DBM FACTS

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Gynecological Cancer Research, with a focus on the development of novel biomarkers, especially in Ovarian Cancer | Laboratory Approaches for New Diagnostics and New Drugs | ¡Vamos a Sevilla!

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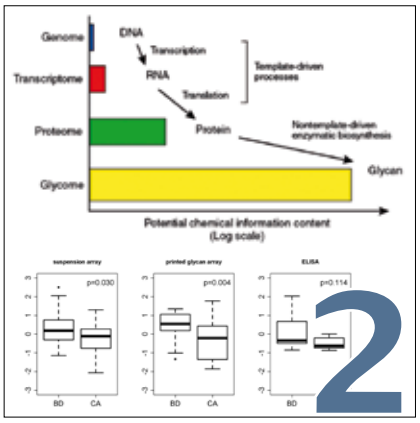
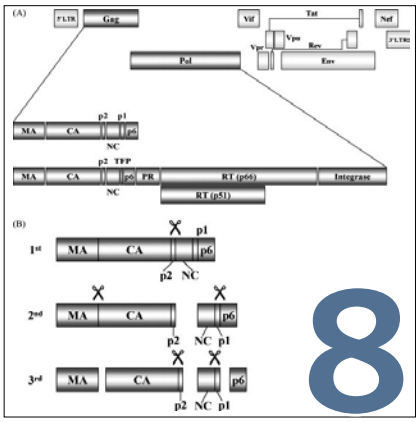


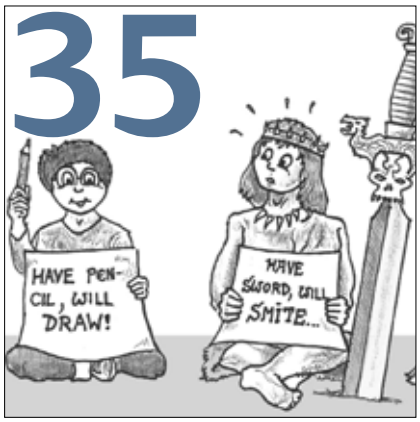
Gynecological Cancer Research, with a focus on the development of novel biomarkers, especially in Ovarian Cancer
from Viola Heinzelmann-Schwarz



Laboratory Approaches for New Diagnostics and New Drugs
from Thomas Klimait

¡Vamos a Sevilla!
from Jana Orellana

Niemand zu klein, um Tennisspieler zu sein
von Marc Bichsel



DBM stellt sich vor
Have pencil, will draw...
from Thorsten Fritzius

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EDITORIAL

Radek Skoda
Leiter DBM

Liebe Leserinnen und Leser

Der lauteste Teil der Umbauarbeiten am DBM-Hebelstrasse liegt nun hinter uns, aber wir müssen weiterhin noch etwas Geduld üben. In den nächsten Monaten werden im zweiten Stock einzelne Wände gezogen, anschliessend werden die Büros und Labors eingerichtet, so dass Anfang nächsten Jahres wie geplant die neuen Räumlichkeiten bezogen werden können. In der ersten Hälfte 2014 folgen dann im dritten Stock der Seminarraum und im vierten Stock die GMP-Facility.

Wir freuen uns zwei neue Professuren am DBM begrüssen zu können. Claudia Lengerke und ihre Mitarbeiter/innen ("Stem cells and Hematopoiesis") haben ihre Tätigkeit am 1. August 2013 aufgenommen. Daniel Pinschewer ("Virology/Immunology") beginnt mit seinem Team am 1. September 2013. Wir wünschen allen viel Erfolg!

Schwerpunkt der nun vorliegenden Spätsommerausgabe der DBM Facts bildet neben den neuesten Publikationen aus dem DBM das Forschungsprojekt von Viola Heinzelmann und ihrem Team. So erfahren wir mehr über "Gynecological Research" (ab Seite 2). Thomas Klimkait stellt uns anschliessend seine Gedanken über "Laboratory Approaches for New Diagnostics and New Drugs" vor (ab Seite 8).

Jana Orellana nimmt uns mit in ihre Heimat Sevilla (ab Seite 30) und Marc Bichsel gibt uns eine kostenlose Tennisstunde (ab Seite 33). Wem das alles zu anstrengend ist, könnte bei Thorsten Fritzius eine Alternative finden, dessen Motto lautet: Have pencil, will draw! (ab Seite 35).

Schöne Spätsommertage und viel Spass bei der Lektüre.

Dear Readers

The noisiest parts of the reconstructions at the DBM-Hebelstrasse are now behind us, but further tolerance will be needed during the coming months. Some walls will be constructed on the second floor, offices and laboratories will be equipped and hopefully the new premises can be occupied as planned at the beginning of next year. Afterwards, and during the first half of 2014, reconstruction of the seminar room on the 3rd floor and of the GMP-Facility on the 4th floor will be undertaken.

We are happy to be able to welcome two new Professors to the DBM. Claudia Lengerke and her colleagues ("Stem cells and Hematopoiesis") began their activities on 1st August 2013. Daniel Pinschewer ("Virology/Immunology") and his team start on 1st September 2013. We wish them all a lot of success.

In addition to the usual presentation of recent DBM publications this late-summer issue of DBM Facts spotlights the research project of Viola Heinzelmann and her team (from page 2), giving us an opportunity to learn more about "Gynecological Research". Thomas Klimkait presents his thoughts on the topic of "Laboratory Approaches for New Dioagnostics and New Drugs" (from page 8).

Jana Orellana takes us for a visit to her hometown Sevilla (from page 30) and Marc Bichsel offers a tennis lesson for free (from page 33). If all this is too arduous, you can find alternative entertainment through Thorsten Fritzius whose motto is "Have pencil, will draw" (from page 35).

Enjoy the beautiful late summer days and have fun reading this issue.

Gynecological Cancer Research
with a focus on the development of novel biomarkers, especially in Ovarian Cancer

Who we are

Our research group was initially founded in 2006 with a Krebsliga grant (Canton of Zurich) when Viola Heinzelmann-Schwarz was a clinical registrar in Gynecology at the University Hospital Zurich. Together with a then PhD student (Francis Jacob) and 3 medical students (Kristjan Ukegjini, Mara Meier, Michelle Bühler) she started off with translational research projects in ovarian cancer, mainly working on target genes identified by transcriptomic data *(Heinzelmann-Schwarz 2004)*. The group established a large biobank in collaboration with various institutions (Department of Gynecology at the University Hospital Zurich and Spital Limmattal, Institute of Pathology at USZ) and assembled a tissue microarray, RNAlater tissues, plasma and ascites based biobank for around 800 Swiss patients, all linked to a comprehensive database. Based on a review on transcriptomic data in ovarian cancer, which was Francis' first publication from the lab *(Jacob 2009)*, we realized, that this method was adequate to differentiate the genetic background between various types of ovarian cancer *(Heinzelmann-*

2013: left to right: Francis Jacob, Reto Kohler, Monica Nunez, Andreas Schötzau, Viola Heinzelmann-Schwarz, Andre Fedier (Missing: Tatiana Pochechuewa and Shahidul Alam).

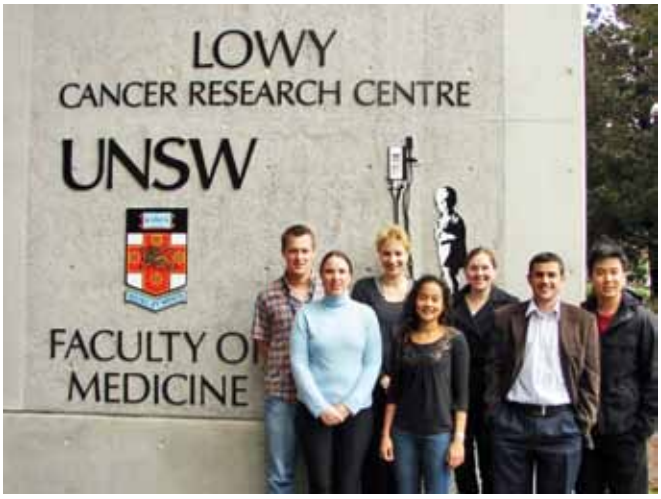
Schwarz 2006, 2007), but not good enough to identify blood-based detection markers for this disease. Due to this limitation we investigated a new field: glycomics, and started collaborations with Professor Nicolai Bovin (Institute of Carbohydrate Chemistry, Russian Academy of Science, Moscow) and Prof. Margaret Huflejt (former Scripps Institute San Diego, now New York State University) in order to screen our Swiss patient cohort with a newly developed printed glycan array (financed by SNF and Oncosuisse). This method allowed the detection of plasma-based anti-glycan antibodies, hereby giving a diagnostic profile to differentiate between ovarian cancer and healthy control patients. These discovery experiments were performed by Francis within his PhD, including a 4 months' stay in San Diego, which was funded by a European Science Foundation Award *(Jacob 2011)*.

Around the time that Francis' finished his PhD, Viola had the chance of subspecializing (in her life as a clinician) in the field of gynecological oncology with one of the world leading experts, Professor Neville Hacker in Sydney. Being research driven, she founded a second research group in Australia within the Lowy Cancer Research Centre, called the Ovarian Cancer Group (Cancer Institute NSW Scholarship, Mary Elisabeth Courier Scholarship, William Maxwell Trust), which consisted of 5 members, including Francis, who meanwhile joined her as a Post-Doc (SNF, prospective researcher grant). We also established a second, independent patient cohort (plasma, ascites, tissue microarrays, RNA later and fresh frozen tissues), connected to a comprehensive clinicopathological database. The advantage of this second 800 patient counting Australian cohort is, that it was established by the same people as the Swiss cohort with the same protocol. Moreover, it is mostly cancer patients, has included high risk control patients like *BRCA* mutation carriers and has longitudinal sample collection, i.e. during chemotherapy treatment, relapse, second-line treatment etc. Meanwhile the Swiss Group continued with one Post-Doc remaining there, Tatiana Pochechueva, who initially joined us coming from the lab of our collaborator Prof. Bovin (Moscow). Both Francis and Tatiana are now also members of the DBM-based Gynecological Cancer Group.

Our research group moved to the laboratories of the DBM in July 2012, as Viola started her tenure track assistant professorship that passes into an Ordinariat in 2014. The group has been strengthened by Andre Fedier, a senior lecturer from the University Hospital Basel, with whom Viola, Francis and Tatiana had worked as colleagues door-to-door in Zurich since 1998. Francis is now Projekt Leader and Andre is Lab Manager; having their expertise in the area of glycobiology and drug resistance, respectively. We have two other colleagues supporting us as Post-Docs, funded by the SNF and Oncosuisse: glycochemist Tatiana and molecular biologist Reto Kohler. Late last year Monica Nunez joined as research assistant, Shahid Alam as PhD Student, and Andreas Schötzau as statistician of the whole Women's Hospital. Rea Gürtler, who was already part of the Australian group as Master student will be joining us in November 2013 as a second PhD student.

As we are interested in translational research this is only possible through interprofessional and interdisciplinary collaborations, both national and international. We have ongoing collaborations with the University of Basel (Ellen Obermann, Alfred Zippelius, Gerhard Christofori), University of Zurich (Holger Moch, Daniel Fink), ETH (Ruedi Aebersold, Bernd Wollscheidt), the University of New South Wales (Kerrie McDonald, Caroline Ford, Pierre Dilda, Neville Hacker), Macquarie University (Nicolle Packer), University of Newcastle (James Scurry), EPF Lausanne (Darlene Goldstein), Russian Academy of Science (Nicolai Bovin), University of Manchester (Sabine Flitsch) and the University of Copenhagen, Denmark (Henrik Claussen).

2006: left to right: Francis Jacob, Mara Meier, Viola Heinzelmann, Kristjan Ukegjini



2009: left to right:
Francis Jacob, Sheri Nixdorf, Viola Heinzelmann, Musfirah Marican, Rea Gürtler, Imad Ben-Hmeda, Brian Tse

Ovarian cancer: the clinical problem

Epithelial ovarian cancer is the fifth most common malignancy in women and the second leading cause of gynecological cancer death worldwide. The majority of patients are diagnosed at an advanced FIGO Stage due to limited screening tools and the non-specific nature of symptoms. The five-year survival rate for women with early pelvic disease is over 70% but less than 30% for those with advanced metastatic disease. Ovarian carcinomas are histologically categorized into serous (75%), mucinous (10%), endometrioid (10%), clear cell (1%) and undifferentiated (1%) subtypes. Maximal cytoreductive therapy and chemotherapy with Carboplatin and Paclitaxel are the two mainstays of adjuvant therapy, but approximately 70% of patients with advanced disease will relapse despite response to initial treatments. Until 5 years ago ovarian cancer was thought to be mainly sporadic. Only a small percentage of about 10% was thought to be due to *BRCA1/2* mutations or – even more rarely – due to Lynch Syndrome, mutations in DNA mismatch genes. Due to patients with *BRCA1/2* mutations, which received prophylactic removals of their ovaries and fallopian tubes, researchers like Crum and colleagues found early tubal cancers or *in situ* metaplastic changes in the distal parts of the fallopian tubes. There is now increasing evidence, that serous ovarian cancers derive for a high frequency from the fimbrial end of the fallopian tubes and that even up to 20% of patients with a serious malignancy have *BRCA1/2* mutations, even if there is no family history of cancer. In these patients, the only preventative method which will result in a 96% risk reduction is the prophylactic removal of ovaries and tubes. Before these findings occurred, literature suggested that due to the continuous interruption of the ovarian surface epithelium after ovulation, inclusion cysts would form and show signs of metaplastic changes.

Ovarian cancer is currently diagnosed in 75% of patients with advanced stage disease where the cancer is not anymore only located in the pelvis but has spread throughout the abdominal cavity. Diagnostically, clinicians either palpate the ovarian mass or detect it with the help of the tumor marker CA125, transvaginal ultrasound and computer tomography. However, all these methods even performed in 3-monthly intervals, have not proven to detect the disease at an earlier time. Therefore, other early detection methods are urgently needed.

Our research: prior achievements

In order to identify other potential diagnostic markers, we investigated the initially as top candidate identified *HE4* (human epididymal 4; *Heinzelmann-Schwarz 2004)*, which is meanwhile the second available tumor marker in ovarian cancer. We compared this new commercially available tumor marker HE4 with CA125 individually, in combination, within the risk of malignancy index (RMI) and the newly defined risk of malignancy algorithm (ROMA). Our prospectively-collected cohort of 160 patients consisted of healthy controls, benign diseases, and borderline tumors/adenocarcinomas of ovarian, tubal, peritoneal and endometrial origin. HE4 and CA125 were measured in serum using standardized ELISA. Both markers showed similar diagnostic performance in the detection of ovarian cancers at clinically defined thresholds (CA125 35 U/ml; HE4 70 pM) but HE4 was not elevated in endometriosis, a benign disease where endometrial cells are spread throughout the abdomen, causing pain through chronic inflammation and its side effects. Comparison of non-malignant diagnoses (n=71) *versus* early stage ovarian and tubal cancers (n=19) revealed that HE4 and ROMA displayed the best diagnostic performance (AUC 0.86 / 0.87, specificity 85.9% / 87.3% and sensitivity 78.9% / 78.9%, respectively).

