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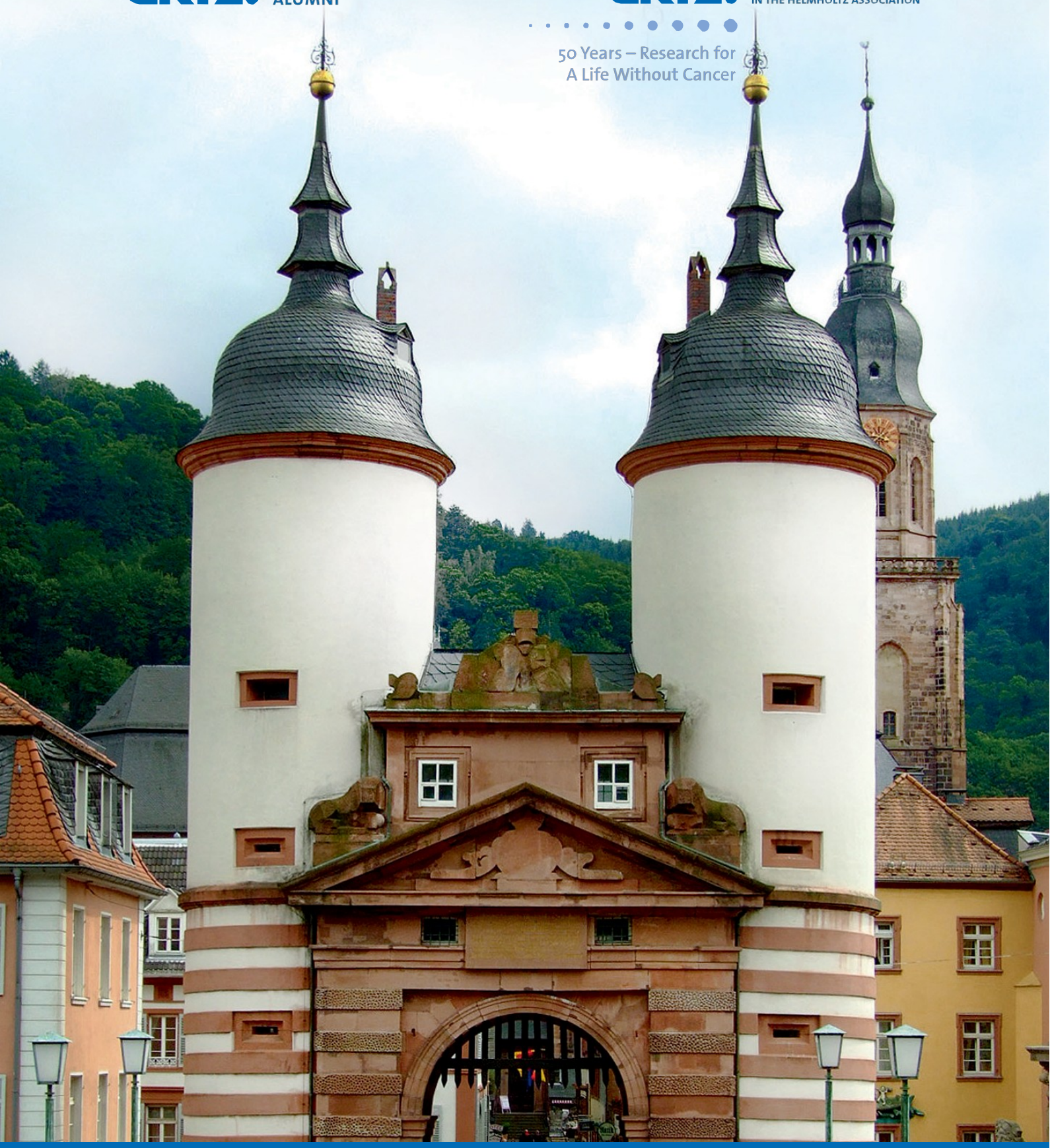
50 Years – Research for A Life Without Cancer



DEUTSCHES KREBSFORSCHUNGSZENTRUM

7th General Alumni Meeting 2016

Final Program and Book of Abstracts

7th General Alumni Meeting on June 09 - 11, 2016

In the session **Mentoring** on Thursday, Adelheid Cerwenka, Susanne Weg-Remers and Hai-kun Liu will focus on different kinds of career opportunities in the cancer field. Additionally, Christian Tidona will introduce BioMed X. The Innovation Center is an exciting new collaboration model at the interface between academia and industry.

The topic of the Scientific Symposium on Friday is "**Clinical Epigenetics**", an area that is attracting increasing attention and importance. The human genome is encoded by the DNA sequence. Alterations in genes (mutations) are primarily responsible for the onset of many diseases including cancer.

The functional status of the genome, i.e. the pattern of genes with on and off status, is controlled by a packaging machinery that regulates accessibility of genetic information to the reading machinery. This control system summed up by the expression epigenetics, is located outside the DNA and essentially consists of chemical modifications of the DNA itself as well as wrapping histone proteins. Sequencing of cancer genomes of different tumor diseases has demonstrated that mutations in components of the epigenetic machinery result in deregulated activity of genes with functions like growth regulation. Further, dysregulation of the activity status of the epigenetic system can lead to reprogramming of normal into cancer cells (dedifferentiation). The molecular understanding of epigenetic dysregulation of the genome is highly relevant from a therapeutic point of view, as there is an increasing number of drugs that are able to interfere with epigenetic control mechanisms. These include, among others, inhibitors of DNA methyltransferases, histone deacetylases and bromodomain-inhibitors.

Based on clinical trials some of these drugs already have been approved for the treatment of several diseases, such as myelodysplastic syndromes (MDS) as well as certain leukemias. Currently the biggest challenge in the field is to identify biomarkers for predicting mechanism-based effectiveness of epigenetically-active drugs to certain tumor diseases. Further, it's pivotal to enable a targeted assignment of patients to epigenetic drugs and to explore a rational combination of this new class of drugs with other therapeutic modalities.

The invited speakers are internationally recognized scientists who will lecture on the relevance of epigenetic research to get more insight into the etiology and treatment of cancer.

Further, you shouldn't miss the **Poster Presentations** of young scientists on recent research findings which will be concluded with an Award Ceremony. Beside six Merck poster prizes of 500 Euro each, the DKFZ Alumni/Cancer Letters Award for International Scientists endowed with 5,000 Euro will be presented during the Reception on Friday afternoon.

You may also take advantage of the opportunity to exchange with current and former colleagues in a less formal way during both the reception and the **Excursion** to regional sites in Darmstadt and Heppenheim (for details, please see social activity) that conclude the 7th General Alumni Meeting on Saturday.

We look forward to welcoming you!

Manfred Schwab

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Thursday, June 9, 2016,
DKFZ, Communication Center

12:15 Registration, mounting of posters (Foyer)

13:00 **WELCOME** (Lecture Hall)

Manfred Schwab, DKFZ Heidelberg

MENTORING

Chair: Barbara Janssens, DKFZ Heidelberg

13:15 Adelheid Cerwenka, DKFZ Heidelberg

The joys and challenges of an international academic scientific career

13:45 Susanne Weg-Remers, DKFZ Heidelberg

Career perspectives in science management and science communication

14:15 Hai-kun Liu, DKFZ Heidelberg

A personal view of how to pursue an academic career in the DKFZ

14:45 Christian Tidona, BioMedX Heidelberg

Biomedical innovation at the interface between academia and industry – the value of mentorship

15:15 *Coffee Break, parallel Round Table Discussions in K1 and K2*

16:00 Poster Session (Foyer) parallel

17.00 Happy Hour (organized by DKFZ PhD Council)

18:30 *Departure for Kulturbrauerei*

19:30 *Dinner at Kulturbrauerei, Heidelberg*

Friday, June 10, 2016,
DKFZ, Communication Center

09:00 **GENERAL ASSEMBLY ALUMNI ASSOCIATION** (Conferene Room K1/K2, for Alumni members)

Agenda

- Approval of the Agenda and of the minutes of the previous General Assembly
- Report on the Activities of the Alumni by the Chairman
- Report by the Treasurer
- Report by the Auditors
- Approval of the Board's Actions
- Election of Board Members
- Election of Auditor
- Honorary members
- Any other business

10:00 *Coffee Break*

CLINICAL EPIGENETICS (Lecture Hall)

Chair: Angela Risch, University of Salzburg

10:30 Olaf Witt, DKFZ, Heidelberg
Dirty drugs meet precision medicine: HDAC inhibitors

11:15 Kristian Pajtler, DKFZ, Heidelberg
DNA methylation fingerprints for accurate tumor classification

12:00 Toshikazu Ushijima, Natl. Cancer Center Res. Inst., Tokyo
Epigenetic field formation by chronic inflammation, and its application to precision risk diagnosis

12:45 *Lunch Break (Poster Session continued)*

Chair: Christoph Plass, DKFZ, Heidelberg

14:30 Jean-Pierre Issa, Temple University, Philadelpha
Epigenetic Therapy

15:15 Manel Esteller, Bellvitge Biomedical Research Inst., Barcelona
Cancer Epigenetics: From knowledge to applications

16:15 *Coffee Break*

RECEPTION (Lecture Hall)

16:30 Welcome Address (Michael Boutros)

16:35 Michael Boutros, DKFZ, Heidelberg
Recent key developments at the DKFZ

16:55 Collegium Musicum of the Heidelberg University Brass Orchestra

17:10 Awarding Ceremony (DKFZ Alumni/Cancer Letters Award for International Scientists)

17:15 Harriet Wikman-Kocher, UKE Hamburg, Scientific Presentation by the Winner 2016,
Identification and functional characterization of metastasis associated genes in breast and lung cancer

17:25 Anne Lloyd, Director Elsevier Publishing

17.30 Awarding Ceremony (6 Merck Poster Awards)

17.45 New Honorary Members

17.55 Awarding Ceremony Alumni Associations' Award

18:00 Marius Schwabenland, Alumni Heidelberg Life-Science Lab e. V., Heidelberg
A sustainable model for supporting young scientists: the Alumni of the Heidelberg Life-Science Lab e.V.

