser gefühlt.» Er steht auf, reckt sich, als wolle er demonstrieren, wie athletisch er gebaut ist. «Ich fühle mich phantastisch.»

Der Gesichtsausdruck des Touristen wird immer unglücklicher, er kann die Frage nicht mehr unterdrücken, die ihm sozusagen das Herz zu sprengen droht: *«Aber warum fahren Sie dann nicht aus?»*
Die Antwort kommt prompt und knapp. «Weil ich heute morgen schon ausgefahren bin.»

«War der Fang gut?»
«Er war so gut, dass ich nicht noch einmal auszufahren brauche, ich habe vier Hummer in meinen Körben gehabt, fast zwei Dutzend Makrelen gefangen...» Der Fischer, endlich erwacht, taut jetzt auf und klopft dem Touristen beruhigend auf die Schultern. Dessen besorgter Gesichtsausdruck erscheint ihm als ein Ausdruck zwar unangebrachter, doch rührender Kümmernis.
«Ich habe sogar für morgen und übermorgen genug», sagt er, um des Fremden Seele zu erleichtern. «Rauchen Sie eine von meinen?»
«Ja, danke.»

Zigaretten werden in die Münder gesteckt, ein fünftes Klick, der Fremde setzt sich kopfschüttelnd auf den Bootsrand, legt die Kamera aus der Hand, denn er braucht jetzt beide Hände, um seiner Rede Nachdruck zu verleihen.

«Ich will mich ja nicht in Ihre persönlichen Angelegenheiten mischen», sagt er, *«aber stellen Sie sich mal vor, Sie führen heute ein zweites, ein drittes, vielleicht sogar ein*

Foto: Ralfsen, Polen Ostsee Frische Nehrung

viertes Mal aus, und Sie würden drei, vier, fünf, vielleicht gar zehn Dutzend Makrelen fangen – stellen Sie sich das mal vor.» Der Fischer nickt.

«Sie würden», fährt der Tourist fort, *«nicht nur heute, sondern morgen, übermorgen, ja, an jedem günstigen Tag zwei-, dreimal, vielleicht viermal ausfahren – wissen Sie, was geschehen würde?»*
Der Fischer schüttelt den Kopf.

«Sie würden sich spätestens in einem Jahr einen Motor kaufen können, in zwei Jahren ein zweites Boot, in drei oder vier Jahren vielleicht einen kleinen Kutter haben, mit zwei Booten und dem Kutter würden Sie natürlich viel mehr fangen – eines Tages würden Sie zwei Kutter haben, Sie würden…», die Begeisterung verschlägt ihm für ein paar Augenblicke die Stimme, *«Sie würden ein kleines Kühlhaus bauen, vielleicht eine Räucherei, später eine Marinadenfabrik, mit einem eigenen Hubschrauber rundfliegen, die Fischschwärme ausmachen und Ihren Kuttern per Funk Anweisungen geben. Sie könnten die Lachsrechte erwerben, ein Fischrestaurant eröffnen, den Hummer ohne Zwischenhändler direkt nach Paris exportieren – und dann…»,* wieder verschlägt die Begeisterung dem Fremden die Sprache.

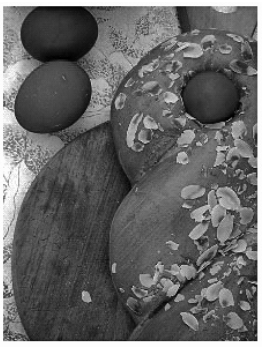
Kopfschüttelnd, im tiefsten Herzen betrübt, seiner Urlaubsfreude schon fast verlustig, blickt er auf die friedlich hereinrollende Flut, in der die ungefangenen Fische munter springen. *«Und dann»,* sagt er, aber wieder verschlägt ihm die Erregung die Sprache.
Der Fischer klopft ihm auf den Rücken, wie einem Kind, das sich verschluckt hat.
«Was dann?» fragt er leise.

«Dann», sagt der Fremde mit stiller Begeisterung, *«dann könnten Sie beruhigt hier im Hafen sitzen, in der Sonne dösen – und auf das herrliche Meer blicken.»*
«Aber das tu› ich ja schon jetzt», sagt der Fischer, «ich sitze beruhigt am Hafen und döse, nur Ihr Klicken hat mich dabei gestört.»

Tatsächlich zog der solcherlei belehrte Tourist nachdenklich von dannen, denn früher hatte er auch einmal geglaubt, er arbeite, um eines Tages einmal nicht mehr arbeiten zu müssen, und es blieb keine Spur von Mitleid mit dem ärmlich gekleideten Fischer in ihm zurück, nur ein wenig Neid.