Whilst RMICA125 detects peritoneal cancer better than all other models (AUC 0.99, specificity 97.2 / %, sensitivity 80.0%), there is no other detection benefit from RMI compared to HE4 alone or included in ROMA. We therefore concluded that the major advantage of HE4 lies in its specificity and improved detection of borderline tumors and early stage ovarian and tubal cancers. HE4 is superior to CA125 with or without RMI and ROMA indices. However, we see no benefit from combining both markers in clinical practice *(Jacob, Meier 2011)*.

Another target which derived from our initial transcriptomic analysis was SFRP4, secreted frizzled-related protein 4, which has been proposed to have an inhibitory activity through binding and sequestering Wnt ligands. Activation of the Wnt signaling pathway is implicated in aberrant cellular proliferation in various cancers. In 40% of endometrioid ovarian cancers, constitutive activation of the pathway is due to oncogenic mutations in β-catenin or other inactivating mutations in key negative regulators. We performed RT-qPCR and Western-blotting in primary cultures and ovarian cell lines for SFRP4 and its key downstream regulators (activated-) β-catenin and GSK3β. SFRP4 was then examined by immunohistochemistry in a cohort of 721 patients and due to its proposed secretory function, in plasma, presenting the first ELISA for SFRP4.

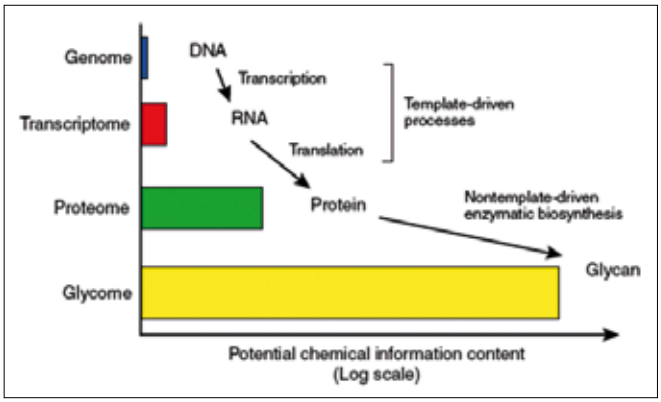
SFRP4 was most highly expressed in tubal epithelium and decreased with malignant transformation, both on mRNA and protein level, where it was even more profound in the membrane fraction ($p<0.0001$). SFRP4 was expressed on the protein level in all histotypes of ovarian cancer but was decreased from borderline tumors to cancers and with loss of cellular differentiation. Loss of membrane expression was an independent predictor of poor survival in ovarian cancer patients (unadjusted $p=0.02$; adjusted $p=0.089$), which increased the risk of a patient to die from this disease by the factor 1.8. Our results therefore support a role for *SFRP4* as a tumor suppressor gene in ovarian cancers via inhibition of the Wnt signaling pathway. This has not only predictive implications but could also facilitate a therapeutic role using epigenetic targets *(Jacob, Ukegjini 2012)*.

In a follow-up investigation we showed that recombinant SFRP4 (rSFRP4) treatment of a serious ovarian cancer cell line results in inhibition of β-catenin dependent Wnt signaling as measured by TOP/FOP Wnt reporter assay and decreased transcription of Wnt target genes, Axin2, CyclinD1 and Myc. In addition, rSFRP4 treatment significantly increased the ability of ovarian cancer cells to adhere to collagen and fibronectin, and decreased their ability to migrate across an inflicted wound. We conclude that these changes in cell behavior may be mediated via mesenchymal to epithelial transition (MET), as rSFRP4 treatment also resulted in increased expression of the epithelial marker E-cadherin, and reduced expression of Vimentin and Twist. Combined, these results indicate that modulation of a single upstream gatekeeper of Wnt signaling can have effects on downstream Wnt signaling and ovarian cancer cell behavior, as mediated through epithelial to mesenchymal plasticity (EMP) *(Ford 2012)*.

Another project within the Wnt signaling pathway is our present investigation into altered Wnt5a expression, which has been linked to numerous human cancers. Nevertheless, some confusion surrounds its role in activating or inhibiting β-catenin dependent and independent Wnt signaling pathways. Aberrant Wnt signaling has previously been associated with gynecological cancers, and in a recent publication we report the specific association of increased Wnt5a expression with epithelial ovarian cancer. Patients expressing Wnt5a have a shorter relapse free and overall survival compared to patients that do not express Wnt5a. Treatment of ovarian surface epithelial cells with recombinant Wnt5a increased proliferation and migration and decreased cell adhesion. In addition, downstream targets of β-catenin dependent Wnt signaling were inhibited, and both β-catenin independent and epithelial to mesenchymal (EMT) targets increased following Wnt5a treatment *(Ford 2013, submitted)*.

Our research: current translational research projects

When Francis wrote his review on the results of transcriptomic data in ovarian cancer *(Jacob 2009)*, he realized that the most commonly asked question "detection of a new tumor marker for ovarian cancer" was not ideally examined by this technique. In contrast, whilst transcriptomic was an ideal platform to examine the expression of genes for specific histological subtypes *(Heinzelmann-Schwarz 2008, 2007)*, there is an issue

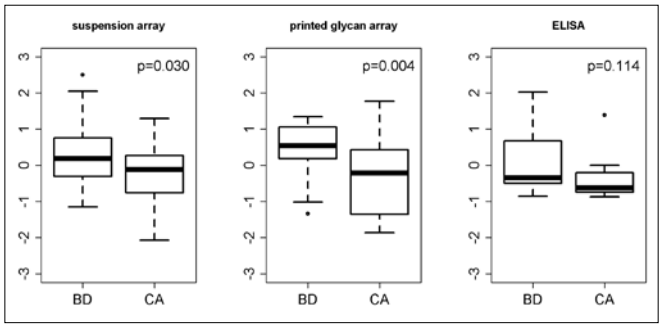


Adopted from Turnbull and Field, Nat Chem Biol, 2007, "Emerging glycomics technologies." 3(2): 74–7.

with potentially blood-based tumor markers. Precisely, whilst the examination on RNA level will give an insight into the genome of the tumor, the biological effect of a disease is best reflected for blood based tests, if they examine post-translational modifications (see Figure above). As the largest group of post-translational modifications is the glycome, we concluded that the examination of carbohydrate (glycan) structures at the surface of cells and their potential binding partners would be the most promising method to detect future markers.

The cellular membrane is covered by carbohydrates, linked to membrane-bound proteins and lipids, with all our current tumor markers being glycoproteins (MUC1, CA125, HE4) or glycans (CA19–9) by itself. The role of anti-glycan antibodies, binding to glycoproteins or glycolipids, is well known for A and B blood groups and is starting to evolve in other inflammatory or malignant conditions. There are well known tumor-associated carbohydrate antigens already known for various cancers.
Through a collaboration with Prof. Nicolai Bovin (Russian Academy of Science, Moscow) and Prof. Margaret Huflejt (then Scripps Institute, GlycoMedical Institute, San Diego) we were able to screen our Swiss ovarian cancer patient cohort as the first group world-wide using a newly developed printed glycan array (PGA). This array allowed the simultaneous detection of anti-glycan antibodies (AGA) against over 203 chemically synthesized carbohydrate structures.
In this discovery approach we demonstrated that the level of naturally occurring AGA in the serum is lower to certain glycan structures in ovarian cancer patients than in healthy women (Swiss discovery cohort). The PGA consists of >200 chemically synthesized carbohydrates mounted on a glass slide, 24 out of these carbohydrates were found to significantly discriminate the cancer patients from healthy women, with P_1 glycan as trisaccharide, as the top candidate with the highest discriminatory power. The largest discriminatory power (specificity and sensitivity) between ovarian cancer patients and healthy controls was found for P_1 trisaccharide and was comparable to that of CA125, the commonly used tumor marker for ovarian cancer. These findings were confirmed with two different methods as "validation approaches" (suspension array and ELISA) in an independent patient cohort (Swiss validation cohort) and were therefore method- and cohort-independent (*Pochechueva 2010, 2011;* Figure below). The discriminatory ability of P_1 glycan was further confirmed in our Australian validation cohort profiled for (specific) anti-P_1-antibodies

Whether P1 or other glycans are expressed on ovarian cancer cells is still unknown as is the biological function of P1 (and related glycans). There are several hypotheses about the function of AGA and glycan-like antigens such as P1 and their significance in ovarian cancer. One attractive idea is that ovarian cancer cells express antigens like P1 on their cell surface which are detected and bound by naturally occurring AGA in body fluids like blood and ascites of cancer patients. These bound AGA are no longer freely circulating in these body fluids, which might explain the observed reduced levels of AGA in the blood and ascites of ovarian cancer patients compared to the healthy individuals. Another idea is that these antigens are also expressed in non-cancer cells, but that these antigens are encrypted and not accessible by naturally occurring AGA.



To verify the presence of P1 antigen and therefore deliver the proof of P1 expression on ovarian cancer cells, we are currently performing negative mode LC-MS/MS on tissue samples and cell lines. We also have a set of in-house well defined (printed glycan array, suspension array, ELISA, competition assays) and characterized monoclonal IgM AGA that will be applied in flow cytometry to identify cancer cell lines positive for our glycans of interest. These cell lines will serve as optimal tools for continuative functional experiments: the use of gene targeting with zinc finger nucleases allows us to investigate cellular characteristics such as proliferation and migration and to evaluate the potential of site-specific targeting for potential drug deliveries. Also, naturally occurring AGA to P1 from ovarian cancer patients will be purified by affinity chromatography to expand knowledge of glycan binding IgM in cancer patients.

Viola Heinzelmann-Schwarz

Publications from our group

Tse BWC, Collins A, Oehler MK, Zippelius A, Heinzelmann-Schwarz VA. Antibody-based immunotherapy for ovarian cancer – where are we at? ***Ann Oncol (2013), accepted for publication.***

Buehler M, Tse B, Leboucq A, Jacob F, Caduff R, Fink D, Goldstein DR, Heinzelmann-Schwarz V. Meta-analysis of microarray data identifies GAS6 expression as an independent predictor of poor survival in ovarian cancer. ***Biomed Research International (2013), accepted for publication.***

Francis J, Guertler, R, Naim, S, Nixdorf, S, Fedier, A, Hacker, NF, Heinzelmann-Schwarz V. Careful Selection of Reference Genes Is Required for Reliable Performance of RT-qPCR in Human Normal and Cancer Cell Lines. ***PLOS ONE (2013), accepted for publication.***

Ford CE, Jary E, Ma SSQ, Nixdorf S, Ward RL, Heinzelmann-Schwarz V. The wnt gatekeeper sFRP4 modulates EMT, cell migration and downstream wnt signaling in serous ovarian cancer cells. ***PLOS ONE 8(1):e54362 (2013).***

Pochechueva T, Jacob F, Fedier A, Heinzelmann-Schwarz V. Tumor-Associated glycans and their role in gynecological cancers: accelerating translational research by novel high-throughput approaches. ***Metabolites 2: 913–9 (2012).***

Cowin PA, George J, Fereday S, Loehrer E, Van Loo P, Cullinane C, Etemadmoghadam, D, Ftouni S, Galletta L, Anglesio MS, Hendley J, Bowes L, Sheppard KE, Christie E, AOCS, Pearson RB, Harnett PR, Heinzelmann-Schwarz V, Friedlander M, McNally O, Quinn M, Campbell P, deFazio A, Bowtell DDL. Deletion of LRP1B is associated with acquired chemotherapy resistance to liposomal doxorubicin in high-grade serous ovarian cancer. ***Cancer Res 72: 4060–73 (2012).***

Jacob F, Ukegjini K, Nixdorf S, Ford CE, Olivier J, Caduff R, Scurry J, Guertler R, Hornung D, Mueller R, Fink DA, Hacker NF, Heinzelmann-Schwarz VA. Loss of secreted frizzled-related protein 4 correlates with a more aggressive phenotype and is an independent predictor of poor outcome in ovarian cancer patients. ***PLOS ONE 7(2):e31885 (2012).***

Jacob F, Goldstein DR, Bovin NV, Pochechueva T, Spengler M, Caduff R, Fink D, Vuskovic MI, Huflejt ME, Heinzelmann-Schwarz V. Serum anti-glycan antibody detection of non-mucinous ovarian cancers by using a printed glycan array. ***Int J Cancer 130: 138–46 (2012).***

Chang CY, Picotti P, Huettenhain R, Heinzelmann-Schwarz V, Iovanovic M, Aebersold R, Vitek O. Statistical framework for protein quantification with Selected Reaction Monitoring (SRM) experiments. ***Mol Cell Proteomics 11:M111.014662 (2011).***

Pochechueva T, Jacob F, Goldstein DR, Huflejt ME, Chinarev, A, Caduff R, Fink D, Hacker N, Bovin NV, Heinzelmann-Schwarz V. Comparison of printed glycan array, suspension array and ELISA in the detection of human anti-glycan antibodies. ***Glycoconj J 28: 507–17 (2011).***

Heinzelmann-Schwarz V. Epithelial ovarian cancer – a change of paradigm. Invited review. ***Ther Umsch 68: 565–72 (2011).***

Jacob F, Meier M, Caduff R, Goldstein D, Pochechueva T, Hacker N, Fink D, Heinzelmann-Schwarz V. No benefit from combining HE4 and CA125 as ovarian tumor markers in a clinical setting. ***Gynaecol Oncol 121:487–91 (2011).***

Theurillat JP, Metzler S, Henzi N, Djouder N, Helbling M, Zimmermann AK, Jacob F, Soltermann A, Caduff R, Heinzelmann-Schwarz V, Moch H, Krek W. URI Is an Oncogene Amplified in Ovarian Cancer Cells and Required for Their Survival. ***Cancer Cell 19: 317–32 (2011).***

Pochechueva T, Chinarev A, Spengler M, Korchagina E, Heinzelmann-Schwarz V, Bovin N, Rieben R. Multiplex suspension array for human anti-carbohydrate antibody profiling. ***Analyst 136: 560–9 (2011).***

Jacob F, Goldstein D, Fink D, Heinzelmann-Schwarz V. Proteogenomic studies in epithelial ovarian cancer: established knowledge and future needs. ***Biomarkers Med 3: 743–6 (2009).***

O'Brien PM, Davies MJ, Scurry JP, Smith AN, Barton, CA, Henderson MJ, Saunders DN, Clancy JL, Heinzelmann-Schwarz VA, Scolyer RA, Zeng, Y, Williams ED, Scurry L, DeFazio A, Quinn DI, Watts CKW, Hacker NF, Henshall SM, Sutherland RL. The E3 ubiquitin ligase EDD is an adverse prognostic factor for serous epithelial ovarian cancer and modulates cisplatin resistance in vitro. ***Br J Cancer 98: 1085–93 (2008).***

Heinzelmann-Schwarz VA, Scolyer RA, Scurry JP, Smith AN, Gardiner-Garden M, VandenBergh P, Baron-Hay S, Scott C, Ward RL, Morey A, Fink D, Hacker NF, Sutherland RL, O'Brien PM. High galectin 4 and low meprin alpha expression differentiates primary mucinous epithelial ovarian carcinoma from mucinous gastrointestinal cancers. ***J Clin Path 60: 622–6 (2007).***

Heinzelmann-Schwarz VA, Gardiner-Garden M, Henshall SM, Scurry JP, Scolyer RA, Smith AN, Bali A, VandenBergh P, Baron-Hay S, Scott C, Fink D, Hacker NF, Sutherland RL, O'Brien PM. A distinct molecular profile associated with mucinous epithelial ovarian cancer. ***Br J Cancer 94: 904–13 (2006).***

Heinzelmann-Schwarz V, Gardiner-Garden M, Henshall S, Scurry J, Scolyer R, Davies M, Heinzelmann M, Kalish L, Bali A, Kench J, VandenBergh P, Hacker N, Sutherland RL, O`Brien P. Overexpression of the cell adhesion molecules DDR1, claudin 3 and Ep-CAM in metaplastic ovarian epithelium and ovarian cancer. ***Clin Cancer Res 10: 4427–36 (2004).***

Laboratory Approaches for New Diagnostics and New Drugs

The history of virology is tightly linked to the development of fascinating indirect detection methods or to the sophisticated electron microscopy. Since its early days virology is characterized by the struggle to make the "invisibly small" visible to the human eye and to modern clinical diagnostics. This may explain the remarkable notion that it took until the year 1983 to describe the cancer-causing human papilloma viruses 16 and 18. Also the dangerous human pathogens HIV and HCV were only discovered in 1983 and 1989, respectively. These dates correspond to a period of an unprecedented molecular revolution and the invention of key molecular methodologies including cloning, culture techniques, and automated sequencing or PCR.