18:20 Collegium Musicum of the Heidelberg University Brass Orchestra

18:35 *Buffet*

We are indebted to Merck for supporting the 7th Alumni Meeting with a total of 6,000 Euro. The sum will be spent for 6 Merck Poster Awards with 500 Euro each and the rest for Merck Travel grants.

Saturday, June 11, 2016

SOCIAL ACTIVITY

09:00	Departure Bus waiting at DKFZ main entrance
10:00	Arrival at the Mathildenhöhe in Darmstadt
	Guided tour of the Jugendstilmuseum - in English and German (1 hour)
	and
	Guided tour of the Art Nouveau District Mathildenhöhe - in English and German (1 hour)
12:00	Departure
12:15	Lunch at the historic Restaurant Bockshaut in Darmstadt
14:00	Departure
14:30	Guided tour of the city of Heppenheim - in English and German
16:00	Departure
	Coffee and Cake at the Starkenburg Castle Heppenheim
17:30	Departure to Heidelberg
18:00	Arrival at DKFZ

Speakers and Chairpersons

7th General Alumni Meeting, June 9 – 11, 2016, DKFZ Heidelberg

Sequence of the Program

Name	**Address**	**E-Mail**
Prof. Dr. Manfred Schwab	Prof. Dr. Manfred Schwab Chairman, Alumni Association German Cancer Research Center Heidelberg	m.schwab@dkfz-heidelberg.de
PD Dr. Adelheid Cerwenka	PD Dr. Adelheid Cerwenka Group-Leader Innate Immunity German Cancer Research Center Heidelberg	a.cerwenka@dkfz.de
Dr. Susanne Weg-Remers	Dr. Susanne Weg-Remers Head of Cancer Information Service German Cancer Research Center Heidelberg	s.weg-remers@dkfz-heidelberg.de
Dr. Hai-kun Liu	Dr. Hai-Kun Liu Group Leader Regulation of normal and neoplastic brain stem cells German Cancer Research Center Heidelberg	L.Haikun@Dkfz-Heidelberg.de
Dr. Christian Tidona	Dr. Christian Tidona Managing Director BioMed X Im Neuenheimer Feld 583 69120 Heidelberg	ct@bio.mx
Prof. Dr. Angela Risch	Prof. Dr. Angela Risch Univ. Salzburg – Mol. Biology Billrothstr. 11 5020 Salzburg Österreich	angela.risch@sbg.ac.at
Prof. Dr. Olaf Witt	Prof. Dr. Olaf Witt Head of Division Pediatric Oncology German Cancer Research Center Heidelberg	o.witt@dkfz.de
Dr. Kristian Pajtler	Kristian Pajtler Pediatric Neorooncology German Cancer Research Center Heidelberg	k.pajtler@dkfz.de

Dr. Toshikazu Ushijima	Dr. Toshikazu Ushijima National Cancer Center Research Institute Tokyo Japan	tushijim@ncc.go.jp
Prof. Dr. Christoph Plass	Prof. Dr. Christoph Plass Head of Division Epigenomics and Cancer Risk Factors German Cancer Research Center Heidelberg	c.plass@dkfz-heidelberg.de
Dr. Jean-Pierre Issa	Dr. Jean-Pierre Issa Temple University Director, Fels Inst. for Cancer Research and Molecular Biology Philadelphia USA	jpissa@temple.edu
Dr. Dr. Manel Esteller	Dr. Dr. Manel Esteller Director, Cancer Epigenetics and Biology Program Bellvitge Biomedical Res. Institute (IDIBELL) Hospital Duran i Reynals Barcelona Spain	mesteller@idibell.cat
Michael Boutros	Prof. Dr. Michael Boutros Chairman Management Board DKFZ (interim) German Cancer Research Center Heidelberg	m.boutros@dkfz.de
Marius Schwabenland	Marius Schwabenland Chairman Alumni of the Heidelberg Life-Science Lab German Cancer Research Center Heidelberg	marius.schwabenland@lab-alumni.de

Names of Speakers are typed in bold

Abstracts Speakers

(sequence of presentation)

MENTORING

The joys and challenges of an international academic scientific career

Adelheid Cerwenka

German Cancer Research Center (DKFZ), Heidelberg, Germany

An academic career harbors many fulfilling moments, but also challenges. As illustrated by my brief CV below, my career was always characterized by mobility (Vienna, USA, Vienna, Heidelberg) before being recruited to the DKFZ. In my presentation, I will discuss the pros and cons of an international academic scientific career and will elucidate my motivations for taking the different steps in my career. I will give a personal view on an academic career in Germany and will in particular discuss the challenges of balancing work and family life.

Short CV:

Adelheid (Heidi) Cerwenka is Head of the Research Group “Innate Immunity” in the German Cancer Research Center in Heidelberg. She received a doctorate from the University of Vienna, Austria, and the Venia Legendi from the Faculty of Medicine, University of Heidelberg. After her postdoctoral research at the Trudeau Institute New York, USA and UCSF, San Francisco, USA, she became group-leader at the Novartis Research Institute, Vienna before being appointed as group-leader at the DKFZ. Adelheid Cerwenka is author of more than 70 publications and member of the German Society for Immunology and the Society for Natural Immunity. She serves as reviewer for several journals (e.g. Nature, Nature Immunology, JEM, Cancer Research) and national and international grant agencies. In 2008 she was awarded with the Georges Koehler Award of the DGfI. She coordinates the NK study group “NK cell biology” of the German Society of Immunology and is the President “elect” of the Society of Natural Immunity. Her research was funded by a Marie Curie Excellence Grant and by several grant agencies (e.g. Dt. Krebshilfe and Carreras foundation).
Her research topics are

- Innate immune responses against tumors
- Natural killer cells and cancer
- Innate lymphoid cell subsets in the tumormicroenvironment

Career perspectives in science management and science communication

Susanne Weg-Remers

Krebsinformationsdienst, German Cancer Research Center (DKFZ), Heidelberg, Germany

Publically funded research institutions in Germany offer many interesting job opportunities, besides a classical adademic career. However, young graduates and PhDs looking for a perspective beyond science are frequenly not aware of all possible options.

In this presentation, an overview will be given of the German science system. Similarities and differences of universities, Helmholtz centers, Max Planck institutes, Fraunhofer institutes and others will be elucidated, in terms of scientific focus, funding mechanism and framework.

Different working areas in science management and science communication will be presented, not only in research institutions, but also in related organisations like funding agencies, governmental bodies, or umbrella organizations.

For those, who are heading towards a career in science management or science communication, helpful information will be provided on career perspectives, entry positions, and further possibilities to get specific academic training besides training on the job.

A personal view of how to pursue an academic career in the DKFZ

Hai-kun Liu

Normal and neoplastic CNS Stem Cells, German Cancer Research Center (DKFZ), Heidelberg, Germany

It is commonly seen that pursuing an academic career is challenging and might be of high risk. I will present my personal view of pursuing a career within the DKFZ and as an international scientist in Germany. Several key aspects including independency, lab management, visibility, collaborations and funding application will be discussed.

Short CV

Hai-Kun Liu (b. 1978) studied biology at the Shandong Normal University in China and earned his doctorate at the Shanghai Institute for Biological Sciences of the Chinese Academy of Sciences. Dr. Liu worked as a postdoc at the DKFZ from 2005 to 2010. He has led the independent Helmholtz Young Investigator Group (tenure track) since 2011 and the research division of Molecular Neurogenetics since 2015 following a positive tenure evaluation. He received several awards including the Helmholtz Young Investigator Award, the EMBO Young Investigator Award, the ERC Consolidator Award and the Chica and Heinz Schaller Award. His research focuses on cellular heterogeneity during neurogenesis and tumorigenesis.

Biomedical innovation at the interface between academia and industry - the value of mentorship

Christian Tidona

BioMed X Innovation Center, Managing Director, Heidelberg, Germany

Translation of biomedical academic research into commercially viable products for the benefit of patients is a global challenge. The BioMed X Innovation Center in Heidelberg, Germany, represents a new collaboration model at the interface between academia and industry. The model is based on global crowdsourcing and local incubation of the best ideas and research talents. At the center, distinguished early career scientists recruited from all over the world are working jointly on novel pre-clinical research projects in the fields of biomedicine, molecular biology, cell biology, diagnostics and bioinformatics. These interdisciplinary project teams are conducting groundbreaking biomedical research in an open innovation lab facility on the campus of the University of Heidelberg, under the guidance of experienced mentors from academia and industry while expanding their scientific network and receiving entrepreneurship and leadership training. Each team is typically sponsored by a corporate pharma or biotech partner of BioMed X. After a fully funded project term, successful projects are either internalized into the development pipeline of the respective pharma or biotech sponsor or spun off into an independent startup company.

CLINICAL EPIGENETICS

Dirty drugs meet precision medicine – HDAC Inhibitors

Olaf Witt, Till Milde and Ina Oehme

German Cancer Research Center (DKFZ), CCU PediatricOncology, Division head, Heidelberg, Germany

Histone deacetylases are a family of enzymes that regulate the acetylation level of nuclear histones but also many cytoplasmatic non-histone proteins that collectively make up the acetylome. Similar to the kinome, the acetylome controls key cellular functions including cell death and survival pathways. HDAC inhibitors have been shown to induce apoptosis, differentiation, inhibit autophagy and sensitize tumor cells to chemotherapy and radiation in cell culture and animal models and several compounds including vorinostat, romidepsin and panobinostat are now approved for treatment of cutaneous T-cell lymphoma and multiple myeloma. However, beyond these indications, HDAC inhibitors have failed to show efficacy in the majority of solid tumors in clinical trials. Modelling of clinical application of HDAC inhibitor application in cell culture indeed explains limited efficacy. This observation is supported by our phase I/II individualized dose escalation trial of HDAC inhibitor vorinostat, demonstrating a significant correlation of tumor response simply with dose applied. In our next generation precision oncology program INFORM, we have analyzed more than 200 pediatric relapsed tumors and did not find mutations or overexpression of one of the 11 HDAC family members suggesting that HDACs are no prototypic oncogenic drivers. In conclusion, in order to take the next step for successful clinical development of HDAC-inhibitors, molecular biomarkers for response prediction that could help identifying the right cohort of patients for clinical trials are urgently required. In that sense we are currently investigating novel functions of individual HDAC family members in controlling autophagy and differentiation as well as their role in controlling MYC oncogenic functions.