Easter Recipes – Osterrezepte

Easter Bread



Ingredients:

- 12 cups of flour
- 3 tablespoons of yeast
- 1 cup of melted butter
- 1 cup of sugar
- 6 eggs
- 1/2 tablespoon of mahlab (or crushed aniseed, or vanilla extract) + 2 cups of water
- 2–3 pieces of mastic, crushed with 1/4 teaspoon of sugar (or grated peel of 1 orange)
- 1 cup of lukewarm milk
- 3/4 teaspoon of sea salt
- 1 egg yolk lightly beaten with 1 tablespoon of water
- sesame seeds or blanched sliced almonds (optional)
- hard boiled dyed red eggs (optional)

Preparation:

Dissolve the yeast in lukewarm milk. Add a few spoonfuls of flour to make a paste. Cover and set aside in a warm place to rise.

In a bowl, combine 10 cups of the flour and salt, and add the melted butter. Add 1 teaspoon of mahlab or other flavoring and 2 cups of liquid (1 cup of orange juice, milk or water.) Add the eggs, mastic, sugar, and the risen yeast mixture. On a floured surface or in a mixer, knead the mixture well, adding in the remaining flour until the dough becomes smooth. Cover and allow it to rise in a warm place until it doubled in bulk (1 1/2 – 2 hours).

Punch down the dough and create shapes:

Twists *(photo):* Shape into ropes 20–24 inches long. Fold each rope in half and twist gently. Tuck a red egg into the top of the twist. Sprinkle with sesame seeds, or blanched almond slices (optional).

Preheat the oven to 350°F (175°C) and bake it for 30 minutes (until it becomes golden brown).

Vaia Stavropoulou

Struffoli

500 g flour
3 whole eggs and 3 egg yolks
1 lemon peel
100 g sugar
70 g Margarine
1 pinch of salt

Make the dough, working fine ingredients. You have to form a paste of medium consistency.

Let stand at least an hour covered in a bowl.

Then make a little «finger-thin» sticks with a diameter of 1 cm maximum, and cut them into pieces of 1/2 cm.

Fry a little at a time in hot oil (in a pan half-full) at moderate heat.

They should become clear blonde (often change oil when it darkens)

250 g honey
100 g sugar
4 tablespoons water

Put everything into a big pot.

Just begins to bubble slightly and foam, that is formed, becomes light yellow, turn off the heat and throw in the struffoli running well.

Then pour them into the tray, giving a shape with wet hands and pour over the sweets.

Valentina Mele

Pääsiäis Pasha
(Easter Pasha)

750 g	curd cheese	2 tsp	vanilla sugar
150 g	melted butter	50 g	crushed almonds
200 g	sugar	150 g	raisins
2	egg yolk		juice of 1 lemon
250 g	whipped cream		

For this desert all ingredients, except some raisins and almonds for decoration, are mixed with a hand blender and filled in a form covered with foil. After cooling over night, the mass is turned out and decorate with the remaining raisins and almonds. In Finland we have a traditional form for this with the letters XB, symbolising the resurrection of Jesus Christ.

Karoliina Pelttari

Osterfladen

Vom Basler Lokalhistoriker Eugen A. Meier erfahren wir, leider ohne genauere Quellenangabe, dass eine Art Osterfladen seit dem Jahre 962 bekannt ist. Im 14. Jahrhundert, so Meier, wurden in Basel an Ostern Fladen durch den Leutpriester gesegnet. Am Osterabend erhielten die Aussätzigen im Siechenhaus zu St. Jakob einen Osterfladen als Geschenk. Im folgenden Jahrhundert führte das Rechnungsbuch der Münsterbauhütte kleinere Beträge für «allerhand Züg zu den Fladen» auf. Die Nonnen im Maria Magdalena-Kloster schmausten im Jahr 1513 an Ostern ebenfalls Osterfladen. Das erste Rezept, das dem heute bekannten Osterfladen nahe kommt, findet man in der Schweiz Ende des 16. Jahrhunderts. Es befindet sich unter dem Namen „Ein dorten oder fladen von Reiss» in Anna Weckers «Ein köstlich new Kochbuch», das erste von einer Frau verfasste gedruckte Kochbuch aus dem Jahr 1598. Eine Verbindung zum Osterfest fehlt jedoch. Die Baslerin Anna Wecker unterstützte ihren Mann, den Colmarer Stadtarzt, mit ihrem Wissen über die Diätik, der Lehre des richtigen Essens. Im 19. Jahrhundert haben verschiedene Kochbuchautorinnen das Rezept des Osterfladens auch mit der Stadt Basel in Beziehung gesetzt: Margareta Spörlein, Autorin des Oberrheinischen Kochbuchs aus dem Jahre 1811 und eine gebürtige Schweizerin, nannte ihn explizit Basler Osterfladen. Und die Autorin der bekannten Basler Kochschule aus dem Jahr 1877 Amalie Schneider-Schlöth bezeichnet das entsprechende Rezept als «Speziell altes Basler Rezept». Der Basler Kulinariker Andreas Morel ist der Meinung, dass das Rezept des heutigen Osterfladens seit dem ausgehenden 16. Jahrhundert in Basel traditionell ist.