Immediately after its discovery, and driven by the dramatic global spread of AIDS, HIV was the first virus to become subject of massive molecular research and drug development in the 1990s. HIV became precedent and role model for "targeted antiviral concepts" and treatment strategies. HIV-disease today represents the first incurable virus infection that is successfully controlled for decades by specific drugs.

My own research interest in retroviruses goes back to the year 1987 at the National Institutes of Health, Bethesda, USA, where, together with Klaus Strebel, we were the first to discover the role of a new HIV protein, "vpu", as coordinator of virus particle release. My research was further strongly imprinted by a productive period in the pharmaceutical industry (until 1997) at Ciba-Geigy/Novartis, where I was member of the research group identifying and developing the HIV protease inhibitor atazanavir, today an important drug in the anti-HIV armamentarium.

Research focus

Since my return to academia I tried to retain, along with the scientific curiosity in virus-driven processes, a touch of applicability and commercial reasoning. Consequently my research group focuses on these key strategies:

Molecular Virology Group
(from left, front to back) Sabrina Steiner (Bachelor); Adelaide Loureiro (Fachmaturpraktikum); Joëlle Bader (PhD student); Konstantin Kletenkov (PhD student); Isabell Seibert (Technician); Sarah Wagner (Technician); Yannick Gerth (Master student); Thomas Klimkait; Frauke Mekolli (Administration)

- Understand aspects in a viral life cycle that associate with clinical therapy escape (therapy resistance and cell tropism of HIV);
- Identify new inhibitory concepts targeting vital virus functions; here our activities focus onto the intriguing source of natural compounds in South African plants, known in traditional medicine to possess disease-fighting properties;
- Develop and improve laboratory diagnostic tools for viral diseases.

Our research tools

In previous years we established an HIV replication system in human reporter cells. These represent the entire viral life, from cell entry via its receptors to virion release from the cell membrane. The chosen adherent human cell host carries the key properties of human lymphocytes (CD4 receptor, chemokine receptors CXCR4 and CCR5) and allows efficient HIV production after infection with free virus or after introduction of plasmid-based HIV DNA. This system enabled us to phenotype viral drug resistance in a clinically meaningful way: drug concentrations for and kinetics of stopping viral replication correlate well with those required in the patient, and clinical drug formulations can be used. This combination of a replication-competent virus assay with a built-in reporter read-out forms our HIV drug-profiling platform. We validated the principles and verified them in clinical settings5. We founded the University spin-off InPheno AG, which, to this day, provides professional services of HIV- and drug profiling, mechanism-of-action studies, or compound screening for pharmaceutical companies. The properties of our HIV-profiling and -test system in a nutshell: Patient-derived virus sequences of the genes protease and reverse transcriptase (RT) are PCR-amplified and inserted into a plasmid, which contains the entire HIV genome without protease and RT. This step reconstitutes a replication-competent HIV-1, which is functionally assessed e.g. in the presence of the various clinical drugs. Although today simpler sequencing-based resistance tests have taken over, the phenotyping system is still in use profiling new drugs and novel inhibitor classes. Of particular interest are newly emerging mutations in today's drug-target genes, which are not represented in the published algorithms.

Our direct phenotyping method can help to identify novel

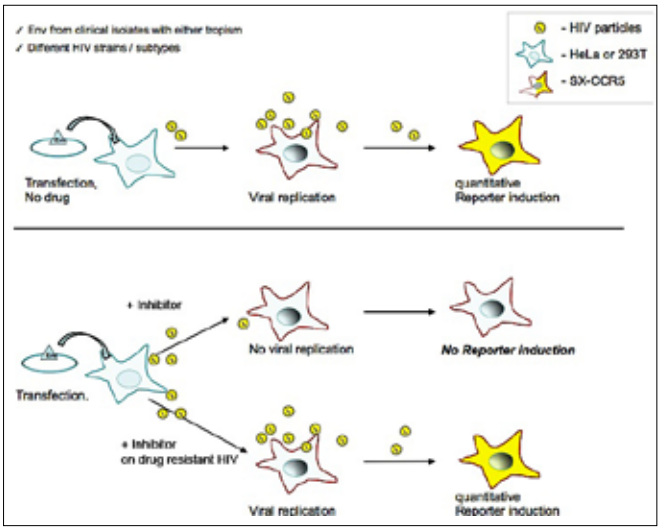


Figure 1: (top graph) Cell-based system for HIV drug profiling and resistance testing. Infectious plasmid DNA is introduced into human cell (left). Virus is produced and passed onto a recipient cell for amplification (center) and to infectible reporter cells (right) turning on an endogenous HIV-driven reporter for quantitative read-out. (Bottom graph) Addition of inhibitor blocks reporter readout unless the drug is not effective (drug-resistant virus).

viral escape routes and therapy failure. In a cohort-wide analysis we validated the unique properties of r-phenotyping versus genotyping and received Swiss-wide approval by the Swiss Federal Office of Health (BAG) for diagnostic use and reimbursement. This "r-phenotyping" system for HIV also assesses the replicative fitness of a virus, and it can reveal mixed virus populations. The latter is critically important for studies of the viral evolution in a given patient, particularly when following the emergence of a minor virus population over time. The role of virus fitness is still not well understood: On the one hand it appears obvious that a virus with higher fitness has a better chance to efficiently reproduce, on the other is a rapidly multiplying virus more likely to be detected by the immune system. This paradox drives studies to better understand how HIV replicates, adapts, and hides in a functional immunological environment. The research of my group focuses on two elements: The structural virus proteins of the gag gene and the tropism-conveying envelope functions.

HIV Gag protein – enzyme substrate and contributor to viral resistance?

The Gag-project bases on the observation that particle maturation can be inhibited in two ways: either by blocking the vital viral protease responsible for the proteolytic processing of precursor proteins, or by inhibiting process-

ing of substrate, the Gag-Pol precursor protein. For particle maturation this precursor pr55 needs to be cleaved into its active components p17, p24, p7, p6. Upon inhibition only non-infectious virions are released. Although the highly selective protease inhibitors effectively block the enzyme, multiple HIV-1 protease mutations have been described allowing the virus to escape drug pressure. Resistance can be largely explained by structural alterations in the enzyme leading to a less perfect fit of the inhibitor in the substrate-binding site. However, in recent years it was discovered that viral resistance to protease inhibitors (PI) seem not to be limited to mutations in the protease gene itself: Amino acid mutations in Gag near protease cleavage sites were reported, preferentially in the context of inhibitor failure. Also, a new inhibitor class was described that is based on preventing Gag processing. Of note, today in a substantial number of patients with failing PI-based therapy no major resistance-associated mutations have been detected. This finding suggests alternative escape mechanisms of the virus outside the protease gene.

Therefore my research group initiated a collaborative study with the SHCS, in which we systematically analyzed

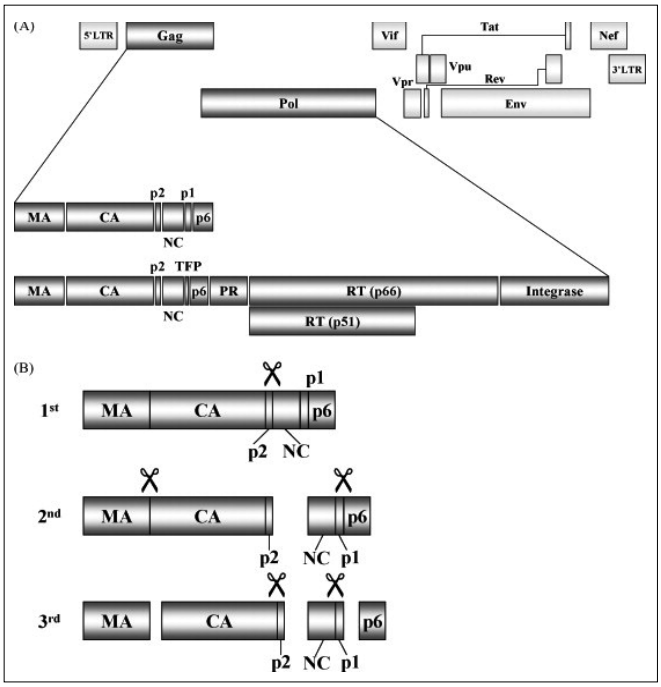


Figure 2: Genomic organization of the retroviral Gag-pol precursor protein. (A) Position in the HIV genome; (B) position of the various functional units and cleavage products MA, CA, p2, NC, p1 and p6. Graph taken from Wensing, A. M., van Maarseveen, N. M. & Nijhuis, M. Fifteen years of HIV Protease Inhibitors: raising the barrier to resistance. Antiviral research 85, 59–74, doi:10.1016/j.antiviral.2009.10.003 (2010).

virus sequences near the 3' end of the gag gene, where key cleavage sites encode. From the anonymized HIV-1 sequence databases in Basel and Zurich (J.Böni) we retrieved some 2000 sequences, stemming from a characterized therapy context or from untreated patients. A side-by-side analysis of these two groups enables us to identify those alterations emerging only under drug pressure, either by enhancing "minor protease mutations", or as new mutations that are by themselves responsible for drug-resistance and clinical treatment failure. We identified some ten mutations associating with clinical PI resistance. Several correspond with earlier publications and thus validate our algorithm. Six mutations have not been described before, neither as HIV subtype-specific polymorphisms nor as treatment-associated escape mutation. Currently we are introducing these exciting mutations into the HIV-1 reference virus NL4-3. Using the r-phenotyping system we will test if they convey PI resistances. This could be crucial for a better understanding of "unexplained virological therapy failures" and would be an important addition to the current resistance algorithms.

HIV tropism – new drug target and driver of therapy response?

A second major project in my research group was the development of a diagnostic test for the cell tropism of HIV: The virus exists as two distinct forms, which differ in their receptor dependence. In the recent past research had yielded a new class of inhibitors targeting the main viral coreceptor of HIV, the T-cell specific chemokine receptor CCR5. Their use would, however, require the existence of laboratory tests that discriminate the tropism of the virus in a patient: Does it use CCXR5 as its receptor or the second option, the CXCR4 chemokine receptor. Therefore, several years ago my research group set out to compete in establishing a reliable rapid test system for HIV tropism. When maraviroc, the first such inhibitor, was introduced, the clinical diagnosis relied solely on a laborious phenotypic test exclusively offered by a US-based diagnostic company.

Together with our University-of-Basel spinoff InPheno AG we built a test, which relies on the hybridization-based properties of DNA-duplexes between a patient-derived HIV sequence and a known probe. The labeled probe represents the tropism-determining sequence "V3" within the

HIV envelope gene. Based on best-matching properties to over 1000 HIV-1 sequences from various HIV-1 subtypes we identified a set of suitable CCR5-tropic probes with maximal overall homology. Today we are able to determine CCR5-tropism with positive predictive values above 86%. Moreover, in a European ring trial for quality control our system passed excellently[12]. Aside from the direct tropism feature our system can identify and dissect mixed virus populations, a main advantage over sequencing-based systems.

Our HIV tropism system XTrack passed the BAG requirements for new diagnostic tests, and received approval in 2012; today test costs are reimbursed by the Swiss health-insurance system.

The figure below depicts the essential features of this "XTrack-system" for the determination of the HIV tropism in clinical specimens. The test principle bases on the difference in electrophoretic mobility of perfectly base-paired DNA duplexes versus less perfect hybrids ("Duplex tracking assay").

It has been observed that most HIV strains, isolated early after infection or during first therapy failures, use the chemokine receptor CCR5. In contrast to these there is a clear trend towards CXCR4-use in advanced disease. Although we and others have seen the link (CCR5 = early; CXCR4 = late + poor prognosis) years ago, it continued to be tricky to clearly identify "hen and egg" in this relationship: It is possible that the emergence of CXCR4-tropic virus causes a poorer outcome of the disease, but it might also well be possible that it is the decaying immune system that only

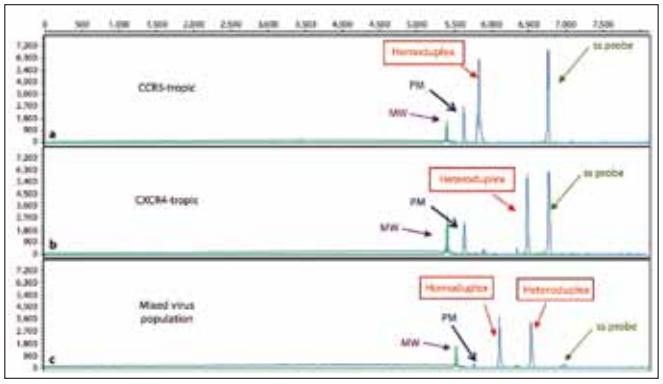


Figure 3: Principle of our system for HIV tropism. Electrophoregram of typical examples: a) imperfect homoduplex of patient-derived HIV sequence with CCR5 probe; b) CXCR4-tropic heteroduplex; c) mixed virus population with 2 peaks. MW: size marker, PM perfect match of probe-duplex; ss probe singles-stranded labeled probe.

permits the rise of CXCR4-tropic HIV. We set out to examine this puzzle. A very useful SHCS study in this context was reported by Kaufmann et al., identifying two principal classes of long term HIV-infected patients: Those, who under successful triple-therapy benefit through a continuous CD4 recovery and those who experiences a CD4 plateau after several years – although in both groups viral replication was continuously suppressed (figure below).