DNA methylation fingerprints for accurate tumor classification

Kristian W. Pajtler[1,2], David T. W. Jones[1], David Capper[3,4], Dominik Sturm[1,2], Marcel Kool[1], Stefan M. Pfister[1,2]

[1]Division of Pediatric Neuro-oncology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[2]Department of Pediatric Oncology, Hematology and Immunology, University Hospital, Heidelberg, Germany
[3]Clinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[4]Department of Neuropathology, University of Heidelberg, Heidelberg, Germany

The current WHO classification of central nervous system (CNS) tumors comprises over 100 entities, most of which are defined by purely histological criteria with varying and often overlapping spectra. Histological diagnosis is often challenging, however, especially in cases with limited or non-representative biopsy material. Molecular technologies that can complement standard pathology testing have the potential to greatly enhance diagnostic precision and improve clinical decision-making. DNA methylation profiling, acting as a 'fingerprint' of cellular origins and molecular alterations, is one such promising technology. Applying methylation profiling to clinically and histologically heterogeneous malignancies, such as ependymomas or primitive neuroectodermal tumors of the CNS (CNS-PNETs), gave rise to novel molecular classification schemes. Ependymal tumors can be classified into nine clinically meaningful molecular subgroups that outperform the current WHO grading system. A large fraction of previously histologically diagnosed CNS-PNETs were re-classified into other CNS tumor entities. Four new tumor entities have emerged from the remaining fraction all of which are associated with distinct histological and clinical features and characterized by a defining recurrent genetic alteration. Through various translational research programs, including routine application of molecular based tumor classification algorithms, we are currently aiming to support

Assessment of Methylation and Mutation Burdens Provides Precision Cancer Risk Diagnosis

Toshikazu Ushijima

National Cancer Center Res Inst, Div of Epigenomics, Tokyo, Japan

Aberrant DNA methylation can be accumulated in normal-appearing tissues. Especially in gastric tissues, aberrant DNA methylation is induced by chronic inflammation triggered by Helicobacter pylori (HP) infection [Niwa, Cancer Res, 70:1430, 2010; Maekita, Clin Cancer Res, 12:989, 2006], and accumulation levels of aberrant methylation (methylation burdens) were correlated with risk of gastric cancer [Nakajima, CEBP, 15:2317, 2016], a typical chronic inflammation-associated cancer. Importantly, a large-scale multicenter prospective cohort study involving 826 patients demonstrated that measurement of methylation burden in a gastric tissue can predict the risk of metachronous gastric cancer [Asada, Gut, 64:388, 2015].

In esophageal tissues, accumulation levels of specific genes were correlated with smoking history [Oka, Cancer, 115:3412, 2009], and those of multiple genes with esophageal squamous cell cancer risk [Lee, Cancer Prev Res, 4:1982, 2011]. Nevertheless, the correlation between methylation levels and cancer risk was much weaker in esophageal tissue than in gastric tissue.

To complement methylation burden by accumulation of point mutations (mutation burden), we developed a novel sequencing technology that enabled us to measure rare mutations in polyclonal tissues. Using the technology, a correlation between mutation burden and cancer risk was clearly demonstrated for esophageal squamous cell cancer. In contrast, even after measurement of mutation burden, gastric cancer risk was mainly determined by methylation burden in gastric mucosae. These showed that the influence of methylation and mutation burdens is highly different among tissues, and their measurement can provide precision cancer risk diagnosis.

Epigenetic therapy

Jean-Pierre Issa

Fels Institute and Fox Chase Cancer Center, Temple University School of Medicine, Philadelphia, USA

Epigenetic reprogramming erases the malignant potential of some transformed cells. The aim of epigenetic therapy is to achieve some degree of reprogramming in-vivo through reversing epigenetic changes and reactivating important genes including tumor-suppressor genes. It is hoped that this strategy will modify the malignant phenotype and induce clearance of the malignant clone by various mechanisms, including apoptosis, differentiation, senescence and an immune response. The DNA methyltransferase inhibitors azacitidine, decitabine and guadecitabine induce clinically meaningful remissions or improvements in 30-60% of patients with myeloid leukemias and prolong survival compared to standard approaches including chemotherapy. This therapy is accompanied by (i) global and gene specific demethylation, (ii) reactivation of silenced gene expression, (iii) delayed responses that correlate with early epigenetic reactivation and result in clonal elimination and (iv) eventual relapses and resistance the mechanisms of which appear to involve genetic evolution in some cases.

I will discuss two strategies to enhance the efficacy of DNMT inhibitors – targeting other epigenetic pathways and unbiased screens for epigenetic activity. Using gene expression as an endpoint, we compared inhibitors of DNMTs (decitabine, DAC), HDACs, G9A, EZH2 and LSD1 in-vitro. We find that DNMT inhibition is the most specific approach to reactivation of genes silenced in cancer. While HDAC inhibition is also very effective, it has the highest rate of non-specific effects, which may explain why adding HDAC inhibitors to DNMT inhibitors has been disappointing clinically to date. Drugs that inhibit G9A, EZH2 or LSD1 have modest effects on their own but showed significant, non-overlapping synergy with DAC, while preserving specificity. Separately, using unbiased live cell screens for epigenetic activity, we discovered multiple new drugs and pathways that can be targeted for gene reactivation in cancer. Clinical trials using these new approaches (e.g. DAC in combination with Arsenic trioxide) are showing promising early results. Thus, our data uncover multiple distinct mechanisms that can be targeted for epigenetic therapy in cancer.

Cancer Epigenetics: From Knowledge to Applications

Dr. Manel Esteller

Bellvitge Biomedical Research Institute, Barcelona, Spain

For the last twenty-five years an increasing amount of evidence has shown the relevance of epigenetics in cell biology and tissue physiology, being DNA methylation aberrations in cancer the flag-ship for the recognition of its disturbance in human diseases. From the candidate gene approaches, new powerful technologies such as comprehensive DNA methylation microarrays and whole genome bisulfite sequencing has recently emerged that have reinforced the notion of epigenetic disruption in the crossroad of many sickness. From the poster-boy cases of MGMT and GSTP1 hypermethylation in the prediction of alkylating drug response and prostate cancer detection, respectively, to the personalized treatment of leukemia with small molecules targeted to fusion proteins involving histone modifiers such as DOT1L and MLL, the field has walked a long path. The current talk will focus in the epigenetic profiling, basically at the level of DNA methylation and histone modifications, that is starting to provide clinical value in the diagnosis, prognosis and prediction of response to drug therapies, with an emphasys in neoplasia, but without forgetting the novel advances in other human disorders. For cancer, we have already a wide view of the undergoing DNA methylation events that expand beyond classical promoter CpG islands of tumor suppressor genes and we have a growing list of mutated chromatin remodeler genes that contributes to the tumorigenesis process. It is time to apply this knowledge in practical clinical situations like the diagnosis of cancers of unknown primary, the screening of malignancies in high-risk populations or a biomarker selection of the patients that should receive treatment with epigenetic or genetic drugs.

Abstracts Posters

(in alphabetical order)

Predicting primary origin of metastatic samples based on DNA methylation profiles

Poster 01: Yassen Assenov and Christoph Plass

German Cancer Research Center (DKFZ), Epigenomics and Cancer Risk Factors, Group Leader, Computational Epigenomics, Heidelberg, Germany

Carcinoma of unknown primary origin (CUP) is a diagnosis encompassing a heterogeneous group of cancers and is defined by the presence of metastatic disease with no identified primary tumor. Patients with CUP face limited treatment options and a poor prognosis. Two techniques assist the identification of tumor type in such cases - genetic and immunohistochemical panels, and targeted search by magnetic resonance imaging. The highly de-differentited state of tumor cells and multiple affected organs render these approaches insufficient.

We and others have shown that the methylome of tumor samples covered by Illumina"s HumanMethylation450 array (Infinium 450k) contains a large fraction of the methylome specific to the cell type of origin. To this end, we trained classifiers (mathematical models) that are able to reliably identify tissue of origin of a metastatic sample given its Infinium 450k methylation profile. The result of a classifier provides valuable information, giving hints for targeted search for the origin of a tumor and assisting pathologists in making an accurate diagnosis.

Genetic Dissection of the c-Myc Super-Enhancer in Homeostatic Hematopoiesis and Leukemia

Poster 2: Carsten Bahr (1,2), von Paleske L* (1,2), Uslu VV* (3), Remeseiro S (3), Petretich M (3), Scognamiglio R (1,2), Zeisberger P (1,2), Benk A (1,2), Dick J. E. (5), Amit I (4), Spitz F (3) and Trumpp A (1,2,6)

(1) Division of Stem Cells and Cancer, Deutsches Krebsforschungszentrum (DKFZ), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

(2) Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM gGmbH), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

(3) Developmental Biology Unit, European Molecular Biology Laboratory (EMBL), D-69117 Heidelberg, Germany.