Aus: www.kulinarischeserbe.ch

Und hier ein heutiges Rezept:

Zutaten

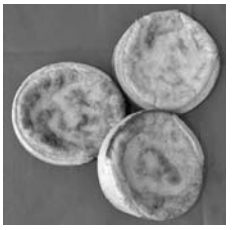
Butter für 12 Förmchen von 9 cm Ø

Teig:

250 g Mehl	125 g Butter, kalt, in Stück geschnitten
1 Prise Salz	1 Ei, verquirlt
2 EL Zucker	1–2 EL Vollrahm
1 Zitrone, abgeriebene Schale	

Füllung:

2 dl Vollrahm	3 Eigelb
2 dl Milch	1/2 Zitrone, abgeriebene Schale
100 g Sultaninen	100 g gemahlene Mandeln
3 EL Hartweizen- oder Maisgriess	3 Eiweiss, steif geschlagen
3 EL Zucker	



Garnitur:

Puderzucker

farbige Zuckereili

Zubereitung

Für den Teig Mehl, Salz, Zucker und Zitronenschale mischen. Butter beifügen und zu einer krümeligen Masse verreiben, eine Mulde formen. Ei und Rahm hineingiessen. Zu einem Teig zusammenfügen, nicht kneten. In Folie gewickelt 30 Minuten kühl stellen. Teig auf wenig Mehl 2–3 mm dick auswallen. In die ausgebutterten Förmchen legen und den Teigboden dicht einstechen, 20 Minuten kühl stellen.

Für die Füllung Rahm, Milch und Sultaninen aufkochen. Griess dazurühren, auf der ausgeschalteten Platte 15 Minuten quellen lassen. Auskühlen lassen, ab und zu rühren. Zucker, Eigelb, Zitronenschale und Mandeln darunter rühren. Eischnee sorgfältig darunter ziehen. Füllung auf dem Teigboden verteilen. Im unteren Teil des auf 200 °C vorgeheizten Ofens 20–25 Minuten backen, auskühlen lassen. Den Osterfladen mit Puderzucker bestäuben. Mit Zuckereili garnieren.