Working hypothesis of our next study is that, if tropism *drives* (rather than results from) the observed immunological difference visible in the CD4 cell population, then we should be able to already observe such a difference early, at the time of treatment initiation. The project with the SHCS used the very samples of Kaufmann's study: One hundred samples were chosen at the time of therapy-initiation, fifty samples from patients who end up in the plateau group and fifty from patients with continuous CD4-cell recovery. Of note, the plateau formed some 3–5 years after treatment start and could therefore not be anticipated at the time of our test.

To our surprise there is a significant correlation between initial virus tropism and immunological outcome (graph above): Despite the overall low number of CXCR4-tropic virus samples more CXCR4-tropism was identified in the plateau group. Vice versa were far more CCR5-tropic vi-

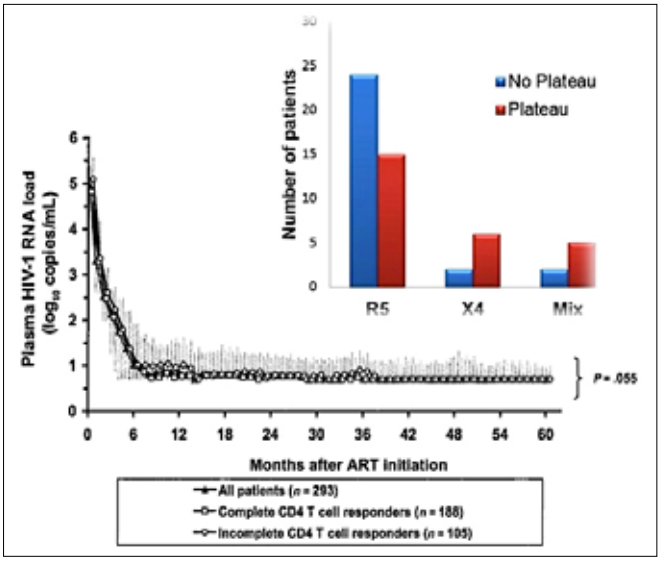


Figure 4: HIV tropism in complete vs. incomplete CD4 T-cell responders, who all were fully HIV-suppressed (black graph, bottom). Inset: (blue bars) = virus isolates form complete responders = "no plateau"; (red bars) = virus isolates from incomplete r. = plateau. HIV tropism was determined at time "0" and assigned to the groups "R5" = CCR5-tropic; "X4" = CXCR4-tropic or of mixed virus tropism ("mix").

ruses found in the patient group with continuous CD4-recovery. A subsequent pilot study compared results at the start of therapy (time 0) with those 48–60 months later. Also this study strongly supports the claim that HIV tropism testing even on stored samples is likely to retain a high diagnostic value: HIV-tropism in free virus at time 0 correlated well with the tropism in cell-associated integrated provirus from infected cells at time 60 mo. In a next step the project will now analyze retrieved cell samples from time 0. This will assess stability and predictive value of the proviral tropism information over the duration of therapy. Several studies claimed that, in the context of therapy with the new class of coreceptor antagonists, proviral information would not be a predictive marker for the tropism of HIV. It is rather suspected that retroviruses such as HIV continuously deposit as provirus any viral genomic information from active virus particles. As a consequence one expects that after years of infection the proviral compartment would heavily over-estimate the proportion of CXCR4-tropic virus in a given patient. Hence proviruses should not be used for testing and for clinical diagnostics. Other clinical reports suggested a certain dynamics in the role and appearance of immune-competent cells yielding overall only a conservative evolution of the coreceptor tropism with rare chances.

These conflicting claims prompted us to initiate an investigation on the study group of patients described above: It is an excellent cohort for studying in detail whether or not fully suppressive therapy will drive viral evolution from one tropism to the other, i.e. from using CCR5 as coreceptor to utilizing CXCR4. We plan to follow the viral tropism over time in each of the patients in the study. As all patients are virologically suppressed, i.e. no free HIV-1 can be isolated from plasma, we will focus on the cellular compartment and analyze the integrated virus genomes. With each round of replication HIV deposits its genome in the next host cell, and it should be possible to follow any tropism evolution. Our preliminary data do not support the expectation that more and more CXCR4-tropic HIV will be found as time goes by. This was based on the observation that with a destroyed immune system HIV-1 loses envelope glycosylation coinciding with a tropism switch towards CXCR4 use or towards viruses using both coreceptors.

When we compared virus at the time of therapy initiation and time points during successful therapy most initially CCR5-tropic virus had not changed. In contrast, initial CXCR4-tropic virus variants were often replaced by CCR5-tropic proviruses. (Small overall patient numbers render these data preliminary and require support by a larger study group and strict side-by-side comparison of matched groups). It is, however, striking that our successfully treated cohort seems to follow the reverse trend: It is rather the CXCR4-tropic virus that diminishes over time. If confirmed this may point towards excitingly new strategies for reducing the proviral reservoir under suppressive HIV-treatment. If possible this should assist long-term improvement of HIV infection in patients or even open new ways towards eradicating retrovirus infections.

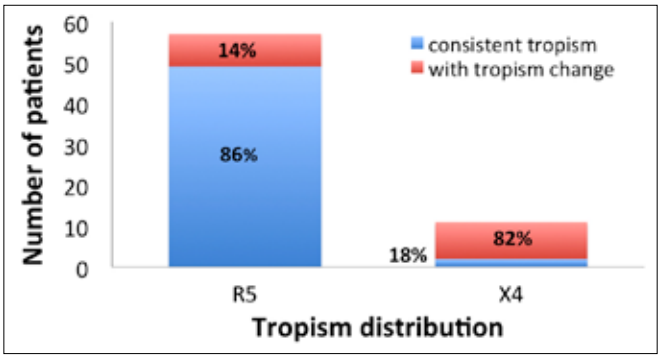


Figure 5: HIV-1 tropism stability in longitudinal samples: virus at baseline versus cell-associated DNA at month 60. (Left bar:) Consistently CCR5-tropic virus (in blue) with percent overall; percentage of samples with change from CXCR4-tropism to CCR5 (in red). (Right bar:) Virus consistently CXCR4-tropic in blue; virus with change from CCR5 to CXCR4 in red, incl. percentage of the respective.

Natural compounds – indigenous sources for new antiviral drug classes?

From my earlier research days in the pharmaceutical industry stems a consistent curiosity towards the discovery of new drugs against pathogenic viruses. We engaged several years ago in an international collaboration with South Africa along these lines. Together with the Swiss "Esperanza Medicines Foundation" (Alex Matter) we were awarded a publicly funded project in the frame of the "Swiss-South-African Joint Research" at the DEZA in Bern. An exciting and fruitful collaboration with the Council for Scientific and Industrial Research (CSIR) in Pretoria and the co-directorship of Vinesh Maharaj began, enabling us to screen hundreds of plant-derived extracts. All extracts were identified, collected and prepared in South Africa, and active ones micro-fractionated by preparative HPLC.

Cellular profiling for anti-retroviral activity was then conducted by my group in Basel. A rich traditional knowledge exists in African on various plants with anecdotal activity in different human diseases including AIDS, we were curious as to whether we would be able to molecularly reproduce activities in support of some of the claims.
At the closure of the three-year funding period by 2012 we had screened close to six hundred plants, extractions or fractions with activity. On some thirty of them we performed deeper analyses that included mechanism of action studies, activity tests for different HIV-1 strains and subtypes, and the potency against known drug-resistant HIV variants. The graph below depicts a typical screen of extracts at a "bio-compatible highest concentration". In order to eliminate unselectively toxic plants the anti-HIV activity was always assessed in parallel to a cytotoxicity test (not shown).

For our most active "hits" we have applied for further funding in order to follow them for further anti-HIV profiling, scale-up and safety studies in rodents. This includes four substances stemming from the Tanga-AIDS-working group in Tanzania (TAWG). The plant-based therapeutic bases on the observations of a clinician working in rural Tanzania in Tanga: Some of his HIV-infected patients did much better than many others, which prompted him to investigate possible reasons. Their traditional healer, whose recipe was based on these very four plants was willing to participate in a scientific investigation, and via the CSIR in Pretoria my research group got involved in the profiling. Very revealing confirmation was our identification of potent anti-HIV activity in extracts from two of the four plants; colleagues in Leiden identified high antimicrobial activity in the other two. The TAWG now plans to scale-up production, to reach a reliable formulation for the required tea-infusion and to perform a clinical study

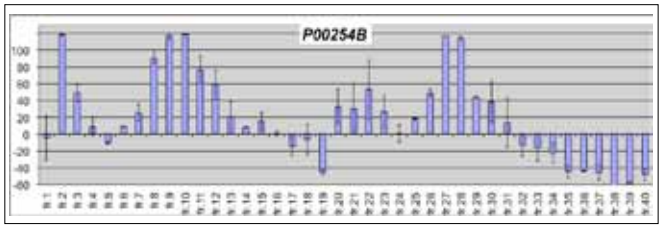


Figure 6: Cellular screen of 40 fractions (fr.1-40) of an extract from South African plants. The y-axis represents percent inhibition of HIV-1 activity in triplicate cultures (w/ error bars). Downward bars indicate a virus induction by the respective fraction.

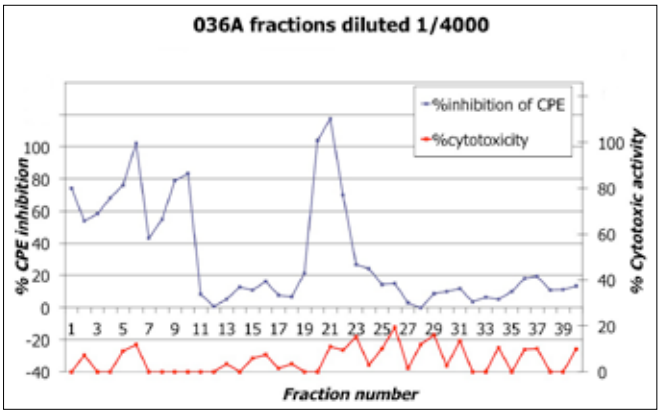


Figure 7: Cell-based screening of the anti-Influenza activity of a plant extract (036A) in a plaque assay. The y-axis on the left indicates the inhibition of the virus-caused cytopathic effect (CPE) for each fraction (blue line); the axis on the right expresses cytotoxic effects of each fraction on uninfected cells (red line).

in Tanzania. It will be exciting to continue participating in the project, as we shall conduct batch-activity testing as well as the characterization of any viral resistance, should it emerge during the study.

A last recently started project aims at widening our collaboration with the CSIR in South Africa. We focus on other infectious diseases highly relevant for the South African population, i.e. respiratory infections caused by RSV and influenza. Also for "flu-like" diseases many African plants have been claimed to possess potent anecdotal activity. Together with the Basel-based InPheno AG we have very recently been able to validate a new phenotypic screening system for these human viruses causing respiratory disease and morbidity. In a first small pilot screen with the CSIR forty extracts were tested from plants with traditional use for flu-like symptoms.

The results of the cellular screening were a confirmation that the reported anti-flu activity of certain plant extracts may have a solid scientific basis. The project is an excellent opportunity for further preparative work and for allowing cell-based molecular profiling of these indigenous plants. We are hopeful that the productive collaboration with the CSIR in South Africa will lead to the discovery of new plant-based principles that could form a starting point for new drug discovery programs that will involve African enterprises but could also become interesting for established international pharmaceutical companies.

Thomas Klimkait

Dissertationen

Seit dem 21. Juni 2013 darf sich **Veronica Sacchi** von der Forschungsgruppe Cell and Gene Therapy (ICFS/Departement Biomedizin Hebelstrasse) Frau Dr. nennen. Sie befasste sich in ihrer Doktorarbeit mit dem Thema: "Highly tunable delivery of matrix-bound growth factors in therapeutic angiogenesis".

Am 24. Juni 2014 stellte sich **Kseniya Maslova** von der Forschungsgruppe Signaling (Departement Biomedizin Hebelstrasse) dem Dissertationskomitee. Der Titel ihrer Dissertation hiess: "Modulatory effects of T-cadherin on cell behavior and growth factor receptor activity in carcinoma cells".

Mit der Doktorprüfung am 25. Juni 2013 schloss **Elena Groppa** von der Forschungsgruppe Cell and Gene Therapy (ICFS/Departement Biomedizin Hebelstrasse) erfolgreich ihre Dissertationszeit ab. Das Thema ihrer Doktorarbeit lautete: "Mechanisms of vascular morphogenesis and stabilization by VEGF dose".

Am 26. Juni 2013 konnte **Cédric Hysek** von der Forschungsgruppe Pyschopharmacology Research (Departement Biomedizin Hebelstrasse) seine Dissertation mit Erfolg beenden. Er befasste sich in seiner Dissertation mit dem Thema "The Role of Norepinephrine in the Pharmacology of 3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy')".

Auszeichnungen

Nicole Pina von der Forschungsgruppe Transplantation and Clinical Virology (Departement Biomedizin Petersplatz) hat ihre Fachmatur «Gesundheit, Soziales und Kunst» mit besonderer Auszeichnung bestanden. Der Titel ihrer Arbeit lautete: «Unterscheiden sich verschiedene NF1-Isoformen in ihren DNA-Bindungseigenschaften und gibt es eine Wechselwirkung zwischen NF1-Expression und der Replikation von BK Polyomavirus in RPTE-Zellen?»

Venia docendi an Andrea Banfi

In ihrer Sitzung am 23. Mai 2013 hat die Regenz der Universität Basel **Andrea Banfi** von der Forschungsgruppe Cell and Gene Therapy (ICFS/Departement Biomedizin Hebelstrasse) die Venia docendi für Experimentelle Medizin erteilt. Er darf nun den Titel eines Privatdozenten führen.

Daniela Finke und Radek Skoda zu Mitgliedern der SAMW gewählt

Daniela Finke von der Forschungsgruppe Developmental Immunology (Departement Biomedizin Mattenstrasse) und **Radek Skoda** von der Forschungsgruppe Experimental Hematology (Departement Biomedizin Hebelstrasse) sind am 28. Mai 2013 zu Mitgliedern der Schweizerischen Akademie der Medizinischen Wissenschaften gewählt worden. Sie werden die Urkunde im Rahmen der Feier zum 70jährigen Jubiläum der SAMW am 28. November 2013 in Empfang nehmen.

Swiss Research Award for Multiple Sclerosis an Claudia Sievers

Claudia Sievers von der Forschungsgruppe Clinical Neuroimmunology (Departement Biomedizin Hebelstrasse) hat am 25. Januar 2013 den mit 20'000 CHF dotierten Swiss Research Award for Multiple Sclerosis von der Schweizerischen Gesellschaft für Multiple Sklerose an deren Jahrestagung entgegen nehmen dürfen.

Bruno Speck Award an Celeste Scotti und Elia Piccinini

Celeste Scotti und **Elia Piccinini** von der Forschungsgruppe Tissue Engineering (ICFS/Departement Biomedizin Hebelstrasse) haben am 26. April 2013 im Rahmen der Tagung des Basel Stem Cell Network für ihre Publikation: "Engineering of a functional bone organ through endochondral ossification". Scotti C, Piccinini E, Takizawa H et al. Proc Natl Acad Sci U S A 110:3997–4002 (2013), den diesjährigen Bruno Speck Award im Bereich Klinische Forschung erhalten.