(4) Department of Immunology, Weizmann Institute of Science, Rehovot, Israel

(5) Princess Margaret Cancer Research Tower, Room 8-301, 101 College Street, Toronto M5G 1L7, Canada

(6) The German Cancer Consortium (DKTK)

*** contributed equally**

German Cancer Research Center (DKFZ), Stem Cells and Cancer, Heidelberg, Germany

c-Myc is an essential regulator of metabolic growth, cellular proliferation and differentiation, whose over-expression is observed in various cancer entities including leukemia. Recently, a super-enhancer (SE) 1.7 Mb telomeric from c-Myc has been reported in a murine acute myeloid leukemia (AML) cell line. We confirmed the conservation and the presence of this SE in primary human AML patient samples by ATAC-Seq. These patient samples were sub-divided into leukemic stem cell (LSC) containing fractions and fractions lacking these cells. Interestingly, two of the enhancer elements showed differential chromatin openness in LSCs and blasts. The openness of these modules correlates with c-Myc expression, suggesting a differential regulation of c-Myc by the SE in LSCs and blasts. Next, we used chromatin profiling of a large set of hematopoietic cells to check if the SE is also active in normal hematopoietic cells. Based on this we identified 9 distinct enhancer modules partially overlapping with the ones observed in AML. These enhancer modules are marked differentially by H3K27ac in distinct hematopoietic cell types and recruit multiple haematopoietic transcription factors in a cell-type specific manner. We genetically dissect the regulatory input of the SE on c-Myc expression in vivo. Deletion of the entire SE results in a complete loss of c-Myc expression in HSCs, progenitors and restricted mature cell types. Furthermore, we observe a robust accumulation of differentiation-arrested multipotent progenitors and associated loss of myeloid and B cells, phenocopying bone marrow-specific inactivation of c-Myc in

adult HSCs. Deletion of individual modules suggests that c-Myc is regulated in a combinatorial manner by the individual enhancer modules. Currently, we are generating AML models from these enhancer mutant mice to explore the relevance of individual enhancer modules for AML development.

Genome-Wide DNA Methylation Profiling Service

Poster 3: Melanie Bewerunge-Hudler, Stefan Wiemann

German Cancer Research Center (DKFZ), Genomics and Proteomics Core Facility, Heidelberg, Germany

The DKFZ Microarray Facility provides access to full service in state-of-the-art molecular profiling, including DNA methylation analysis, gene expression profiling, microRNA analysis, and genotyping.

Over the last years, the demand for our methylation analysis service using Illumina's 450k or EPIC Methylation array has rapidly grown and has currently reached >6500 samples that are processed in every year. The full service covers DNA quantity and quality control, bisulfite treatment, chip analysis, data analysis identifying the methylation status on all tested CpGs, and data return. To exploit the full potential of the methylation array technology, we have implemented a protocol enabling compatibility with DNA from FFPE tissue and optimized the process for small amounts of sample. In addition, we can specifically detect both 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) in a single workflow. This is meaningful, as 5'hydroxy methylcytosine (5'hmC) is functionally different from conventional methylation (5'mC).

Data collected in the Microarray Unit is increasingly utilized in clinical studies aimed at patient stratification and even guiding therapy decision. In the INFORM ("INdividualized Therapy FOr Relapsed Malignancies in Childhood")-Study, for example, the Microarray Unit participates with "fast track" - gene expression profiling and 450k-methylation analysis. Along these lines, the Microarray Unit is currently building up a quality management system for laboratories to achieve accreditation of the 450k/EPIC-methylation array technology.

DNMT and HDAC inhibitors globally induce cryptic TSSs encoded in long terminal repeats

Poster 4: David Brocks 1,14, Christopher R. Schmidt1,14, Michael Daskalakis1,12,14, Daofeng Li2, Jing Li2, Hyo S. Jang2, Bo Zhang2, Daniel B. Lipka1, Johanna Schott3, Holger Bierhoff4,5, Yassen Assenov1, Monika Helf1, Alzbeta Ressnerova1, Anders M. Lindroth1,6, Simon Haas7, Marieke Essers7, Charles D. Imbusch8, Benedikt Brors8,12, Ina Oehme9, Olaf Witt9,12, Michael Lübbert10,12, Georg Stoecklin3, Clarissa Gerhäuser1, Christopher C. Oakes1, 11,15, Ting Wang2,15, Christoph Plass1,12,13,15

1Division of Epigenomics and Cancer Risk Factors, DKFZ, Heidelberg, Germany.

2Department of Genetics, Center for Genome Sciences and Systems Biology, Washington University School of Medicine, St. Louis, MO, USA

3Division of Biochemistry I, Center for Biomedicine and Medical Technology Mannheim (CBTM), Medical Faculty Mannheim, Heidelberg University, and Center for Molecular Biology of Heidelberg University (ZMBH), DKFZ-ZMBH Alliance, Mannheim, Germany

4Division of Molecular Biology of the Cell II, DKFZ, DKFZ-ZMBH Alliance, Heidelberg, Germany.

5Present address: Department of RNA Bioinformatics and High Throughput Analysis, Faculty of Mathematics and Computer Science, Friedrich Schiller University Jena, Jena, Germany

6Present address: Graduate School of Cancer Science and Policy, National Cancer Center, Goyang-si, Republic of Korea.

7Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM gGmbH), Heidelberg, Germany; Junior Research Group Stress-induced Activation of Hematopoietic Stem Cells, Division of Stem Cells and Cancer, DKFZ, Heidelberg, Germany

8Division of Applied Bioinformatics, DKFZ, Heidelberg, Germany

9Department of Pediatric Oncology, Hematology and Immunology, University Hospital, Heidelberg, Germany; Clinical Cooperation Unit Pediatric Oncology, DKFZ, Heidelberg, Germany

10Department of Medicine, Division of Hematology/Oncology, University of Freiburg, Medical Center, Freiburg, Germany.

11Present address: Division of Hematology, Department of Internal Medicine, The Ohio State University, Columbus, USA

12German Cancer Research Consortium (DKTK)

13German Center for Lung Research (DZL)

14Co-first author

15Co-senior author

Several mechanisms of action have been proposed for DNA methyltransferase and histone deacetylase inhibitors (DNMTi and HDACi); mainly based on candidate gene approaches. However, less is known about their genome-wide transcriptional and epigenomic consequences. By mapping global transcription start site (TSS) and chromatin dynamics, we observed the activation of thousands of cryptic, currently non-annotated TSSs (TINATS) following DNMTi and/or HDACi treatment. The resulting transcripts encode truncated or chimeric open reading frames translated into products with predicted abnormal functions or immunogenic potential. TINAT activation after DNMTi coincided with DNA hypomethylation and gain in H3K4me3, H3K9ac, and H3K27ac histone marks. In contrast, HDACi induced only canonical TSSs in association with histone acetylation, but TINATs via a yet unknown mechanism. Nevertheless, both inhibitors convergently induced unidirectional transcription from identical sites since TINATs are encoded in solitary long-terminal repeats of the endogenous retrovirus-9 family, epigenetically repressed in virtually all normal cells.

Risk of second primary cancers in women diagnosed with endometrial cancer in German and Swedish cancer registries

Poster 05: Tianhui Chen 1,2*, Hermann Brenner 3,4,5, Mahdi Fallah 2,3, Lina Jansen 3, Felipe A. Castro 3, Kristina Sundquist 6,7, Kari Hemminki 2,6

1 Institute of Occupational Diseases Prevention, Zhejiang Academy of Medical Science, Hangzhou, China;
2 Division of Molecular Genetic Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany;
3 Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, Germany;
4 Division of Preventive Oncology, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Heidelberg, Germany;
5 German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany;
6 Center for Primary Health Care Research, Lund University, Malmö, Sweden;
7 Stanford Prevention Research Center, Stanford University School of Medicine, Stanford, California 94305-5705, USA

We aimed at investigating risk of second primary cancers (SPCs) in women diagnosed with endometrial cancer in Germany and Sweden to provide etiology of SPCs after endometrial cancer and to offer opportunities for counseling and uptake of second cancer prevention strategies. Endometrial cancer patients diagnosed at age =15 years for Germany during 1997-2011 (from 12 German cancer registries) and for Sweden nationwide during 1997-2012 were selected. Standardized incidence ratios (SIRs) were used to assess risk of a specific SPC compared to risk of the same first cancer in the corresponding background population. Among 46,929 endometrial cancer survivors in Germany and 18,646 in Sweden, overall 2,897 and 1,706 SPCs were recorded, respectively. Significantly elevated SIRs of specific SPCs were observed in Germany for ovarian cancer (SIR=1.3; 95%CI:1.1-1.5), kidney cancer [1.6 (1.3-1.8)], and other uterus cancer [3.9 (2.3-6.1)], while in Sweden SIRs were 5.4 (4.6-6.3),1.4 (1.0-1.9) and 2.8 (1.3-52), respectively. Elevated risk for ovarian cancer was more pronounced for younger (<55 years) onset of first endometrial cancer in Germany [3.3 (2.2-4.8)] and Sweden [9.1 (6.6-12)]. In Sweden, elevated risks for those cancers were more pronounced by some histological subtypes of first endometrial cancer, reaching 4.9 (1.6-11) for kidney cancer by clear cell subtype, 3.9 (1.8-7.4) for other uterus cancer by endometrioid subtype, and 2.8 (1.2-5.4) for ovarian cancer by adenosqumous subtype. We found elevated SPC risk for ovarian, kidney and other uterus cancers in both populations, suggesting cancer prevention strategies shall focus on those cancers after diagnosis of endometrail cancer.

Cell-free circulating DNA Integrity as Marker for prediction of Breast Cancer Recurrence

Poster 6: Jie Cheng (1,2), Katarina Cuk (1,2), Jörg Heil (3), Michael Golatta (3), Sarah Schott (3), Christof Sohn (3), Andreas Schneeweiss (3,4), Barbara Burwinkel (1,2), Harald Surowy (1,2)

(1) Division of Molecular Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany;

(2)Molecular Biology of Breast Cancer, Department of Gynecology and Obstetrics, University of Heidelberg, Heidelberg, Germany;

(3) Department of Gynecology and Obstetrics, University Women's Clinic, Heidelberg, Germany;

(4) National Center for Tumor Diseases, University of Heidelberg, Heidelberg, Germany;

Non-invasive blood-based molecular biomarkers are promising these days. Here we investigated the potential of cell-free circulating DNA Integrity (cfDI) as blood-based marker for the prediction of recurrence during the fellow-up of breast cancer patients within a prospective study cohort. cfDI was determined in plasma of 156 individuals, by measuring Alu and LINE1 repetitive DNA elements using quantitative PCR. A significant decrease of cfDI in recurrent breast cancer patients was observed. The group of recurrent patients had significant lower cfDI compared to the group of non-recurrent patients ($P<0.001$ for Alu and LINE1 cfDI). cfDI could differentiate recurrent breast cancer patients from non-recurrent breast cancer subjects (area under the curve, AUC = 0.71 for Alu and 0.72 for LINE1). Univariate and multivariate analysis confirmed a significant association of recurrence and cfDI. Breast cancer patients with a lower cfDI had a much higher risk to develop recurrence than the patients with a higher cfDI ($p= 0.035$ for Alu cfDI and $p= 0.024$ for LINE1 cfDI, respectively). In this study we showed that cfDI is an independent predictor of breast cancer recurrence. In combination with other molecular markers, cfDI might be a useful biomarker for the prediction for breast cancer recurrence in clinic utility.