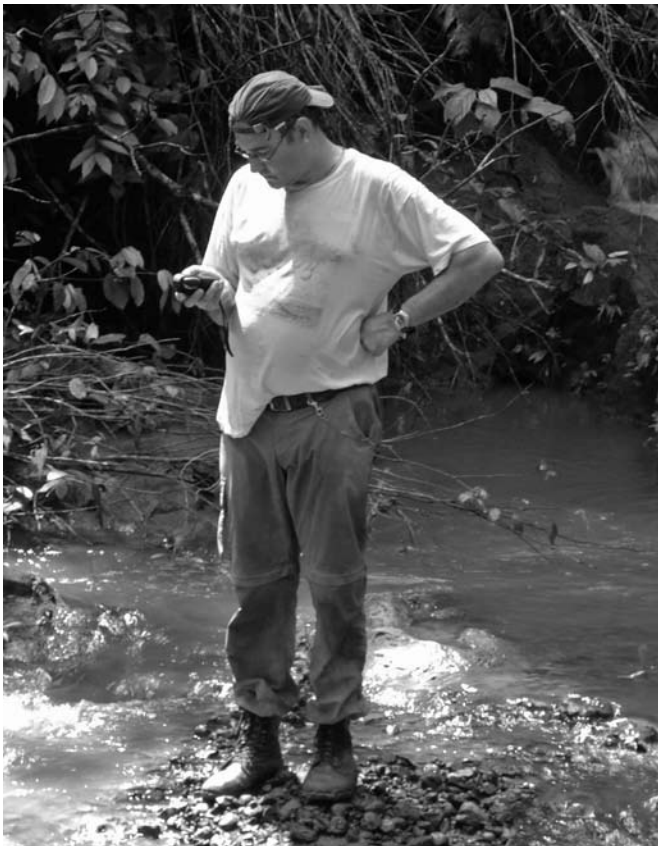
Verena Jäggin

Mit Phyllobates terribilis auf Du und Du

Schon als kleiner Junge war ich von den bunten Pfeilgiftfröschen, welche hier und da in den Zoohandlungen angeboten wurden, fasziniert. Meine ersten Pfeilgiftfrösche waren dann auch Goldbaumsteiger aus Costa Rica. Anfänglich wusste man noch wenig über die Haltung und Zucht dieser farbigen Tiere. So war es auch nicht verwunderlich, dass die meisten Tiere kein hohes Alter erreichten. Nach diesem ersten Versuch folgte dann eine längere froschlose Zeit. Erst als ich meine spätere Frau kennenlernte, wurde mein Interesse an diesen Tieren wieder geweckt. Bei ihr stand im Wohnzimmer ein grosses Terrarium, in welchem mehrere Arten von Pfeilgiftfröschen lebten. Sie hält mir heute noch vor, dass ich mich damals fast mehr für die Frösche als für sie interessiert habe. Jetzt betreiben wir unser Hobby gemeinsam.

Was sind aber Pfeilgiftfrösche und wie kommen sie zu dem doch eher furchterregenden Namen? Die etwas mehr als 250 Arten der Pfeilgiftfrösche leben im tropischen Süd- und Mittelamerika. Davon werden tatsächlich drei Arten von den Indianern in Kolumbien zum Vergiften von Pfeilspitzen verwendet. Die giftigste von diesen Arten hat auch den treffenden Namen, *Phyllobates terribilis*, der schreckliche Pfeilgiftfrosch. Wie alle Amphibien besitzen auch Pfeilgiftfrösche giftige Hautsekrete, diese dienen primär der Infektabwehr. Zusätzlich bauen sie Gifte aus ihrer Nahrung, in der Natur besteht diese vor allem Ameisen, in ihre Haut ein. Durch grelle Warnfarben teilen sie möglichen Fressfeinden mit, dass sie ungeniessbar sind. Unsere einheimischen Ameisen scheinen ihnen aber nicht zu schmecken. Wir füttern sie mit verschiedenen Insekten, wie Grillen, Fruchtfliegen, Springschwänzen und Läusen. Da diese Futtertiere nicht über Gifte verfügen, geht die Toxizität der im Terrarium gehaltenen Frösche relativ schnell zurück und Terrariennachzuchten sind nicht giftiger als unsere einheimischen Amphibien.

Phyllobates terribilis, der schreckliche Pfeilgiftfrosch



Auf Froschsuche im Regenwald

Uns hat es vor allem der Erdbeerfrosch *Oophaga pumilio* angetan. Diese Art kommt in Nicaragua, Costa Rica und



Verschiedene Varianten der Erdbeerfrösche aus Panama

Panama vor und bildet unzählige Farbvarianten aus. Vor allem auf den der Karibikküste vorgelagerten Bocas-Inseln in Panama findet man auf jeder Insel eine andere Farbform. Mehrere Reisen führten uns auch in diese Gegend. Jetzt versuchen wir die auf diesen Reisen gesammelten Eindrücke in unseren Terrarien umzusetzen. Wir versuchen dabei, das Klima sowie die Bepflanzung möglichst naturnah nachzubauen.

Ein Pärchen Erdbeerfrösche aus Costa Rica

In 30 Terrarien halten und züchten wir mehrere Varianten des Erdbeerfrosches. Jedes Terrarium wird mehrmals täglich beregnet und belüftet. Auch das Züchten der verschiedenen Futtertiere benötigt einiges an Zeit.

Rufender Erdbeerfrosch aus Costa Rica

Terrarium für Pfeilgiftfrösche

Bei guter Pflege kann man auch mit Nachzuchten rechnen. Gerade die Fortpflanzung ist bei den knapp 2 cm grossen Fröschen extrem spannend. Anders als bei unseren Fröschen besteht ein Gelege aus nur 5–10 Eiern. Diese werden auf Blättern abgelegt. Nach etwa 1 Woche schlüpfen die Kaulquappen und werden vom Weibchen auf den Rücken genommen und in kleinste Gewässer, meisten Blattachseln von Pflanzen verteilt. Dort werden sie regelmässig mit unbefruchteten Eiern gefüttert, bis nach etwa 2 Monaten ein winziger Frosch die Pflanze verlässt. Wir ziehen mehrere Varianten regelmässig nach. Diese werden meist an befreundete Froschhalter abgegeben oder auf speziellen Froschbörsen getauscht und verkauft. So kann man bei vielen Arten auf Naturentnahmen verzichten.