Herzliche Gratulation an alle!

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 October 31, 2013.

Cell Metabolism Cell Metabolism **17, 448–455, March 5, 2013** IF 14,6

Identification of a SIRT1 Mutation in a Family with Type 1 Diabetes

Anna Biason-Lauber[1,*], Marianne Böni-Schnetzler[2,*], Basil P. Hubbard[3,*], Karim Bouzakri[4,*], Andrea Brunner[2], Claudia Cavelti-Weder[2], Cornelia Keller[2], Monika Meyer-Böni[1], Daniel T. Meier[2], Caroline Brorsson[5], Katharina Timper[2], Gil Leibowitz[6], Andrea Patrignani[7], Remy Bruggmann[7], Gino Boily[9], Henryk Zulewski[2], Andreas Geier[10], Jennifer M. Cermak[13], Peter Elliott[13], James L. Ellis[13], Christoph Westphal[13], Urs Knobel[2], Jyrki J. Eloranta[11], Julie Kerr-Conte[14], François Pattou[14], Daniel Konrad[15], Christian M. Matter[12], Adriano Fontana[8], Gerhard Rogler[10], Ralph Schlapbach[7], Camille Regairaz[16], José M. Carballido[16], Benjamin Glaser[6], Michael W. McBurney[9], Flemming Pociot[5], David A. Sinclair[3], Marc Y. Donath[2]

Summary

Type 1 diabetes is caused by autoimmune-mediated β cell destruction leading to insulin deficiency. The histone deacetylase SIRT1 plays an essential role in modulating several age-related diseases. Here we describe a family carrying a mutation in the *SIRT1* gene, in which all five affected members developed an autoimmune disorder: four developed type 1 diabetes, and one developed ulcerative colitis. Initially, a 26-year-old man was diagnosed with the typical features of type 1 diabetes, including lean body mass, autoantibodies, T cell reactivity to β cell antigens, and a rapid dependence on insulin. Direct and exome sequencing identified the presence of a T-to-C exchange in exon 1 of *SIRT1*, corresponding to a leucine-to-proline mutation at residue 107. Expression of SIRT1-L107P in insulin-producing cells resulted in overproduction of nitric oxide, cytokines, and chemokines. These observations identify a role for SIRT1 in human autoimmunity and unveil a monogenic form of type 1 diabetes.

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[*] These authors contributed equally to this work

Cell Metabolism | Cell Metabolism | 17, 1–14, May 7, 2013 | IF 14,6

Sustained Activation of mTORC1 in Skeletal Muscle Inhibits Constitutive and Starvation-Induced Autophagy and Causes a Severe, Late-Onset Myopathy

Perrine Castets[1,2,*], Shuo Lin[1,*], Nathalie Rion[1], Sabrina Di Fulvio[2], Klaas Romanino[1], Maitea Guridi[1], Stephan Frank[3], Lionel A. Tintignac[1,4], Michael Sinnreich[2], Markus A. Rüegg[1]

Summary

Autophagy is a catabolic process that ensures homeostatic cell clearance and is deregulated in a growing number of myopathological conditions. Although FoxO3 was shown to promote the expression of autophagy-related genes in skeletal muscle, the mechanisms triggering autophagy are unclear. We show that TSC1-deficient mice (TSCmKO), characterized by sustained activation of mTORC1, develop a late-onset myopathy related to impaired autophagy. In young TSCmKO mice, constitutive and starvation-induced autophagy is blocked at the induction steps via mTORC1-mediated inhibition of Ulk1, despite FoxO3 activation. Rapamycin is sufficient to restore autophagy in TSCmKO mice and improves the muscle phenotype of old mutant mice. Inversely, abrogation of mTORC1 signaling by depletion of raptor induces autophagy regardless of FoxO inhibition. Thus, mTORC1 is the dominant regulator of autophagy induction in skeletal muscle and ensures a tight coordination of metabolic pathways. These findings may open interesting avenues for therapeutic strategies directed toward autophagy-related muscle diseases.

Introduction

Muscle wasting, a hallmark of genetic and acquired muscle pathologies, is also associated with aging, cancer, AIDS, and chronic diseases of the heart, lung, or kidney. Muscle size depends on the balance between protein synthesis and protein degradation, the latter being ensured by the ubiquitin-proteasome pathway and the autophagy process (Rüegg and Glass, 2011). Despite the identification of the main molecular pathways involved in the regulation of this homeostatic balance, our understanding of the integrated signaling network remains limited.

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[*] These authors contributed equally to this work

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PKCβ Phosphorylates PI3Kγ to Activate It and Release It from GPCR Control

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Abstract

All class I phosphoinositide 3-kinases (PI3Ks) associate tightly with regulatory subunits through interactions that have been thought to be constitutive. PI3Kγ is key to the regulation of immune cell responses activated by G protein-coupled receptors (GPCRs). Remarkably we find that PKCβ phosphorylates Ser582 in the helical domain of the PI3Kγ catalytic subunit p110γ in response to clustering of the high-affinity IgE receptor (FcεRI) and/or store-operated Ca^{2+}-influx in mast cells. Phosphorylation of p110γ correlates with the release of the p84 PI3Kγ adapter subunit from the p84–p110γ complex. Ser582 phospho-mimicking mutants show increased p110γ activity and a reduced binding to the p84 adapter subunit. As functional p84-p110γ is key to GPCR-mediated p110γ signaling, this suggests that PKCβ-mediated p110γ phosphorylation disconnects PI3Kc from its canonical inputs from trimeric G proteins, and enables p110γ to operate downstream of Ca^{2+} and PKCβ. Hydrogen deuterium exchange mass spectrometry shows that the p84 adaptor subunit interacts with the p110γ helical domain, and reveals an unexpected mechanism of PI3Kγ regulation. Our data show that the interaction of p110γ with its adapter subunit is vulnerable to phosphorylation, and outline a novel level of PI3K control.

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Constitutive Notch2 Signaling Induces Hepatic Tumors in Mice

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Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCC) are the most common liver tumors and a leading cause for cancer-related death in men. Notch2 regulates cellular differentiation in the developing and adult liver. Although aberrant Notch signaling is implicated in various cancers, it is still unclear whether Notch2 regulates proliferation and differentiation in liver carcinogenesis and thereby contributes to HCC and CCC formation. Here, we investigated the oncogenic potential of constitutive Notch2 signaling in the liver. We show that liver-specific expression of the intracellular domain of Notch2 (N2ICD) in mice is sufficient to induce HCC formation and biliary hyperplasia. Specifically, constitutive N2ICD signaling in the liver leads to up-regulation of pro-proliferative genes and proliferation of hepatocytes and biliary epithelial cells (BECs). Using the diethylnitrosamine (DEN) HCC carcinogenesis model, we further show that constitutive Notch2 signaling accelerates DEN-induced HCC formation. DEN-induced HCCs with constitutive Notch2 signaling (DEN^{N2ICD} HCCs) exhibit a marked increase in size, proliferation, and expression of pro-proliferative genes when compared with HCCs from DEN-induced control mice (DEN^{ctrl} HCCs). Moreover, DEN^{N2ICD} HCCs exhibit increased Sox9 messenger RNA (mRNA) levels and reduced Albumin and Alpha-fetoprotein mRNA levels, indicating that they are less differentiated than DEN^{ctrl} HCCs. Additionally, DEN^{N2ICD} mice develop large hepatic cysts, dysplasia of the biliary epithelium, and eventually CCC. CCC formation in patients and DEN^{N2ICD} mice is accompanied by re-expression of hepatocyte nuclear factor 4α(HNF4α), possibly indicating dedifferentiation of BECs.
Conclusion: Our data establish an oncogenic role for constitutive Notch2 signaling in liver cancer development.

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Engineering of a functional bone organ through endochondral ossification

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Embryonic development, lengthening, and repair of most bones proceed by endochondral ossification, namely through formation of a cartilage intermediate. It was previously demonstrated that adult human bone marrow-derived mesenchymal stem/stromal cells (hMSCs) can execute an endochondral program and ectopically generate mature bone. Here we hypothesized that hMSCs pushed through endochondral ossification can engineer a scaled-up ossicle with features of a "bone organ," including physiologically remodeled bone, mature vasculature, and a fully functional hematopoietic compartment. Engineered hypertrophic cartilage required IL-1β to be efficiently remodeled into bone and bone marrow upon subcutaneous implantation. This model allowed distinguishing, by analogy with bone development and repair, an outer, cortical-like perichondral bone, generated mainly by host cells and laid over a premineralized area, and an inner, trabecular-like, endochondral bone, generated mainly by the human cells and formed over the cartilaginous template. Hypertrophic cartilage remodeling was paralleled by ingrowth of blood vessels, displaying sinusoid-like structures and stabilized by pericytic cells. Marrow cavities of the ossicles contained phenotypically defined hematopoietic stem cells and progenitor cells at similar frequencies as native bones, and marrow from ossicles reconstituted multilineage long-term hematopoiesis in lethally irradiated mice. This study, by invoking a "developmental engineering" paradigm, reports the generation by appropriately instructed hMSC of an ectopic "bone organ" with a size, structure, and functionality comparable to native bones. The work thus provides a model useful for fundamental and translational studies of bone morphogenesis and regeneration, as well as for the controlled manipulation of hematopoietic stem cell niches in physiology and pathology.

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Sequential induction of type I and II interferons mediates along-lasting gene induction in the liver in response to a novel toll-like receptor 9 agonist

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Background & Aims: The toll-like receptor 9 (TLR9) agonist IMO-2125 is currently evaluated in clinical trials for chronic hepatitis C therapy. The aim of this study was to investigate the *in vivo* mode of action of a closely related compound, referred to as immunomodulatory oligonucleotide (IMO).

Methods: We analyzed the Jak-STAT pathway activation and induction of interferon-stimulated genes in the liver of wild type, interferon-α/β receptor-deficient and interferon-γ-deficient mice, after administration of IMO.

Results: IMO induced a prolonged activation of the Jak-STAT pathway and upregulation of interferon-stimulated genes in the mouse liver. Contrary to the response observed after interferon-α injection, the signalling induced by IMO was not abrogated following repeated administration.

At early time points after IMO injection, STAT1 phosphorylation and interferon-stimulated gene induction required a functional interferon-α/β receptor, whereas at the later time points, the activation was type I interferon-independent. Microarray analysis revealed that IMO induced a broad transcriptional response in the mouse liver. This included upregulation of cytokine and chemokine genes responsible for recruitment of IFN-γ producers, such as T cells and natural killer cells. Interferon-γ-deficient mice showed a transient response to IMO, demonstrating the central role of interferon-γ in sustained activation of Jak-STAT pathway by IMO.

Conclusions: The bimodal kinetics of response to IMO in the mouse liver are driven by the sequential endogenous production of type I and II interferons. The lack of refractoriness to IMO, combined with the long-lasting induction of interferon-stimulated genes, reveals a favourable pharmacodynamics profile of this novel TLR9 agonist for the treatment of chronic viral hepatitis.

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An International Multicenter Performance Analysis of Cytomegalovirus Load Tests

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Background. Quantification of cytomegalovirus (CMV) load is central to the management of CMV infections in immunocompromised patients, but quantitative results currently differ significantly across methods and laboratories.

Methods. The COBAS AmpliPrep/COBAS TaqMan CMV Test (CAP/CTM CMV test), developed using the first World Health Organization CMV standard in the calibration process, was compared to local assays used by 5 laboratories at transplant centers in the United States and Europe. Blinded plasma panels (n = 90) spiked with 2.18–6.7 $\log_{10}$ copies/mL and clinical plasma samples from immunocompromised patients (n = 660) were tested.

Results. Observed mean panel member concentrations by site and 95% confidence intervals (CIs) of the data combined across sites were narrower for CAP/CTM CMV test compared with local assays. The 95% CI in $\log_{10}$ copies/mL of the combined data per panel member for CAP/CTM CMV test vs comparator assays was .17 vs 1.5 at 2.18 $\log_{10}$ copies/mL; .14 vs .52 at 2.74 $\log_{10}$ copies/mL; .16 vs .6 at 3.3 $\log_{10}$ copies/mL; .2 vs 1.11 at 4.3 $\log_{10}$ copies/ mL; .21 vs 1.13 at 4.7 $\log_{10}$ copies/mL; and .18 vs 1.4 at 6.7 $\log_{10}$ copies/mL. In clinical specimens, constant and variable quantification differences between the CAP/CTM CMV test and comparator assays were observed.

Conclusions. High interlaboratory agreement and precision of CAP/CTM CMV test results across 5 different laboratories over 4 orders of magnitude suggest that this assay could be valuable in prospective studies identifying clinical viral load thresholds for CMV treatment.

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Differential effects of hydroxyurea and INC424 on mutant allele burden and myeloproliferative phenotype in a *JAK2*-V617F polycythemia vera mouse model

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To establish a preclinical animal model for testing drugs with potential effects on myeloproliferative neoplasms (MPNs), we first performed a detailed phenotypic characterization of Cre-inducible transgenic *JAK2*-V617F mice. Deleting the conditional mouse *Jak2*-knockout alleles increased erythropoiesis and accentuated the polycythemia vera phenotype, but did not alter platelet or granulocyte levels. In a transplantation assay, *JAK2*-V617F^{+} BM cells had an advantage over wild-type competitor cells. Using this competitive repopulation assay, we compared the effects of INC424 (ruxolitinib), a dual Jak1/Jak2 inhibitor, and hydroxyurea (HU). HU led to weight loss, but did not reduce spleen weight. The hematologic parameters were lowered and a slight decrease of the mutant allele burden was noted. INC424 had little effect on body weight, but strongly decreased spleen size and rapidly normalized RBC and neutrophil parameters. No significant decrease in the mutant allele burden was observed. INC424 reduced the phospho-Stat5 levels, whereas HU strongly increased phospho-Stat5, most likely because of the elevated erythropoietin levels in response to the HU-induced anemia. This compensatory increase in JAK/STAT signaling may counteract the beneficial effects of cytoreduction at higher doses of HU and represents an adverse effect that should be avoided.

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Osteogenic graft vascularization and bone resorption by VEGF-expressing human mesenchymal progenitors

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Rapid vascularisation of tissue-engineered osteogenic grafts is a major obstacle in the development of regenerative medicine approaches for bone repair. Vascular endothelial growth factor (VEGF) is the master regulator of vascular growth. We investigated a cell-based gene therapy approach to generate osteogenic grafts with an increased vascularization potential in an ectopic nude rat model *in vivo*,by genetically modifying human bone marrow-derived stromal/stem cells (BMSC) to express rat VEGF. BMSC were loaded onto silicate-substituted apatite granules, which are a clinically established osteoconductive material. Eight weeks after implantation, the vascular density of constructs seeded with VEGF-BMSC was 3-fold greater than with control cells, consisting of physiologically structured vascular networks with both conductance vessels and capillaries. However, VEGF specifically caused a global reduction in bone quantity, which consisted of thin trabeculae of immature matrix. VEGF did not impair BMSC engraftment *in vivo*, but strongly increased the recruitment of TRAP-and Cathepsin K-positive osteoclasts. These data suggest that VEGF over-expression is effective to improve the vascularization of osteogenic grafts, but also has the potential to disrupt bone homoeostasis towards excessive degradation, posing a challenge to its clinical application in bone tissue engineering.