Hippo/YAP signaling contributes to neural crest cell fate and migration

Poster 7: Alexandra-Larisa Condurat(1,2,3), Christopher J. Hindley(1), Vishal Menon(1,2,3), Ria Thomas(1,2,3), Luis M. Azmitia(1), Jason A. Davis(1), Jan Pruszak(1,4)

(1)Emmy Noether-Group for Stem Cell Biology, Department of Molecular Embryology, Institute of Anatomy and Cell Biology, University of Freiburg, Germany; (2)Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, Germany; (3)Faculty of Biology, University of Freiburg, Germany; (4)Center for Biological Signaling Studies (BIOSS), University of Freiburg, Germany

The Yes-associated protein (YAP) is a transcriptional regulator that acts downstream of the Hippo pathway, a highly conserved tumor suppressor signaling cascade involved in tissue homeostasis. The nuclear activity of YAP as a transcriptional coactivator is limited by the Hippo core kinases, mammalian STE20-like protein kinase 1 and 2 (MST1/2) and large tumor suppressor 1 and 2 (LATS1/2), which promote YAP phosphorylation and thus its subsequent sequestration into the cytoplasm. YAP plays a major role in the regulation and maintenance of various stem cell niches. In our study, we aim to investigate the role of Hippo/YAP-signaling in the development of neural crest cells, a transient population of multipotent and highly migratory cells that emerge at the edge of the neural plate. To this end, we used multiple human in vitro stem cell models, including human embryonic stem (hES) and human induced pluripotent stem (hIPS)-derived cell systems, together with different neural differentiation models. Our results show that YAP expression is associated with immature neural precursors and decreases upon neural differentiation. Furthermore, YAP expression is enhanced in low density-conditioned neural stem cells (NSCs), presenting a neural crest (NCR) phenotype with enriched expression of specific surface antigens, CD44 and CD49d. Further analysis, using gain and loss-of function approaches, reveal that Hippo/YAP signaling and retinoic acid (RA) signaling jointly participate in the maintenance of the CD44+/CD49d+/YAP+ population and the regulation of its migratory potential. Together, our results illustrate the importance of YAP modulation and its contribution to the complex signaling network that directs neural crest cell development.

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Biotop Heidelberg: Do-It-Yourself Biology to promote citizen science

Poster 8: Marco Raffaele Cosenza

German Cancer Research Center (DKFZ), Molecular Hematology/Oncology, Heidelberg, Germany

Do-It-Yourself Biology (DIYbio) is a global movement that brings biology outside the academic or industrial labs and to the lay public.

The DIYbio community is a heterogeneous group of citizen scientists including enthusiasts, amateurs, designers, artists, entrepreneurs, students and trained scientists.

DIYers are often organized in shared spaces known as "community labs", carrying common projects focused on education, personal exploration, discovery but also technology, entrepreneurship, health and art.

The ultimate scope of DIYbio is to promote democratization of science through access to biotechnology and learn-by-doing education.

Community labs often foster alternative and creative solutions thanks to their creative and stimulating environment and the unique background of each DIYers. Indeed, DIYbio has already contributed to public awareness and education, and has produced significant technical and scientific achievements, as well as new business ideas.

The most famous DIYbio labs are born in eclectic, international cities with a flourishing student community such as San Diego, New York, Paris, London.

In Germany, with few exceptions the scene is still quiet: there are many DIYers working alone and only one group in Berlin called “Biotinkering”, which is in the process of building its own community lab.

Thanks to the large number of international students and research institutes, Heidelberg is a place where biology and society constantly interact, with potential DIYbio enthusiasts arriving every year. It is the perfect city where a DIYbio community lab could thrive and act as a bridge between the residents, industry and academia.

However, such a lab would first need enthusiast, motivated DIYers.

If you also believe in democratization of science, do-it-together and love biologyJoin the community! Colonize the Biotop!

Activation of Telomerase by enhancer hijacking in high-risk neuroblastoma

Poster 9: Daniel Dreidax1, Moritz Gartlgruber1, Sebastian Steinhauser2, Larissa Savelyeva1, Ron Schwessinger2, Martin Peifer3,4, Matthias Fischer4,5,6, Stefan Gröschel7, Kai-Oliver Henrich1, Young-Gyu Park1, Carl Herrmann2, Frank Westermann1

1Division Neuroblastoma Genomics (B087), German Cancer Research Center (DKFZ), Heidelberg, Germany, 2Division Theoretical Bioinformatics (B080), German Cancer Research Center (DKFZ), Heidelberg, Germany, 3Department of Translational Genomics, Center of Integrated Oncology Cologne–Bonn, Medical Faculty, University of Cologne, Cologne, Germany, 4Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany, 5Department of Pediatric Oncology and Hematology, University Children's Hospital of Cologne, Medical Faculty, University of Cologne, Cologne, Germany, 6Max Planck Institute for Metabolism Research, Cologne, Germany, 7Department of Translational Oncology (G100), German Cancer Research Center (DKFZ), Heidelberg, Germany

Background: Neuroblastoma, a neural crest-derived tumor of the sympathetic nervous system, is the most common extracranial solid tumor in children. Exome sequencing studies revealed that neuroblastomas (NBs) harbor a low overall mutation rate with only few recurrently mutated genes leaving the molecular etiology of a large proportion of NBs elusive. In the present study, we applied a whole genome sequencing (WGS) approach to uncover structural rearrangements in noncoding regions that could potentially drive NB.

Methods: WGS was applied to search for structural rearrangements in NB tumors and cell lines. A protocol for chromatin immunoprecipitation sequencing (ChIP-seq) of tumors was established and used to identify active enhancer elements in NB. Circular chromatin conformation capture sequencing (4C-seq) was used to assay for physical promoter-enhancer interactions.

Results: WGS analyses revealed that chromosomal rearrangements are common in NB with the most frequent event encompassing the telomerase gene (TERT) which is rearranged in 25% of high-risk cases. ChIP-seq analyses confirmed that rearrangements commonly juxtapose active enhancer elements to TERT in NB tumors and cell lines. 4C-seq analyses provided evidence for physical interactions of translocated enhancer elements with the TERT promoter. This is in line with elevated TERT expression in rearranged cases which form a NB subgroup of particularly poor outcome. Currently, we test therapeutic concepts targeting enhancer-mediated gene deregulation with CDK7- or BET- inhibitors.

Conclusions: The study reveals that structural rearrangements frequently juxtapose strong enhancers to TERT, leading to de novo physical promoter-enhancer interactions and TERT overexpression, thus defining a new molecular subgroup of

high-risk neuroblastomas. The mechanism of oncogene activation by enhancer hijacking may open a therapeutic window for epigenetic drugs, including BET inhibitors or CDK7 inhibition, in high-risk NBs.

From lymphoma rolling to rapid arrest: picoNewton resolution of homing receptors and activation by chemokines

Poster 10: Robert H. Eibl

(1) Stanford University, Irving L. Weissman lab, Stanford, CA, USA,
(2) Veterans Hospital, Eugene C. Butcher lab, Palo Alto, CA, USA,
(3) Technical University of Munich, Pathology, Munich, Germany,
(4) University of Munich, Biophysics (guest), Munich, Germany,
(5) University of Miami, Physiology and Biophysics, Miami, FL, USA

Chemokines can activate integrin receptors on a lymphocyte: under conditions of flow the cells stop rolling on the blood vessel wall. I compare these mechanisms with the steps involved in organ-specific metastasis; therefore, I further elucidated my findings from the flow chamber assay with a custom-made atomic force microscope (AFM). I became the first to 1) transfer my metastasis model of rolling B16 melanoma cells to molecular resolution, i.e. I was able to measure the inter-molecular binding forces of single bonds of adhesion receptors (VLA-4/VCAM-1) between two living cells; 2) to detect similar adhesion events between living lymphoma and of either endothelial cells, or of immobilized VCAM-1 fusion protein, respectively; and 3) to detect with his system the immediate activation of VLA-4 integrin receptors by a chemokine (CXCL12/SDF-1) at the single-molecule level and at the same time on a living cell. This very unique tool of measuring the activation state of metastasis-supporting cell adhesion receptors will be used to finally cure cancer.