Peter Zimmermann

Roxane Tussiwand, Developmental and Molecular Immunology

Ciao a tutti!

Let me introduce myself.
As you might guess I am Italian, or more precisely half Italian and half Iranian. And just like everybody else I am proud of my origins. I can only recommend a visit to both countries. They are beautiful and were niches of culture and history. Unfortunately the political situation of both countries is rather questionable in terms of democracy. But I am sure that nobody is interested in listening to my obvious complaints. Reading newspapers would be enough to get a more or less clear picture about what is going on in these countries and share my disappointment.

I was asked by Ton Rolink, my supervisor at the DBM (molecular and developmental immunology) to write something for the DBM facts. And there I started to struggle: What shall I say? What could be interesting for the readers? I have to admit that I just had a quick glance at this journal when it was lying in our coffee place. Therefore writing something for an audience that I do not know is complicated. - And we Italians just love to say "it's complicated". Because you can in these words stop a discussion and declare defeat in finding solutions. We are actually masters in using this. All our problems can be postponed just by saying it's complicated! But I am again moving away from the task I was asked to undertake, namely writing something interesting for the DBM facts journal.

After having thought about what I could tell you, and since I should somehow introduce myself, let's talk about me (women love to do that!). And more precisely what brought me to Basel.

I was born in Iran but at the age of two years my family moved back to Italy (thank God or Insallah!), where I grew up. And then the story becomes similar to many others. I went to school and did my "Abitur". Right after that the time arrived for me to choose university. Or better, to think about what I would like to become as an adult. I do not know if somebody has a clear view at that age about what to do in the future. Astronauts probably do, but for me it was not easy to decide. Anyway I knew I wanted to do research. It is a word, which comprises

many worlds! But at that time I thought I should go for biological science. And what cleared all my doubts was the story of my little brother. Hence forgive this little digression and just keep reading.

At the age of three and a half he got diagnosed with leukemia. I was 11 and I could not understand this complicated and meaningless word. What was clear to me was that everybody in the house started to cry. No words or gestures could help. And the strength of my mother was deeply shaken. I felt far too little and the earth collapsed under my feet, and I cried, overwhelmed by the sadness, which condensed the air of the room. Right after that everything became complicated, my mother disappeared with my little brother. Me and my older brother had to grow up in a few months. Learning what a transfusion, infection, tumor and all these words mean. The smell of hospitals and of disinfecting solutions became familiar.

Gustav Klimt: TheTree of Life

But besides all this we learned probably the most important rule in the game of life: We learned from my little brother that there could be an end, anytime. We learned that you are meaningless in this game. But we also learned that you can fight and you should never give up, and that there is always space for hope.
After about 10 years of therapy, after many relapses, when he already had entered the unit for terminal patients, the doctors tried to rescue him by autologous bone marrow transplantation. And beyond all clinical beliefs he made it through, he was cured.

It was not easy. But these things change your life, your vision of the world and of the people. And smiling at life, I now know that I will try never to give up. I will always stand up again and fight. I will remember that it can be over in no time, therefore "carpe diem" as the Roman's would say. Life is worth being lived, with tears and smiles.

After this experience also my scale of values did change and I realized that there are few things, which are important, and too many things, where we waste our energy. It is hard in every day life to see it but a little effort is worth it. Try to look at your glass as half full and not empty, since I believe that no time should be wasted on emptiness.

This is my story and the story of my brother and my family. This is my reason and motivation for doing research.
After University I decided to work for the research department at the hospital where my brother was treated. I did my best, always having in mind, that behind each sample, each name, each cell to be analyzed there is a patient, a child, a mother, a father, brothers and sisters.

I moved afterwards to basic research since I wanted to understand the rules that drive differentiation of heamatopoietic cells in normal conditions. And here, in Basel, I had the privilege to get to know Ton. Rarely, in my short career had I met anyone like him; so enthusiastic, motivated and always in good spirit. The curiosity that moves him every day is never extinguished and will never move him away from the bench. And for all this I am grateful. Grateful to see that this way of doing research is possible.

What more can I tell you: It is a beautiful profession and if I could choose again, I would do the same again and again.