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Enhanced dihydropyridine receptor calcium channel activity restores muscle strength in JP45/CASQ1 double knock out mice

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Muscle strength declines with age in part due to a decline of Ca^{2+} release from sarcoplasmic reticulum calcium stores. Skeletal muscle dihydropyridine receptors ($Ca_v1.1$) initiate muscle contraction by activating ryanodine receptors in the sarcoplasmic reticulum. $Ca_v1.1$ channel activity is enhanced by a retrograde stimulatory signal delivered by the ryanodine receptor. JP45 is a membrane protein interacting with $Ca_v1.1$ and the sarcoplasmic reticulum Ca^{2+} storage protein calsequestrin (CASQ1). Here we show that JP45 and CASQ1 strengthen skeletal muscle contraction by modulating Cav1.1 channel activity. Using muscle fibres from JP45 and CASQ1 double knockout mice, we demonstrate that Ca^{2+} transients evoked by tetanic stimulation are the result of massive Ca^{2+} influx due to enhanced $Ca_v1.1$ channel activity, which restores muscle strength in JP45/CASQ1 double knockout mice. We envision that JP45 and CASQ1 may be candidate targets for the development of new therapeutic strategies against decay of skeletal muscle strength caused by a decrease in sarcoplasmic reticulum Ca^{2+} content.

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Chemical Development of Intracellular Protein Heterodimerizers

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Summary

Cell activation initiated by receptor ligands or oncogenes triggers complex and convoluted intracellular signaling. Techniques initiating signals at defined starting points and cellular locations are attractive to elucidate the output of selected pathways. Here, we present the development and validation of a protein heterodimerization system based on small molecules cross-linking fusion proteins derived from HaloTags and SNAP-tags. Chemical dimerizers of HaloTag and SNAP-tag (HaXS) show excellent selectivity and have been optimized for intracellular reactivity. HaXS force protein-protein interactions and can translocate proteins to various cellular compartments. Due to the covalent nature of the HaloTag-HaXS-SNAP-tag complex, intracellular dimerization can be easily monitored. First applications include protein targeting to cytoskeleton, to the plasma membrane, to lysosomes, the initiation of the PI3K/mTOR pathway, and multiplexed protein complex formation in combination with the rapamycin dimerization system.

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IDH/MGMT-driven molecular classification of low-grade glioma is a strong predictor for long-term survival

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Background. Low-grade gliomas (LGGs) are rare brain neoplasms, with survival spanning up to a few decades. Thus, accurate evaluations on how biomarkers impact survival among patients with LGG require long-term studies on samples prospectively collected over a long period.

Methods. The 210 adult LGGs collected in our databank were screened for *IDH1* and *IDH2* mutations (*IDH*mut), MGMT gene promoter methylation (*MGMT*met), 1p/19q loss of heterozygosity (1p19qloh), and nuclear TP53 immunopositivity (TP53pos). Multivariate survival analyses with multiple imputation of missing data were performed using either histopathology or molecular markers. Both models were compared using Akaike's information criterion (AIC). The molecular model was reduced by stepwise model selection to filter out the most critical predictors. A third model was generated to assess for various marker combinations.

Results. Molecular parameters were better survival predictors than histology (ΔAIC = 12.5, P < .001). Forty-five percent of studied patients died. *MGMT*met was positively associated with *IDH*mut (P < .001). In the molecular model with marker combinations, *IDH*mut/*MGMT*met combined status had a favorable impact on overall survival, compared with *IDH*wt (hazard ratio [HR] = 0.33, P < .001), and even more so the triple combination, *IDH*mut/*MGMT*met/ 1p19qloh (HR = 0.18, P < .001). Furthermore, *IDH*mut/*MGMT*met/TP53pos triple combination was a significant risk factor for malignant transformation (HR = 2.75, P < .05).

Conclusion. By integrating networks of activated molecular glioma pathways, the model based on genotype better predicts prognosis than histology and, therefore, provides a more reliable tool for standardizing future treatment strategies.

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RyR1 Deficiency in Congenital Myopathies Disrupts Excitation–Contraction Coupling

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Abstract: In skeletal muscle, excitation–contraction (EC) coupling is the process whereby the voltage-gated dihydropyridine receptor (DHPR) located on the transverse tubules activates calcium release from the sarcoplasmic reticulum by activating ryanodine receptor (RyR1) Ca^{2+} channels located on the terminal cisternae. This subcellular membrane specialization is necessary for proper intracellular signaling and any alterations in its architecture may lead to neuromuscular disorders. In this study, we present evidence that patients with recessive *RYR*1-related congenital myopathies due to primary RyR1 deficiency also exhibit downregulation of the alfa 1 subunit of the DHPR and show disruption of the spatial organization of the EC coupling machinery. We created a cellular RyR1 knockdown model using immortalized human myoblasts transfected with RyR1 siRNA and confirm that knocking down RyR1 concomitantly downregulates not only the DHPR but also the expression of other proteins involved in EC coupling. Unexpectedly, this was paralleled by the upregulation of inositol-1,4,5-triphosphate receptors; functionally however, upregulation of the latter Ca^{2+} channels did not compensate for the lack of RyR1-mediated Ca^{2+} release. These results indicate that in some patients, RyR1 deficiency concomitantly alters the expression pattern of several proteins involved in calcium homeostasis and that this may influence the manifestation of these diseases.

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T-cadherin loss promotes experimental metastasis of squamous cell carcinoma

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Abstract

T-cadherin is gaining recognition as a determinant for the development of incipient invasive squamous cell carcinoma (SCC). However, effects of T-cadherin expression on the metastatic potential of SCC have not been studied. Here, using a murine model of experimental metastasis following tail vein injection of A431 SCC cells we report that loss of T-cadherin increased both the incidence and rate of appearance of lung metastases. T-cadherin-silenced SCC metastases were highly disordered with evidence of single cell dissemination away from main foci whereas SCC metastases overexpressing T-cadherin developed as compact, tightly organised sheets. SCC cell adhesion to vascular endothelial cells (EC) in culture was increased for T-cadherin-silenced SCC and decreased for T-cadherin-overexpressing SCC. Confocal microscopy showed that T-cadherin-silenced SCC adherent on EC display an elongated morphology with long thin extensions and a high degree of intercalation within the EC monolayer, whereas SCC overexpressing T-cadherin formed poorly-spread multicellular aggregates that remain on the outer surface of the EC monolayer. T-cadherin-deficient SCC or human keratinocyte cells exhibited increased transendothelial migration *in vitro* which could be attenuated in the presence of EGFR inhibitor gefitinib. Our data suggest that loss of T-cadherin can increase metastatic potential and aggressiveness of SCC, possibly due to facilitating arrest and extravasation through the vascular wall and/or more efficient establishment of metastases in the new microenvironment.

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Clinical impact of programmed cell death ligand 1 expression in colorectal cancer

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Abstract **Background:** Programmed cell death 1 (PD-1) receptor triggering by PD ligand 1 (PD-L1) inhibits T cell activation. PD-L1 expression was detected in different malignancies and associated with poor prognosis. Therapeutic antibodies inhibiting PD-1/PD-L1 interaction have been developed.

Materials and methods: A tissue microarray ($n = 1491$) including healthy colon mucosa and clinically annotated colorectal cancer (CRC) specimens was stained with two PD-L1 specific antibody preparations. Surgically excised CRC specimens were enzymatically digested and analysed for cluster of differentiation 8 (CD8) and PD-1 expression.

Results: Strong PD-L1 expression was observed in 37% of mismatch repair (MMR)-proficient and in 29% of MMR-deficient CRC. In MMR-proficient CRC strong PD-L1 expression correlated with infiltration by $CD8^+$ lymphocytes ($P = 0.0001$) which did not express PD-1. In univariate analysis, strong PD-L1 expression in MMR-proficient CRC was significantly associated with early T stage, absence of lymph node metastases, lower tumour grade, absence of vascular invasion and significantly improved survival in training ($P = 0.0001$) and validation($P = 0.03$) sets. A similar trend ($P = 0.052$) was also detectable in multivariate analysis includingage, sex, T stage, N stage, tumour grade, vascular invasion, invasive margin and MMR status. Interestingly, programmed death receptor ligand 1 (PDL-1) and interferon (IFN)-γ gene expression, as detected by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) in fresh frozen CRC specimens ($n = 42$) were found to be significantly associated ($r = 0.33$, $P = 0.03$).

Conclusion: PD-L1 expression is paradoxically associated with improved survival in MMRproficient CRC.

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Induction of Aberrant Vascular Growth, But Not of Normal Angiogenesis, by Cell-Based Expression of Different Doses of Human and Mouse VEGF Is Species-Dependent

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Abstract

Therapeutic angiogenesis by vascular endothelial growth factor (VEGF) gene delivery is an attractive approach to treat ischemia. VEGF remains localized around each producing cell *in vivo*, and overexpression of mouse $VEGF_{164}$ ($mVEGF_{164}$) induces normal or aberrant angiogenesis, depending strictly on its dose in the micro-environment *in vivo*. However, the dose-dependent effects of the clinically relevant factor, human $VEGF_{165}$ ($hVEGF_{165}$), are unknown. Here we exploited a highly controlled gene delivery platform, based on clonal populations of transduced myoblasts overexpressing specific VEGF levels, to rigorously compare the *in vivo* dose-dependent effects of $hVEGF_{165}$ and $mVEGF_{164}$ in skeletal muscle of severe combined immune deficient (SCID) mice. While low levels of both factors efficiently induced similar amounts of normal angiogenesis, only high levels of $mVEGF_{164}$ caused widespread angioma-like structures, whereas equivalent or even higher levels of $hVEGF_{165}$ induced exclusively normal and mature capillaries. Expression levels were confirmed both in vitro and *in vivo* by enzyme-linked immunosorbent assay (ELISA) and quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR). However, *in vitro* experiments showed that $hVEGF_{165}$ was significantly more effective in activating VEGF receptor signaling in human endothelial cells than $mVEGF_{164}$, while the opposite was true in murine endothelial cells. In conclusion, we found that, even though hVEGF is similarly efficient to the syngenic mVEGF in inducing angiogenesis at lower doses in a widely adopted and convenient mouse preclinical model, species-dependent differences in the relative activation of the respective receptors may specifically mask the toxic effects of high doses of the human factor.

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Careful Selection of Reference Genes Is Required for Reliable Performance of RT-qPCR in Human Normal and Cancer Cell Lines

Francis Jacob[1,2], Rea Guertler[2], Stephanie Naim[2], Sheri Nixdorf[2], André Fedier[1], Neville F. Hacker[3], Viola Heinzelmann-Schwarz[1,2,3]

Abstract

Reverse Transcription -quantitative Polymerase Chain Reaction (RT-qPCR) is a standard technique in most laboratories. The selection of reference genes is essential for data normalization and the selection of suitable reference genes remains critical. Our aim was to 1) review the literature since implementation of the MIQE guidelines in order to identify the degree of acceptance; 2) compare various algorithms in their expression stability; 3) identify a set of suitable and most reliable reference genes for a variety of human cancer cell lines. A PubMed database review was performed and publications since 2009 were selected. Twelve putative reference genes were profiled in normal and various cancer cell lines (n = 25) using 2-step RT-qPCR. Investigated reference genes were ranked according to their expression stability by five algorithms (geNorm, Normfinder, BestKeeper, comparative ΔCt, and RefFinder). Our review revealed 37 publications, with two thirds patient samples and one third cell lines. qPCR efficiency was given in 68.4% of all publications, but only 28.9% of all studies provided RNA/cDNA amount and standard curves. GeNorm and Normfinder algorithms were used in 60.5% in combination. In our selection of 25 cancer cell lines, we identified *HSPCB, RRN18S,* and *RPS13* as the most stable expressed reference genes. In the subset of ovarian cancer cell lines, the reference genes were *PPIA, RPS13* and *SDHA*, clearly demonstrating the necessity to select genes depending on the research focus. Moreover, a cohort of at least three suitable reference genes needs to be established in advance to the experiments, according to the guidelines. For establishing a set of reference genes for gene normalization we recommend the use of ideally three reference genes selected by at least three stability algorithms. The unfortunate lack of compliance to the MIQE guidelines reflects that these need to be further established in the research community.

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Klf4 Is a Transcriptional Regulator of Genes Critical for EMT, Including Jnk1 *(Mapk8)*

Neha Tiwari[1,•], Nathalie Meyer-Schaller[1], Phil Arnold[2], Helena Antoniadis[1], Mikhail Pachkov[2], Erik van Nimwegen[2], Gerhard Christofori[1]

Abstract

We have identified the zinc-finger transcription factor Kruppel-like factor 4 (Klf4) among the transcription factors that are significantly downregulated in their expression during epithelial-mesenchymal transition (EMT) in mammary epithelial cells and in breast cancer cells. Loss and gain of function experiments demonstrate that the down-regulation of Klf4 expression is required for the induction of EMT *in vitro* and for metastasis *in vivo*. In addition, reduced Klf4 expression correlates with shorter disease-free survival of subsets of breast cancer patients. Yet, reduced expression of Klf4 also induces apoptosis in cells undergoing TGFβ-induced EMT. Chromatin immunoprecipitation/deep-sequencing in combination with gene expression profiling reveals direct Klf4 target genes, including E-cadherin *(Cdh1)*, N-cadherin *(Cdh2)*, vimentin *(Vim)*, β-catenin *(Ctnnb1)*, VEGF-A *(Vegfa)*, endothelin-1 *(Edn1)* and Jnk1 *(Mapk8)*. Thereby, Klf4 acts as a transcriptional activator of epithelial genes and as a repressor of mesenchymal genes. Specifically, increased expression of Jnk1 *(Mapk8)* upon down-regulation of its transcriptional repressor Klf4 is required for EMT cell migration and for the induction of apoptosis. The data demonstrate a central role of Klf4 in the maintenance of epithelial cell differentiation and the prevention of EMT and metastasis.

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Noninvasive Ultrasound Molecular Imaging of the Effect of Statins on Endothelial Inflammatory Phenotype in Early Atherosclerosis

Elham Khanicheh[1], Martina Mitterhuber[1], Lifen Xu[1], Stéphanie P. Haeuselmann[1], Gabriela M. Kuster[1,2], Beat A. Kaufmann[1,2]

Abstract

Background/Objectives: Inflammatory changes on the endothelium are responsible for leukocyte recruitment to plaques in atherosclerosis. Noninvasive assessment of treatment-effects on endothelial inflammation may be of use for managing medical therapy and developing novel therapies. We hypothesized that molecular imaging of vascular cell adhesion molecule-1 (VCAM-1) with contrast enhanced ultrasound (CEU) could assess treatment effects on endothelial phenotype in early atherosclerosis.