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Discovery of histone demethylase KDM5C inactivation as a novel mechanism for tumors resistant to VEGF RTKi via genome-wide in-vivo RNAi

Poster 11: Azadeh Fahim Golestaneh, Maoyun Sun (2), Christian Schwager (1), Zili Tang (1,2), Stephan Macher-Goeppinger (3), Lili Ma (2), Philip Hahnfeldt (2), Mahmoud Moustafa (1,4), Wilko Weichert (3), Sascha Pahernik (5), Carsten Grüllich (6), Wilfried Roth (3), Jürgen Debus (1), Lynn Hlatky (2) and Amir Abdollahi (1,2)

(1) German Cancer Consortium (DKTK), Molecular- and Translational Radiation Oncology, Heidelberg Ion-beam Therapy Center (HIT), Heidelberg Institute of Radiation Oncology (HIRO), National Center for Tumor Diseases (NCT), Heidelberg University Medical School and German Cancer Research Center (DKFZ), Heidelberg, Germany; (2) Center of Cancer Systems Biology, Tufts University School of Medicine, Boston, MA, USA; (3) Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany ; (5) Department of Urology, University of Heidelberg, Heidelberg, Germany; (6) Medical Oncology, Heidelberg University Hospital, Heidelberg, Germany

Acquired tumor resistance to VEGF receptor tyrosine kinase inhibitors (RTKi) constitutes a major obstacle for antiangiogenic cancer therapy. We sought to decipher the molecular landscape of tumor resistance to VEGF-RTKi sunitinib (SU) treatment via genome-wide shRNA library. After three in-vivo panning rounds tumor cells containing kd of H3K4 histone demethylase (Kdm5c) were enriched compared to the in-vivo passaged library without the SU pressure. Further depletion of Kdm5c in mouse and human cell lines confirmed the role of it in emerging resistance to Sunitinib in vivo. Expression profiling of sh Kdm5c cells suggested that the development of resistance is partly due to modulation of cellular pathways as well as alteration of interaction between tumor and its micro-environment. Clonogenic survival assay showed that cells lacking Kdm5c are more resistant to hypoxic situations, suggesting one possible mechanism of resistance to Sunitinib. In addition, Cd31 staining in tissue samples recovered from mice indicated induction of micro-vessel formation in sh Kdm5c tumors. This was further shown to be due to up-regulation of a panel of angiogenic factors. In addition, expression profiling of cells under hypoxia illustrated a network of genes under Kdm5c control, which loss of Kdm5c result in an interruption in the natural break imposed by Kdm5c in hypoxia. These genes which are normally expressed hypoxia include several key factors important in cell survival as well as epigenetic modulating components Ezh2, EED and SUZ12 comprising PRC2. A plethora of studies has indicated aberrant regulation of PRC2 in different cancers and has linked over expression of EZH2 to aggressive tumor stage. In conclusion, we have shown that loss of Kdm5c induce resistance to sunitinib at least partly by increasing expression of other epigenetic elements under hypoxic condition which in turn empower the tumor to survive under hypoxia, while inducing angiogenesis in them as well.

Family history of colorectal cancer in half-siblings as important as in siblings

Poster 12: Mahdi Fallah, MD, PhD (1,2); Elham Kharazmi, MD, PhD(2); Kristina Sundquist, MD, PhD(3); Hermann Brenner, MD, PhD(1,‡) ; Kari Hemminki, MD, PhD(2,3,‡)

(1) Division of Preventive Oncology, National Center for Tumor Diseases (NCT), Heidelberg, Germany; (2) Division of Molecular Genetic Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany; (3) Center for Primary Health Care Research, Lund University, Malmö, Sweden
(‡) Equal contribution

Background: None of population-based epidemiological studies to date have investigated the familial risk of colorectal cancer (CRC) in half-siblings.

Design: A nationwide cohort of relatives and half-siblings of CRC patients diagnosed in 1958–2012 were extracted from the Swedish Family-Cancer Database, the world's largest of its kind, with >15.7 million individual records.

Results: The overall lifetime (0-79 years) cumulative risk (LCR) of CRC in siblings of a patient with CRC was 7.2% (men 8.3%; women 6.0%), which represents 1.8-fold (95%CI=1.7–1.1.9, n=1333) increase over the risk in those without any family history of CRC (men 4.5%; women 3.4%). Similarly, significantly increased LCR (5.9%) was found among half-siblings of CRC patients [standardized incidence ratio (SIR)=1.7, 1.3–2.0, n=88; maternal half-sibling: 1.6, 1.1–2.1; paternal half-sibling: 1.7, 1.3–2.3]. If a parent & a half-sibling both had CRC, the risk in other half-siblings (SIR=4.4, 2.8–6.5, n=24) was closer to that of those with both an affected parent & an affected sibling (SIR=3.2, 2.9–3.6, n=281) rather than an affected parent alone (SIR=1.6, 1.5–1.7, n=4250). Highly increased risk of CRC was also found in those with two (SIR=2.4, 1.8–3.1, n=59) or three (SIR=9.8, 5.1–17, n=12) affected siblings, in twin brothers (SIR=4.0, 2.2–6.6, n=14), and presumably in men with two affected half-siblings (SIR=5.3, 1.1–16, n=3). Grandparents (SIR=1.2, 1.1–1.3, n=318) or aunts/uncles (SIR=1.3, 1.0–1.5, n=107) without an affected first-degree relative showed minor contributions to the familial risk of CRC, but we found higher risks for those with both an affected first-degree relative and a grandparent (SIR=3.2, 2.3–4.2, n=46) or an aunt/uncle (SIR=2.3, 1.2–4.0, n=13).

Conclusions: This study provides novel information, useful for the genetic counseling. A family history of CRC in a half-sibling (even in absence of an affected first-degree relative) is as important as a family history of CRC in a sibling.

Identification of an ubiquitin E3 ligase as mTOR pathway regulator from a lung cancer epigenome-wide association study (EWAS)

Poster 13: C Faltus[1], Y Assenov[1], M Barrdahl[2], A Hüsing[2], O Bogatyrova[1], M Laplana[1], T Johnson[2], M Meister[3,4], T Muley[3,4], C Plass[1, 4], R Kaaks[2,4] and A Risch[1,4,5]

1 Division of Epigenomics and Cancer Risk Factors, DKFZ Heidelberg, 2 Division of Cancer Epidemiology, DKFZ Heidelberg, 3 Translational Research Unit, Thoraxklinik-Heidelberg gGmbH, University of Heidelberg, 4 Translational Lung Research Center Heidelberg (TLRC-H), Member of the German Center for Lung Research (DZL), Heidelberg, Germany, 5 University of Salzburg, Department of Molecular Biology, Salzburg, Austria

Lung cancer (LC) is the leading cancer-related death worldwide with an only 8% 5-year survival rate. Smoking is the main risk factor for lung malignancies and accounts for about 70% of LC. Epigenetic alterations like DNA methylation in tumor tissue have been extensively studied. However, the potential of blood DNA methylation as biomarker for LC has not been functionally explored. Hence, in this nested case-control study we used Illumina 450K genome-wide arrays to identify DNA methylation changes in prediagnostic blood samples from smokers in the European Prospective Investigation into Cancer and Nutrition (EPIC) Heidelberg cohort. The top candidate CpGs were found to be differentially methylated in lung tumor and adjacent normal tissue from The Cancer Genome Atlas (TCGA). The top gene-associated candidate CpGs were identified to be contained in differentially methylated regions in lung tumors and that these genes were differentially expressed. Thereby, an ubiquitin E3 ligase was identified which regulates functional lung cancer cell characteristics through the mTOR-AKT pathway.

Chromatin remodeler Chd7 is essential for mammalian brain development

Poster 14: Weijun Feng (1), Daisuke Kawauchi (2); Huiqin Qu (1); Stefan Pfister (2); Hai-Kun Liu (1);

(1) German Cancer Research Center (DKFZ), Molecular Neurogenetics, Heidelberg, Germany

(2) German Cancer Research Center (DKFZ), Pediatric Neurooncology, Heidelberg, Germany

Mutations in chromatin modifiers are frequently associated with developmental diseases and cancer. We herein demonstrate that the chromodomain helicase DNA-binding protein 7 (Chd7), frequently mutated in several genetic diseases such as CHARGE syndrome and medulloblastoma, is indispensable for normal cerebellar development. Genetic inactivation of Chd7 in cerebellar granule neuron progenitors leads to cerebellar hypoplasia in mice, due to the impairment of granule neuron differentiation, induction of apoptosis and abnormal localization of Purkinje cells, which fully recapitulates clinical features in the cerebella of CHARGE patients. ATAC-seq revealed that Chd7 is required to maintain open chromatin of genes essential for granule neuron differentiation. Moreover, Chd7 interacts with DNA topoisomerase Top2b to specifically activate neural genes. Besides these neurological phenotypes, genetic loss of Chd7 accelerates medulloblastoma formation in vivo by blocking neural progenitor differentiation. Our comprehensive analyses reveal an epigenetic mechanism with chromatin remodelers governing brain development via a core transcriptional program for cell-specific differentiation, which sheds light on the significant influence of epigenetic alterations on human disease.

DNA methylation changes in response to active smoking exposure are associated with leukocyte telomere length among older adults

Poster 15: Xu Gao (1), Ute Mons (1), Yan Zhang (1), Lutz Philipp Breitling (1,4), Hermann Brenner (1,2,3)

(1)Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 581, D-69120 Heidelberg, Germany

(2)Division of Preventive Oncology, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Im Neuenheimer Feld 460, D-69120 Heidelberg, Germany

(3)German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

(4)Pneumology and Respiratory Critical Care Medicine, Thoraxklinik, University of Heidelberg, Röntgenstraße 1, D-69126 Heidelberg, Germany

Telomere length (TL) is associated with an increased risk of aging-related diseases. As a preventable environmental hazard of morbidity and mortality, smoking has been reported to promote TL attrition by producing a variety of oxidants and free radicals. Since DNA methylation has been demonstrated to play an important role in the pathways of smoking and smoking-induced diseases, this study aimed to address whether the smoking-induced DNA methylation changes could be involved in accelerated TL shortening. We obtained DNA methylation profiles in whole blood samples by Illumina Infinium Human Methylation 450 Beadchip array in two independent subsamples of the ESTHER study and measured their relative TL by quantitative PCR. Terminal Restriction Fragment analysis was additionally performed in a sub-sample to obtain absolute TL in base pairs. The TL measurements across panels were standardized by z-transformation. Even after correction for multiple testing, we successfully confirmed that seven out of 151 smoking-related CpG sites were associated with the absolute and relative TL (FDR <0.05). A smoking index based on the seven loci showed monotonic associations with TL, cumulative smoking exposure and time after smoking cessation. In conclusion, our study supports suggestions that smoking might contribute to the disproportionate aging as reflected by TL through epigenetic pathways. Further research is required to examine whether the identified epigenetic signatures of smoking can be of value in clinical practice to assess individual aging across the lifespan.