If you had the patience to through to the end I can just thank you and wish you a good day. And just never forget that if your soil is fertile your dreams will grow.

Baci,
Roxane

Die blauen Frühlingsaugen

Die blauen Frühlingsaugen
schau'n aus dem Gras hervor;
das sind die lieben Veilchen,
die ich zum Strauss erkor.

Ich pflücke sie und denke,
und die Gedanken all',
die mir im Herzen seufzen,
singt laut die Nachtigall.

Ja, was ich denke, singt sie
lautschmetternd, dass es schallt;
mein zärtliches Geheimnis
weiss schon der ganze Wald.

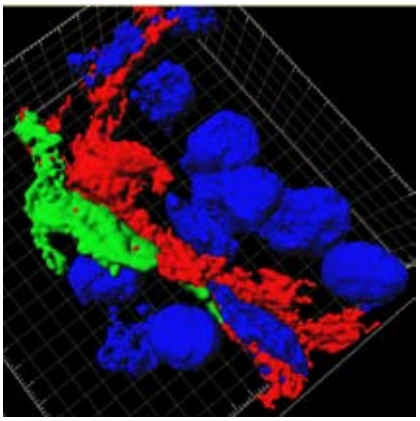
Heinrich Heine

Die Redaktion wünscht allen Leserinnen und Lesern schöne und erholsame Osterfeiertage!

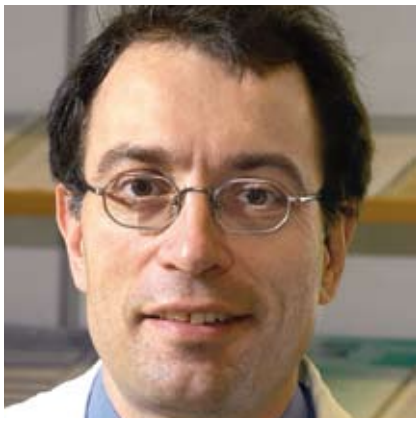
The Editorial team of DBM Facts wishes all its readers Happy Easter Holidays!

VORSCHAU PREVIEW

In der nächsten Ausgabe . . .



... erfahren wir von Gerhard Christofori mehr über die Forschungsaktivitäten seines Labors Tumor Biology



... führt uns Patrick Hunziker in die Welt der Nanomedizin ein

... nimmt uns Jürg Schwaller mit auf Weinreise in die Pfalz

... «watscheln» wir mit Nicole Schaeren-Wiemers auf den Spuren von Konrad Lorenz

... stellen wir die «Route de la Carpe frite» vor

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1460 – 2010:
550 JAHRE UNIVERSITÄT BASEL
Jubiläumsprogramm

17.–19. SEPTEMBER

FEST DER WISSENSCHAFTEN
Ein Fest bewegt Basel und die Region

Am Petersplatz und an der Flaniermeile am Petersgraben gibt es drei Tage lang für Gross und Klein allerlei zum Thema Wissenschaft zu entdecken und aktiv mitzumachen. Die älteste Universität der Schweiz richtet ein grosses Fest aus, das den Besucherinnen und Besuchern faszinierende Einblicke in die Welt der Wissenschaft bietet. Im Rahmen des begleitenden Musik- und Unterhaltungsprogramms treten nationale Pop- und Showgrössen auf. Weitere Höhepunkte setzen Fasnachtscliquen, Vereine und Zünfte, die auch um das leibliche Wohl der Gäste besorgt sind. Ein unterhaltsames Kinderprogramm rundet das Fest ab.

Basel, Petersplatz, 17. bis 19. September 2010

SCHATZKAMMERN DER UNIVERSITÄT BASEL

Die Anfänge einer 550-jährigen Geschichte
Nicht nur Tore zur Zukunft werden anlässlich des Jubiläumsjahres der ältesten Universität der Schweiz geöffnet, sondern auch Türen zu ihren verborgenen Schatzkammern.
In der einzigartigen Atmosphäre des Basler Münsters als Ort der feierlichen Gründung dokumentiert eine Ausstellung die frühe Entwicklung der Universität. Dabei werden Schriftstücke und Objekte aus dem ersten Jahrhundert der «hohen Schule» zum Teil erstmalig der Öffentlichkeit zugänglich gemacht. Sie führen auf eine Reise zu den Anfängen eines Unternehmens, das im Spannungsfeld von religiösen, politischen und wirtschaftlichen Interessen Identität findet und stiftet.

Hochchor des Basler Münsters, 25. April bis 26. November 2010