Methods: Mice with atherosclerosis produced by gene deletion of the LDL-receptor and Apobec-1-editing protein were studied. At 12 weeks of age, mice received 8 weeks of regular chow or atorvastatin-enriched chow (10 mg/kg/day). At 20 weeks, CEU molecular imaging for aortic endothelial VCAM-1 expression was performed with VCAM-1-targeted (MB_{VCAM}) and control microbubbles (MB_{Ctr}). Aortic wall thickness was assessed with high frequency ultrasound. Histology, immunohistology and Western blot were used to assess plaque burden and VCAM-1 expression.

Results: Plaque burden was reduced on histology, and VCAM-1 was reduced on Western blot by atorvastatin, which corresponded to less endothelial expression of VCAM-1 on immunohistology. High frequency ultrasound did not detect differences in aortic wall thickness between groups. In contrast, CEU molecular imaging demonstrated selective signal enhancement for MB_{VCAM} in non-treated animals (MB_{VCAM} 2±0.3 vs MB_{Ctr} 0.7±0.2, p<0.01), but not in statin-treated animals (MB_{VCAM} 0.8±0.2 vs MB_{Ctr} 1.0±0.2, p =ns; p,0<01 for the effect of statin on MBV_{CAM} signal).

Conclusions: Non-invasive CEU molecular imaging detects the effects of anti-inflammatory treatment on endothelial inflammation in early atherosclerosis. This easily accessible, low-cost technique may be useful in assessing treatment effects in preclinical research and in patients.

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Low Levels of Mannan-Binding Lectin or Ficolins Are Not Associated with an Increased Risk of Cytomegalovirus Disease in HIV-Infected Patients

Adrian Egli[1,2,•], Juliane Schäfer[3], Michael Osthoff[2], Steffen Thiel[4], Christina Mikkelsen[4], Andri Rauch[5], Hans H. Hirsch[1,6], Heiner C. Bucher[3], James Young[3], Jens C. Jensenius[4], Manuel Battegay[1], Marten Trendelenburg[2,7], the Swiss HIV Cohort Study

Abstract

Background: In HIV-infected patients, prediction of Cytomegalovirus (CMV) disease remains difficult. A protective role of mannan-binding lectin (MBL) and ficolins against CMV disease has been reported after transplantation, but the impact in HIV-infected patients is unclear.

Methods: In a case-control study nested within the Swiss HIV Cohort Study, we investigated associations between plasma levels of MBL/ficolins and CMV disease. We compared HIV-infected patients with CMV disease (cases) to CMV-seropositive patients without CMV disease (controls) matched for CD4 T-cells, sampling time, and use of combination antiretroviral therapy. MBL and M-ficolin, L-ficolin, and H-ficolin were quantified using ELISA.

Results: We analysed 105 cases and 105 matched controls. CMV disease was neither associated with MBL (odds ratio [OR] 1.03 per $\log_{10}$ ng/mL increase (95% CI 0.73–1.45)) nor with ficolins (OR per $\log_{10}$ ng/mL increase 0.66 (95% CI 0.28–1.52), 2.34 (95% CI 0.44–12.36), and 0.89 (95% CI 0.26–3.03) for M-ficolin, L-ficolin, and H-ficolin, respectively). We found no evidence of a greater association between MBL and CMV disease in patients with low CD4 counts; however in the multivariable analysis, CMV disease was more likely in patients with an increased HIV RNA (OR 1.53 per $\log_{10}$ copies/mL; 95% CI 1.08–2.16), or a shorter duration of HIV-infection (OR 0.91 per year; 95% CI 0.84–0.98).

Conclusions: CMV disease is not associated with low levels of MBL/ficolins, suggesting a lack of a protective role in HIV-infected patients.

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Cellular Signalling Cellular Signalling 25 (2013) 1044–1053 IF 4,0

Cross-talk between EGFR and T-cadherin: EGFR activation promotes T-cadherin localization to intercellular contacts

Emmanouil Kyriakakis[1], Kseniya Maslova[1], Audrey Frachet[1], Nicola Ferri[2], Alessandro Contini[3], Dennis Pfaff[1], Paul Erne[4], Therese J. Resink[1], Maria Philippova[1]

Abstract

Reciprocal cross-talk between receptor tyrosine kinases (RTKs) and classical cadherins (e.g. EGFR/E-cadherin, VEGFR/VE-cadherin) has gained appreciation as a combinatorial molecular mechanism enabling diversification of the signalling environment and according differential cellular responses. Atypical glycosylphosphatidylinositol (GPI)-anchored T-cadherin (T-cad) was recently demonstrated to function as a negative auxiliary regulator of EGFR pathway activation in A431 squamous cell carcinoma (SCC) cells. Here we investigate the reciprocal impact of EGFR activation on T-cad. In resting A431 T-cad was distributed globally over the cell body. Following EGF stimulation T-cad was redistributed to the sites of cell–cell contact where it colocalized with phosphorylated $EGFR^{Tyr1068}$. T-cad redistribution was not affected by endomembrane protein trafficking inhibitor brefeldin A or *de novo* protein synthesis inhibitor cycloheximide, supporting mobilization of plasma membrane associated T-cad. EGF-induced relocalization of T-cad to cell–cell contacts could be abrogated by specificinhibitorsof EGFR tyrosine kinase activity (gefitinib or lapatinib), lipid raft integrity (filipin), actin microfilament polymerization (cytochalasin D or cytochalasin B), p38MAPK (SB203580) or Rac1 (compound4). Erk1/2 inhibitor PD98059 increased phospho-$EGFR^{tyr1068}$ levels and not only amplified effects of EGF but also per se promoted some relocalization of T-cad to cell–cell contacts. Rac1 activation by EGF was inhibited by gefitinib, lapatinib or SB203580 but amplified by PD98059. Taken together our data suggest that T-cad translocation to cell–cell contacts is sensitive to the activity status of EGFR, requires lipid raft domain integrity and actin filament polymerization, and crucial intracellular signalling mediators include Rac1 and p38MAPK. The study has revealed a novel aspect of reciprocal cross-talk between EGFR and T-cad.

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CMV antigenemia and quantitative viral load assessments in hematopoietic stem cell transplant recipients

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Abstract

Background: Sensitive and reliable diagnostic tests are essential for the prevention of cytomegalovirus(CMV) disease after hematopoietic stem cell transplantation (HSCT). pp65 antigenemia and polymerase chain reaction (PCR) assays are commonly used to monitor CMV in HSCT recipients. However, there is considerable intra- and inter-laboratory variability in the results, which impact comparability and clinical practice.

Objectives/study design: Using 380 samples from 135 HSCT recipients, we compared the new FDA approved quantitative PCR assay, COBAS® AmpliPrep/COBAS® TaqMan® CMV test (CAP/CTM CMV test) developed and standardized using the 1st WHO International Standard for CMV with pp65 antigenemia and COBAS® AMPLICOR MONITOR CMV tests.

Results: The median time between transplantation and testing samples was 57 days (range, 0–207 days). The median CMV load (log_{10}) was 3.17 IU/mL (3.21 copies/mL). Among samples with detectable CMV load, 52% were negative by pp65 antigenemia. CMV loads were higher in pp65 antigenemia-positive than in negative samples. One pp65-antigenemia-positive cell per 100,000 leukocytes corresponded to a median CMV load of 1200 IU/mL. CMV loads determined by the CAP/CTM CMV test were slightly lower than the ones by the AMPLICOR MONITOR CMV test (–0.15 [95% CI, –0.18 to –0.13] copies/mL), but slope differences indicated only limited co-linearity.

Conclusions: The CAP/CTM CMV test is more sensitive than pp65 antigenemia and the AMPLICOR MONITOR CMV test in HSCT recipients. The lower limit of quantification and co-linearity with the international WHO standard renders the CAP/CTM CMV test suitable for future clinical trials defining viral load thresholds of CMV therapy.

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REVIEWS

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When metabolism met immunology

Marc Y Donath

Obesity induces metabolic stress and is associated with inflammation. A cellular pathway now links SIRT2, a deacetylase involved in metabolic processes, to cytoskeleton remodelling and activation of the NLRP3 inflammasome.

Traditionally, metabolism and immunity have been perceived as two distinct entities with distinct functions: metabolism regulates the disposal and transformation of nutrients, whereas the immune system is responsible for host defense. However, in a broader sense, these two processes are both an organism's response to stressors, with the aim of restoring homeostasis. For immunity, pathogen, mechanical or chemical insults are the stressors. However, with the increasing abundance of food and the deleterious consequences of overnutrition, it has become apparent that that these can also be important stress factors for the body. Thus, it seems reasonable that, in a manner analogous to the response to microbes, metabolic stress also triggers inflammation. An increasing amount of evidence has demonstrated that the chronic activation of the immune system associated with obesity may be deleterious. Such failure of the immune system may be due to the duration and magnitude of the metabolic stress, along with genetic predispositions. The interactions between immunity and metabolism described above have generated a new field called 'immunometabolism'. However, the precise molecular pathways that link the two systems remain to be clarified. In this issue of *Nature Immunology*, Misawa *et al.* describe a connection among mitochondrial stress, cytoskeletal remodeling and proinflammatory production of interleukin-1β (IL-1β)[1].

The NLRP3 inflammasome is central to the maturation of IL-1β, and evidence increasingly suggests that dysregulated activation of this multiprotein complex has an important role in a variety of autoinflammatory diseases, including metabolic disorders. By means of still rather poorly understood mechanisms, the NLRP3 inflammasome is activated after assembly of NLRP3 proteins, the adaptor ASC and procaspase-1 into a multimolecular complex. Activation of the NLRP3 inflammasome can be triggered by various means, including extra-cellular ATP, reactive oxygen species, K^+ efflux and uric acid crystals, but again it remains unclear how such wide-ranging stimuli can converge on the same activation event.

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Congratulations

Das DBM gratuliert ganz herzlich!

Lisa Wieckowski
Geboren am 10.05.2013

Louise Seguin-Mereau
Geboren am 19.05.2013

Herzlich willkommen, allerseits!

Olga Tureckova
Geboren am 12.02.2013

Flora Helene Allenstein-Bethge
Geboren am 07.05.2013

Ferienfoto – oder, wie es ist, in Ilijas Händen zu schlafen.

¡Vamos a Sevilla!

Barrio de Santa Cruz

Why should I visit Seville? And when? How can I possibly enjoy a city which, instead of four seasons, has only two: the burning sun and the endless rain?

I'd better start explaining where Seville is located. Seville is the capital city of the south of Spain (the region of Andalucía). As with any capital, Seville has had great importance in History. It was in Sevilla where the first expedition to the Americas was organized, in the times when most people thought that crossing the Atlantic Ocean was simply a crazy idea. Over the years, Seville has been home to many different civilizations: Phoenicians, Romans, Arabs, Visigoths... maybe we are just easy to conquer! Even the inhabitants of planet Naboo walked along Seville's Plaza de España in a couple of episodes of the Star Wars saga.

Due to this cultural diversity, Seville's architecture is a mixture of numerous styles, such as Gothic, Mudéjar, Renaissance, Baroque, Neoclassical, Romantic, etc. Little wonder, therefore, that its Old Town contains three UNESCO World Heritage Sites: the Alcázar palace, the Cathedral and the General Archive of the Indies. But you can probably read all this in tourist brochures, so I will write about something different.

One of the wonderful aspects of Seville is that, even though it is one of the biggest cities in Spain, people sometimes behave as if they lived in a small village. It is very common to know most of your neighbours, and receive news about everyone's family while you walk along your own street, as well as telling your stories to others. It is nice to feel some love from your neighbours, isn't it? That's probably one of the most notable characteristics of southern cities: it is really easy to blend in with the community, as we are always open to new people coming.

So, even if you don't speak Spanish and can't find anyone to communicate in English, use the few (or many) words you know in Spanish. People will love to help you or just talk for a while; you will have a very nice surprise. We, generally, love foreigners and trying to communicate is always fun for us.

Alcazar

Alcazar

Another charm of Seville is that biking is just a fantastic way to get to know the city, unlike what normally happens in Spanish towns. The council provides rental bikes on a per diem basis and it is an exceptionally flat city with bike lanes everywhere! What else could you ask for?

Maybe after a long morning of biking and sightseeing you are starving and then you will think about looking for a place to have lunch... but restaurants are still closed. You have probably heard this before, but until you face it you don't realize how serious it is that lunch starts at 14h. Our schedules are totally different, so if you feel hungry before lunch time, why don't you go to have a glass of wine with tapas and ibéricos? Not really a bad idea, don't you think?

In addition, lunch in Spain can last hours. There is a saying that states that if you meet a Spaniard for lunch, you should book a full day in your agenda. You can easily expect to be with that person at least until 18h, – and that's if you only met for lunch!

Accordingly, dinner is not served before 20h, and on summer nights you will probably find all bars and restaurants full until midnight, even on weekdays. Walking around the city at night during summer is a wonderful experience; it is the best time to enjoy the city. In fact, during the summer we live more at night than during the day.

However, there is something I have to warn you about: summer is very hot in Seville. I mean really hot, like 25 degrees at midnight and over 40 degrees at noon. So if you decide to visit during our summer, please drink a lot of water and use sunscreen, unless you want to hear, in a friendly way, that you look like a shrimp. If this happens, do not worry, it is just a joke. We love joking.

Some of my friends from Seville visited me in Basel, and they pointed out that Basel (on sunny

Juderia

Cathedral

days) looks like Seville, as both cities are crossed by a river. The Guadalquivir River flows between Seville and Triana, which in the past used to be independent cities with only a boat bridge between both sides. On the Triana side you will still find traditional ceramist workshops making pottery and traditional tiles. The streets are narrow with stone pavement. There, the old ladies still bring chairs from their homes to sit outside in the street and chit-chat with the neighbours about their lives, and everyone else's lives... Such a bad place to have secrets!

Finally, I have to say that not only is Seville a gorgeous city but all of Andalucía is beautiful and diverse. In Andalucía you will find virgin beaches, snowed mountains, desert, small villages isolated from modern life and all kind of paradises where you can get lost if you do not want to be found.

Jana Orellana

Niemand zu klein, um Tennisspieler zu sein

Marc in jungen Jahren

Auch ich habe einmal angefangen zu spielen und bin nicht mit dem Tennisschläger in der Hand auf die Welt gekommen. Deshalb erkläre ich kurz, was es braucht, um Tennis spielen zu lernen oder auch zu den Top-Spielern zu gehören.

Ich habe begonnen als 4jähriger Tennis zu spielen und es hat mich ab dann nicht mehr los gelassen. Wer schlägt schon nicht gern einen Ball mit einem Schläger?

In diesem Alter kann man es noch nicht richtiges Tennis nennen, aber ab dann können Grundlagen geschaffen werden, die für Kinder in Zukunft viele Vorteile bringt.

Training (4–8 Jahre)
In diesem Alter ist es ideal, mit Tennis zu beginnen, denn jetzt kann man Schwächen und Stärken von Kindern sehr gut erkennen, besonders weil die Schwächen noch nicht von den Stärken verdeckt werden.