Chromosomal rearrangements juxtapose active enhancer elements to proto-oncogenes in high-risk neuroblastoma

Poster 16: Moritz Gartlgruber[1], Daniel Dreidax[1], Sebastian Steinhauser[2], Ron Schwessinger[2], Elena Afanasyeva[1], Larissa Savelyeva[1], Martin Peifer[3,4], Matthias Fischer[4,5,6], Stefan Gröschel[7], Kai-Oliver Henrich[1], Young-Gyu Park[1], Carl Herrmann[2], Frank Westermann[1]

[1]Division Neuroblastoma Genomics (B087), German Cancer Research Center (DKFZ), Heidelberg, Germany, [2]Division Theoretical Bioinformatics (B080), German Cancer Research Center (DKFZ), Heidelberg, Germany, [3]Department of Translational Genomics, Center of Integrated Oncology Cologne–Bonn, Medical Faculty, University of Cologne, Cologne, Germany, [4]Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany, [5]Department of Pediatric Oncology and Hematology, University Children's Hospital of Cologne, Medical Faculty, University of Cologne, Cologne, Germany, [6]Max Planck Institute for Metabolism Research, Cologne, Germany, [7]Department of Translational Oncology (G100), German Cancer Research Center (DKFZ), Heidelberg, Germany

Background: Neuroblastoma (NB) is a childhood tumor derived from precursor cells of the sympathetic nervous system. We have previously shown that genomic rearrangements frequently juxtapose active enhancer elements to the proto-oncogenic TERT. In the present study, we applied a comprehensive approach to identify further oncogenes by combining whole genome sequencing (WGS), chromatin immunoprecipitation sequencing (ChIP-seq) and RNA sequencing.

Methods: WGS was applied to search for structural rearrangements in NB tumors and cell lines. RNA sequencing was used to find outlier expression of oncogenes involved in potential enhancer hijacking events. Furthermore, we used ChIP-seq to identify active enhancer elements in NB tumors and cell lines. Finally, physical promoter-enhancer interactions were confirmed by circular chromatin conformation capture sequencing (4C-seq).

Results: WGS analyses revealed that chromosomal rearrangements are common events in NB and frequently affect regions harboring proto-oncogenes. ChIP-seq profiles of the enhancer associated chromatin mark histone 3 lysine 27 acetylation (H3K27ac) confirmed that these rearrangements recurrently juxtapose active enhancer elements to oncogenes including TERT, MYCN and MYC in NB cells. Intriguingly, quantification of H3K27ac ChIP-seq profiles uncovered that the enhancer elements translocated to MYC were among the most active ones within the cell lines' genomes. 4C-seq analyses proofed physical interactions between translocated enhancer elements and promoters of the respective oncogenes which is in line with their elevated expression in rearranged cases.

Conclusions: The study identifies key neuroblastoma oncogenes, including *MYCN* and *MYC*, being involved in enhancer hijacking events, leading to their activation by *de novo* physical promoter enhancer interactions. This mechanism of oncogene activation by enhancer hijacking may open a therapeutic window for epigenetic drugs, including BET inhibitors or CDK7 inhibition, in high-risk NBs.

A genome-wide approach to study resistance to BET inhibitors in AML

Poster 17: Aoife Gahlawat (1), Pierre Khoueiry (1,2), Enid Lam (3), Mark A. Dawson (3), Vladimir Benes (4), Rab K. Prinjha (5), Gerard Drewes (1) and Paola Grandi (1)

(1) Cellzome GmbH, a GSK company, Heidelberg, Germany

(2) American University of Beirut, Faculty of Medicine, Beirut, Lebanon

(3) Peter MacCallum Cancer Center, Melbourne, Australia

(4) Genomics Core Facility, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

(5) Epinova Epigenetics DPU, GSK Medicines Research Centre, Stevenage, UK

Bromodomain and extraterminal (BET) proteins (BRD2,3,4 and BRDT) are chromatin readers essential for maintaining proper gene transcription. Early clinical trials with BET inhibitors (I-BETs) have shown great promise in acute myeloid leukemia (AML) and therefore evaluation of resistance mechanisms is necessary to optimize the clinical efficacy of these drugs.

To uncover mechanisms of I-BET resistance, we have used novel technologies to map genome wide binding of BET proteins after treatment with I-BET in recently described sensitive and resistant in vitro models of AML. We performed chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) using antibodies against BRD2,3 and 4 to analyze differential binding of the three proteins. Additionally, we generated a novel biotinylated I-BET probe to directly map genome-wide binding of the compound (Chem-Seq). While global BET displacement was observed in both cell systems upon I-BET treatment, the corresponding gene expression was only differentialy affected in the sensitive compared to resistant cells. For example, the expression of Myc, a known target gene of I-BET, was significantly downregulated in sensitive cells but minimally affected in the resistant cell line. We are currently proceeding with global differential analysis.

With this approach, we hope to uncover insights into resistance mechanisms which may guide better treatment regimes to enhance the clinical utility of these targeted therapies in AML and potentially other BET driven diseases.

Development of fluorogenic thioredoxin reductase (TrxR2) probes

Poster 18: Magalie Géraldy and Aubry Miller

German Cancer Research Center (DKFZ), Cancer Drug Discovery, Heidelberg, Germany

The selenoprotein thioredoxin reductase, which presents two subtypes (TrxR1 & TrxR2), plays a key role in regulating cellular redox homeostasis and has attracted increasing attention as a promising anticancer drug target . The first published fluorescent probe for mammalian TrxR is TRFS-green . This compound is composed of a 1,2-dithiolane moiety attached to a naphthalimide fluorophore. TRFS-green displays a green fluorescence off-on change induced by the TrxR-mediated disulfide cleavage and subsequent intramolecular cyclization to liberate the masked fluorophore. It was demonstrated in vitro that TRFS-green is highly selective toward TrxR. A specific improvement of these results toward TrxR2 would consist in the synthesis of a TRFS-green derivative containing a mitochondrially targeted lipophilic triphenylphosphonium (TPP) cation . The synthesis of some other fluorescent probes derived from fluorescein or Tokyo green and containing a 1,2-dithiolane and TPP scaffolds is ongoing

The Effect of APOBEC3A-mediated Mutations on Cancer Cells

Poster 19: Juliane O. Hafermann (1), Susanne Gröbner (2), Marc Zapatka (2), Karin Müller-Decker (3), Hauke Busch (4), Melanie Börries (4), Jochen Heß (5), Sreejith Rajasekharan (1), Martin Löchelt (1), Axel Szabowski (1)

(1) Molecular Diagnostics of Oncogenic Infections, German Cancer Research Centre, Heidelberg, Germany

(2) Molecular Genetics, German Cancer Research Centre, Heidelberg, Germany

(3) Tumour Models, German Cancer Research Centre, Heidelberg, Germany

(4) Institute of Molecular Medicine and Cell Research, University of Freiburg, Freiburg, Germany; German Cancer Research Center (DKFZ), Heidelberg, Germany; German Cancer Consortium (DKTK), Heidelberg, Germany

Inflammation and cancer are often linked, but so far the causal relation has not been established. APOBEC3A (A3A), a cytidine deaminase whose expression is induced by infections and pro-inflammatory stimuli, might be one of the linking factors. A3A-mediated mutational signatures were found in the genomes of various epithelial cancer types, some of which can be driven by HPV infection. Little is known about the long-term effects of the resulting mutations. Here, we address whether A3A-mediated mutations alter tumour growth of already immortalised, transformed cells.

In this study, HEK293 cells were subjected to sustained A3A-mediated mutagenic pressure for up to three months. During this time, A3A-induced mutations arise and the affected cells are expected to undergo selection. We observe that the mutational pressure and selection lead to loss of A3A expression by several different mechanisms. Subsequently, genetically defined single cell clones were generated from the A3A-exposed populations and characterised for altered proliferation and migration. Several A3A-exposed clones showed changes in proliferation and/or migration in comparison to the controls. Interestingly, deregulation of proliferation and migration appear to co-occur in the A3A-mutated cell clones. Several of these clones were selected for more detailed in vitro characterisation concerning drug resistance, metabolic activity, cell death and invasiveness.

The in vivo relevance of the alterations observed in vitro will be proven in a xenograft mouse model. A network analysis will be applied to identify A3A-induced network disturbances underlying the observed phenotypic alterations, and all findings will be correlated to patient data of head and neck squamous cell carcinoma samples stratified for A3A mutation signature and human papillomavirus status.

tRNAs and RNA-processing enzymes in mitotic chromosome segregation

Poster 20: Mark Hartmann (1,2), Merrit Romeike (1), Sebastian Bender (2), Frank Lyko (2), Sylvia Erhardt (1)

(1) Zentrum für Molekulare Biologie Heidelberg (ZMBH), University of Heidelberg and CellNetworks Excellence Cluster, Heidelberg, Germany

(2) German Cancer Research Center (DKFZ), Division of Epigenetics, Heidelberg, Germany

The centromere is a highly specialized chromatin domain essential for kinetochore formation and spindle attachment necessary for chromosome segregation during mitosis. Centromeric DNA is not conserved and epigenetic mechanisms are regulating centromere identity. In various organisms ncRNAs have been described to functionally associate with centromeres, and their misregulation can lead to aneuploidy, a hallmark of many cancer cells.

Here we show that tRNA genes and transcripts, RNA-polymerase III transcription machinery, and RNA processing factors are present at mitotic centromeres in Drosophila. For example, depletion of tRNA methyltransferase Dnmt2 leads to altered chromatin states and chromosome segregation defects. We propose a model where active RNAPIII transcription and modification of tRNAs adjusts chromatin states at Drosophila centromeres, providing a substantial base for kinetochore formation and mitotic chromosome segregation.