So kann es gut aussehen, wenn ein Kind Kraft hat und stark zu schlägt, aber es lenkt von einer fehlerhaften Bewegung ab. Je älter ein Kind ist, desto schwieriger wird es, das zu sehen und neu anzupassen.

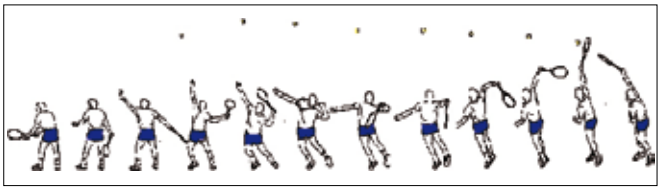
Förderung in diesem Alter: Geschicklichkeit --> Koordination, Reaktion, Differenzierung, Orientierung, Rhythmisierung und Gleichgewichtfähigkeit. Hier gilt, je besser die Basis, desto besser der zukünftige Spieler.

Training (8–12 Jahre)
Ab 8 Jahren ist meiner Meinung nach die Zeit, in der man sieht, wo die Talente der Kinder versteckt sind. Fähigkeiten wie Reaktionsfähigkeit, Spring- und Stopp-Geschwindigkeit, Beweglichkeit, strategisches Denken, Ausdauer und vieles mehr entwickeln sich bei jedem Kind immer verschieden. Auch wenn es nur in der Ausholbewegung ist, die Konzentration auf den Ball mit den Augen oder dann im Schlag, das sind alles Details, welche den Profi dann ausmachen.

Talente
Ich sage gleich im Voraus, egal wie viel Talent jemand hat, wenn der Wille und die Motivation nicht geweckt werden können, dann wird auch niemand zu einem Profi.

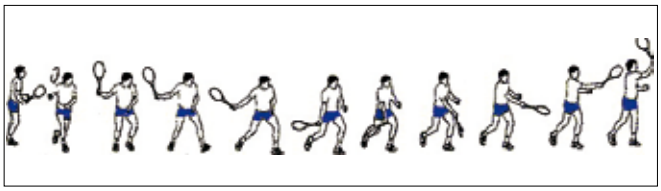
Der Profi
Falls es wirklich zum Profi reicht, dann ist das wirklich harte Arbeit und noch härter ist es, auf dem Level zu bleiben.

Alle diese Automatismen, die perfekt nacheinander funktionieren müssen, müssen jeden Tag trainiert werden. Wenn dann noch Zeit bleibt, geht es daran, sie noch zu verbessern.



Aufschlag

Kinder lernen vieles spielerisch selber, aber wenn es in den professionellen Bereich geht, dann wird jede Bewegung analysiert und psychologisch (wenn möglich spielerisch) beigebracht.
z. B.: Die Vorhand → Ist die Position auf dem Platz richtig? Stimmt der Griff? War die Vorbereitung für die Höhe, Geschwindigkeit und Rotation des Balles angepasst (im besten Fall bevor der Ball über dem Netz ist)? Ausholbewegung richtig? Stimmt die Fussstellung (Gleichgewicht)? Augen auf den Ball fixiert? Fussstellung nach dem Schlag? War der Schlag für die Spielsituation richtig?
Alles Details, die den Profi am Schluss bei nur einem Schlag ausmachen.



Vorhand

Meine Profi-Formel dazu lautet:
Am Tag 2–3 Stunden Training, 2–3 Stunden Ausdauer, 1–2h Analyse, der Rest für die Regeneration, 1–2 Ruhetage pro Woche.

Kosten:
Platzmiete/h ca 35.–, Tennislehrer Privatstunden 55.–, Material 10.–. Also ca. 1500.– bis 2000.–/Monat, je nach Vergünstigungen.
Ich hatte leider nicht so viel Taschengeld, als ich klein war, aber bin mit dem Ergebnis eigentlich ganz zufrieden, und mit 32 Jahren ist die Zeit als Profi sowieso schon fast vorbei. Es reicht zum Glück, seit bald 10 Jahren bei TAFD Reinach, fürs Tennisstunden geben und nicht zu vergessen, muss ich natürlich auch Tennisstunden nehmen.

Weltrekorde:
Schnellster Aufschlag: 263 km/h (ich 205 km/h)
Längster Match: 11 h (ich 4h)
Längster Ballwechsel: 3h 33 Min. (ich ca. 4–5 Min.)
Höchste Preisgeldsumme: Roger Federer 1998 bis 2012 → 72.9 Millionen (ich ca. CHF 2000.–)

Grundsätzlich ist es einfach wichtig, Sport zu machen, denn wie gesagt, der Körper passt sich dem an, für was er gebraucht wird und wenn er nichts leisten muss, dann wird er es irgendwann auch nicht mehr können.

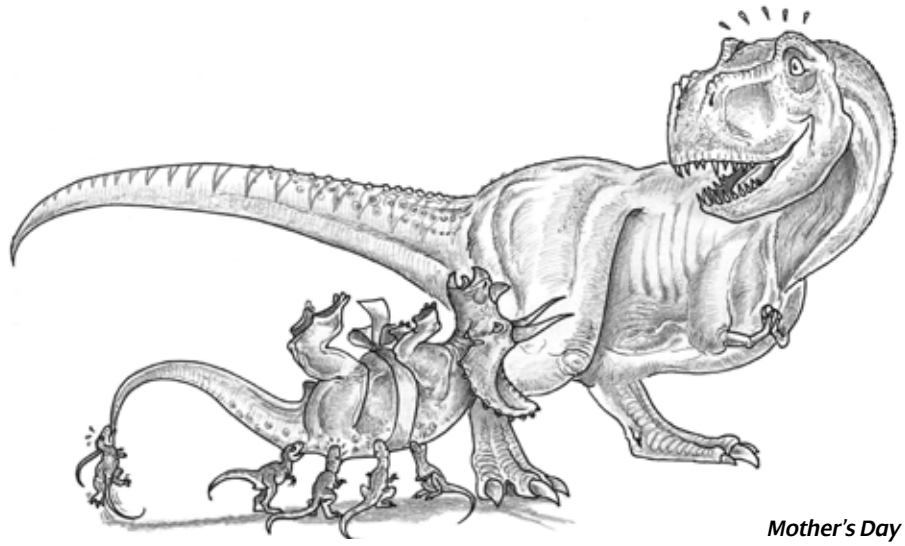
Marc Bichsel

Have pencil, will draw...

Have pencil, will draw

I have been drawing for as long as I can remember – like most kids do! No books or walls were safe from the combined power of colourful crayons and a little boy's fingers. As I grew older, I continued drawing whenever there was time – although often enough there wasn't much. Though I never felt proficient or bold enough to aspire to a professional career as an illustrator or designer, I enjoy drawing to this very day.

Drawing is for me not so much an escape, but rather used for recovery and recreation. It is also an endless source of inspiration and happiness – that is to say when a drawing comes out the way as planned. However, all this doesn't come without a price, as even lab equipment and storage boxes become occasionally decorated by strange ornaments and descriptions in fictional writing systems, much to the confusion of my coworkers.



Mother's Day

Given that there are many other things to do of late, most of the drawings in the last couple of years were done for colleagues, friends and family members, for births and birthdays, weddings and celebrations, and for the many people coming to and leaving work. All these events gave me a very welcome excuse to further pursue my hobby on evenings or weekends. A few of those drawings are shown here – some of them are more cartoonish, others more realistic. It is that kind of total freedom that I also highly appreciate.

As well as these drawings, a little bit of time occasionally remains to concentrate on a small number of larger projects.

One of them is entitled the "Amazing Adventures of Captain Tree". Captain Tree is a superhero, whose life is fully dedicated to the protection of Mother Nature. The Guard-

Captain Tree

Various drawings

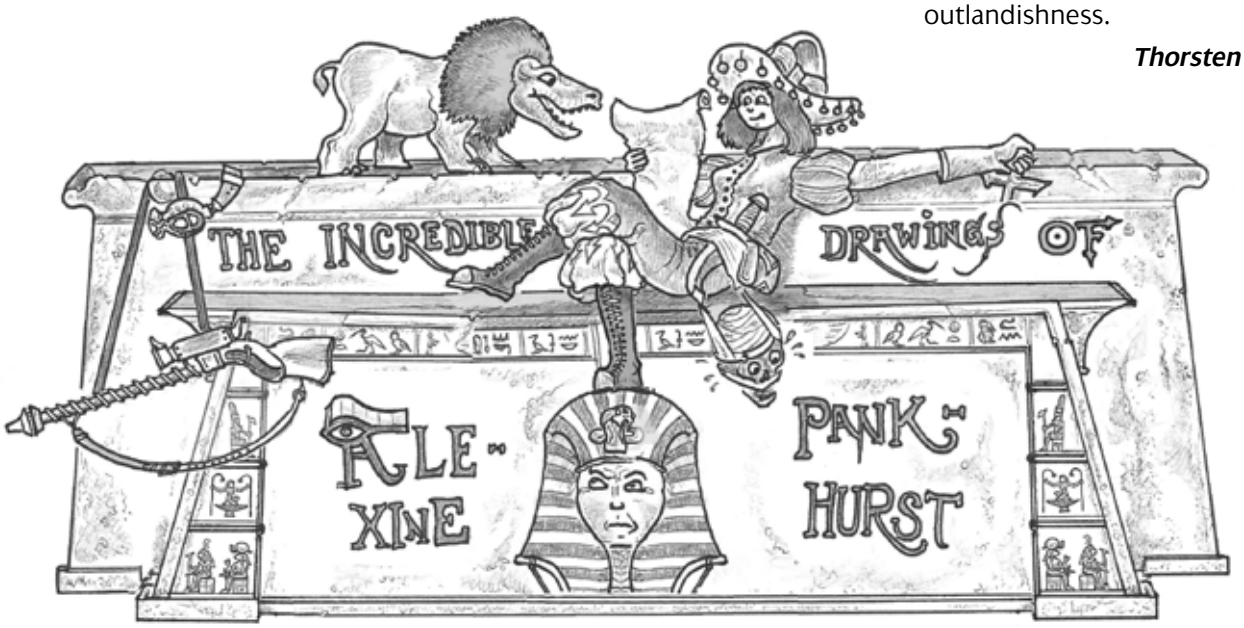
ian of the Good Green Earth, the Friend of Forests and Brother of Bushes is an upright hero with a heart as pure as an unpolluted creek. He is supported by his best friend and most faithful assistant, a girl called Leaf, unknowing however that Leaf is an eco-terrorist who persistently performs acts of sabotage against multinational supercorporations. His loyal side-kick and fellow combatant is Sergeant Shrub, a talking beaver who hides a terrible (but kind of obvious) secret – he eats trees! Over the years, a long series of adventures have accumulated in which Captain Tree constantly tries to save the world and to deal with the many pitfalls of modern life.

Another project is the "Incredible Adventures of Alexine Pankhurst", a Victorian age archaeologist who searches for priceless treasures and long-forgotten scrolls in the dusty tombs and haunted pyramids of Ancient Egypt. Alexine Pankhurst defeats grave-robbers and hungry scarabs, bad-tempered mummies and the occasional Pharaoh's curse with nothing more than her wits and her dreaded Parasol of Pain. This project has been inspired by a variety of movies and books: from "Tomb Raider" to "Indiana Jones" and "The Mummy", as well as from Elizabeth Peter's books, to real-life archaeologists such as Howard Carter, Emile Chassinat and Theodore Davis.

Under the name of "Alexine Pankhurst", all of these drawings are currently available on a website for aspiring artists – and people who like to doodle – as a repository for everybody who would like a glimpse of one of my many hobby horses: http://alexine-pankhurst.deviantart.com/.

So if you have a moment or two, you are always welcome to have a look at this strange hodgepodge of outlandishness.

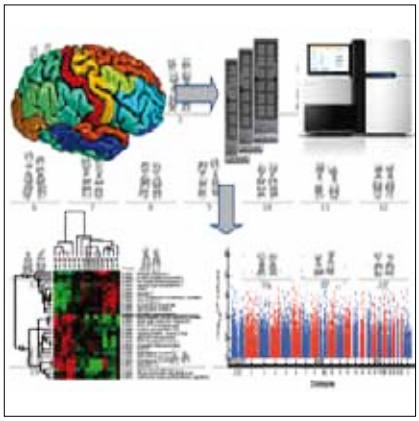
Thorsten Fritzius



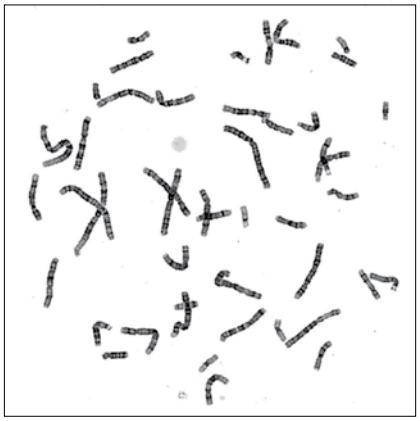
Alexine Pankhurst

VORSCHAU PREVIEW

In der nächsten Ausgabe . . .



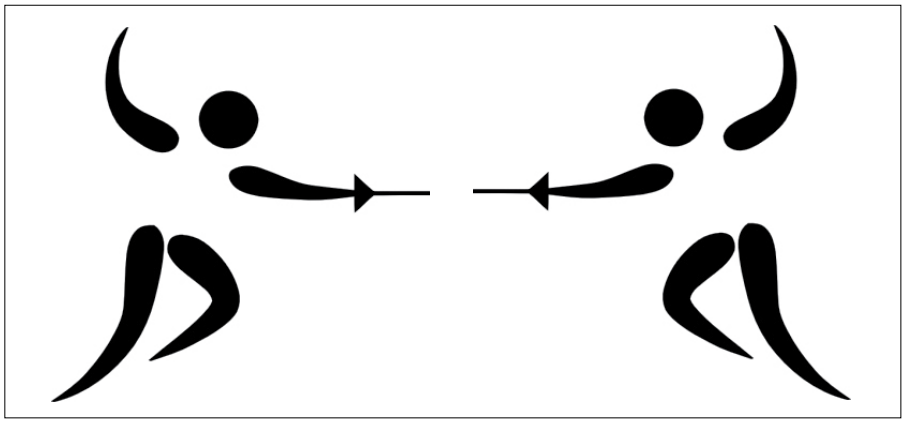
. . . widmen wir uns ganz der Medizinischen Genetik... zuerst den Forschungsaktivitäten...



. . . und anschliessend der klinischen Dienstleistung in diesem dynamischen Bereich

. . . wagen wir uns mit Toni Krebs vor in das Reich der fleischfressenden Pflanzen

. . . erleben wir mit Karol Czaja, wie man in Polen Weihnachten feiert



. . . duellieren wir uns mit Susan Treves

In den Dünen

Weite, möwenüberkreiste
Dünentäler, menschenlose;
rechts die See und ihr Getöse,
links das Haff, das sturmverwaiste.

Alte Dörfer in den Watten,
in der Flur und unterm Sande …
Sonnenleuchten, Wolkenschatten
über einem Märchenlande …

Christian Morgenstern