The liver ECM impacts gene expression in metastasing pancreatic cancer cells

Poster 21: Khamael Kadhim, Martin M. Berger, Hassan Adwan

German Cancer Research Center (DKFZ), Toxicology and Chemotherapy Unit, Heidelberg, Germany

The extracellular matrix (ECM) provides the physical microenvironment which influences cellular properties. Our hypothesis was that liver ECM will influence metastasizing cancer cells and facilitate liver colonization. We used ASML rat pancreatic cancer cells, which were inoculated into the portal vein of isogenic BDX rats. These cells had been marked by GFP, which allowed their re-isolation following liver perfusion by FACS sorting after various periods of growth in the liver, corresponding to early, intermediate, advanced and terminal stages of liver metastasis. Re-isolated ASML cells were used for total RNA isolation and subsequently expression for mRNAs and miRNAs were investigated by Illumina chip array. Pending on the time span following re-isolation, 7–15% of all known genes and 10% of miRNA species were modulated significantly in expression. These genes included gene families as chemokines, transforming growth factor, metallopeptidase Domain, matrix metallopeptidases, collagens and others. From all chemokines investigated (n=59) 18 and 11 mRNAs were significantly up and down regulated. Similarly, from 21 MMPs, 6 and 2 mRNAs were significantly up and down modulated. Finally, 39 collagens 16 (up) and 7 (down) were significantly altered in expression. Correspondingly the respective miRNAs species were modulated accordingly. In case of collages the miR-29b-3p species was significantly down modulated in accord with the upregulation of respective target collagens. Also, the miRNA species regulating chemokines were altered as shown, e.g. for CCR5, which was more than 10 fold increased at mRNA level with, corresponding miR-125b-5p being decreased. The ECM of rat liver significantly altered the expression profile of metastasizing pancreatic cancer cells and thus contributed to successful colonization of the liver. Clear knowledge of these alterations in gene expression might suggest targets which could be addressed for combatting metastasis successfully.

Immunity, the colonic environment and colon cancer

Poster 22: Mohammad Wasim Khan (1), Shingo Tsuji (2), Mengxi Tian (1), Nairika Meshgin (1), Shea Grenier (1), Mathew Giacalone (2), Kathleen McGuire (1)

(1) Department of Biology, Molecular Biology Institute, San Diego State University, San Diego, USA

(2) Vaxiion Therapeutics Inc, San Diego, USA

Colorectal cancer (CRC) is a leading cause of cancer-related deaths in the developed countries. Animal and epidemiological studies suggest that early life changes can lower the risk of CRC. Bacterial minicells (MC) are particles generated from genetically engineered E. coli (Vaxiion Therapeutics, Inc) that have all of the components of E. coli except chromosome, making them non-infectious. MC display anti-tumor activity in various cancer mouse models via integrin-specific cytotoxic effects, but require a functional immune system to be most effective. We used a novel FABP-CreXApcfl/+ mouse model of CRC that develops spontaneous colonic polyps due to targeted deletion of Apc in colon. MC-treatment during the 14th-19th week of age significantly attenuates the mean polyps number at 26 weeks (2.4±1.5) than PBS only (9.3±1.4, p=0.03) or 24 week-old untreated mice (8.0±1.9, p=0.02). Moreover, MC-treatment significantly blocks the development of colonic polyps >4mm in size (0.6±0.2) in comparison to PBS (3.7±0.8, p=0.03) or 24 week untreated (2.0±0.2, p=0.01) mice. Further, as per qPCR studies, MC promote TH1/CTL-associated immunity (CD8a, Tbet, Ifng, perforin) as well as the TH17 (Il17a) in tumor adjacent areas. MC-treatment lowers the pro-inflammatory (il23, Il6), immunosuppressive (Il10, Foxp3) and immune cell recruitment (Cx3cl1) markers in large tumors of MC-treated mice. Also, our data suggests the presence of ‘low’ and ‘high’ responders in MC-treated mice. ‘Low responders’ are MC-treated mice that have at least one large tumor at 26 weeks of age and lower correlation of TH1 and CTL-associated immunity than ’high responders’. ‘High responders’ are MC-treated mice that have no large tumors and display multi-fold higher levels of TH1 and CTL-associated immune responses than PBS-treated mice. This mouse model will be appropriate for the study of ‘low’ and ‘high’ responder anti-tumor immune phenotypes in mice, shedding light on what may be occurring in human CRC patients.

Cancer Risk in Relatives of Testicular Cancer Patients by Histology Type and Age at Diagnosis: A Joint Study from Five Nordic Countries

Poster 23: Elham Kharazmi MD, PhD[1*]; Kari Hemminki, MD, PhD[1,2]; Eero Pukkala, PhD[3,4]; Kristina Sundquist, MD, PhD[2,5]; Laufey Tryggvadottir, MSc[6,7]; Steinar Tretli, PhD[8]; Jörgen H. Olsen, MD, DMSc[9]; Mahdi Fallah, MD, PhD[1]

[1] Division of Molecular Genetic Epidemiology, German Cancer Research Center, Heidelberg, Germany

[2] Center for Primary Health Care Research, Lund University, Malmö, Sweden

[3] School of Health Sciences, University of Tampere, Tampere, Finland

[4] Finnish Cancer Registry, Institute for Statistical and Epidemiological Cancer Research, Helsinki, Finland

[5] Stanford Prevention Research Center, Stanford University School of Medicine, Stanford, California 94305-5705, USA

[6] Icelandic Cancer Registry, Reykjavik, Iceland

[7] Faculty of Medicine, University of Iceland, Reykjavik, Iceland

[8] Norwegian Cancer Registry, Oslo, Norway

[9] Institute of Cancer Epidemiology, Copenhagen, Denmark

BACKGROUND: None of the population-based epidemiologic studies to date has had a large enough sample size to show the familial risk of testicular cancer (TC) by age at diagnosis for patients and their relatives or for rare histologic subtypes.

OBJECTIVE: To estimate absolute and relative risks of TC in relatives of TC patients by age at diagnosis in patients and their relatives and histological subtypes.

DESIGN, SETTING, AND PARTICIPANTS: In a joint population-based cohort study, 97 402 first-degree relatives of 21 254 TC patients who were diagnosed between 1955 and 2010 in five European countries were followed for cancer incidence.

OUTCOME MEASUREMENTS AND STATISTICAL ANALYSIS: Standardized incidence ratios (SIRs) were estimated using histology-, age-, period-, and country-specific incidence rates as references. Lifetime cumulative risks were also calculated.

RESULTS AND LIMITATIONS: The lifetime cumulative risk of TC in brothers of a patient with TC was 2.3%, which represents a fourfold increase in risk (SIR 4.1, 95% confidence interval [CI] 3.6-4.6) compared to the general population. TC in a father increased the risk by up to twofold in his son (95% CI 1.7-2.4; lifetime risk 1.2%) and vice versa. When there were two or more TC patients diagnosed in a family, the lifetime TC risk for relatives was 10-11%. Depending on age at diagnosis, twins had a 9-74% lifetime risk of TC. Family history of most of the histologic subtypes of TC

increased the risk of concordant and most discordant subtypes. There was a tendency toward concordant age at diagnosis of TC among relatives.

CONCLUSIONS: This study provides clinically relevant age-specific cancer risk estimates for relatives of TC patients. Familial TC patients tended to develop TC at an age close to the age at diagnosis of TC among their relatives, which is a novel finding of this study.

Delineating the role of sulforaphane (SFN) in phenotype and functional switching in induced in vitro monocyte model

Poster 24: V Badireenath Konkimalla and Sanjima Pal

National Institute of Science Education and Research (NISER), Bhubaneswar, Orissa, India

Differentiation of monocytes into classically activated M1 macrophages or regulation of tissue macrophage plasticity (by switching existing default polarized, Mo or partially M2 polarized or alternatively activated M2 type to classically activated M1 type) at the site of inflammation is one of the pathogenic outcomes during the development of chronic inflammatory diseases e.g, Rheumatoid arthritis (RA), osteoarthritis (OA), arthrosclerosis, fibrosis, type I diabetes and other autoimmune diseases. In rheumatoid condition, soluble human collagen present as an abundant degradation product of the extracellular matrix plays an important role in inflammation and as a biomarker in vitro. In the current study from PMA-differentiated monocytic THP1 cell line, we established the M1 macrophage mediated inflammation using soluble collagen that developed similar inflammatory autoimmune responses. Further, with this inflammatory model system, the immune suppressive effect of SFN due to the modulation of monocytes/macrophages polarization as well as switching of phenotypes was studied. From the data we conclude that at a non cytotoxic dose, hormetic SFN blocks mounting inflammatory responses involving both MEK1/2 and JNK-1/2 MAP-kinases during monocyte to macrophage differentiation. Such signals induced by SFN eventually shifts macrophage polarization and concomitant activation in an alternative direction (M2 macrophages with CD36high CD197 extremely low CD206 mild high). This modulatory ability of SFN provides a clear indication for its ability to alleviate chronic inflammatory diseases by targeting monocytes/macrophages. The results obtained from collagen-induced inflammatory condition followed by SFN treatment showed that SFN can be a potential molecule to counteract autoimmune responses.

The Epstein-Barr Virus BART miRNA Cluster of the M81 Strain Modulates Multiple Functions in Primary B Cells

Poster 25: Xiaochen Lin, Ming-Han Tsai, Anatoliy Shumilov,Henri-Jacques Delecluse

German Cancer Research Center (DKFZ), Pathogenesis of Virus Associated Tumors, Heidelberg, Germany

EBV is a human tumor-associated virus that infects over 90% of global population. EBV encodes multiple microRNAs (miRNAs) from two primary transcripts, BHRF1 and the BARTs. Whilst the BHRF1 miRNAs are restricted to cells in type III latency, the BART miRNAs are expressed in all EBV-infected cells whatever their latency status. Although the targets of the BART miRNAs have been characterized in various high-throughput studies, the function of most BART miRNAs remains unclear.

M81, an EBV strain isolated from a Chinese patient with nasopharyngeal carcinoma (NPC), shows spontaneously lytic replication in B cells in vitro and in vivo at unusually high levels. By using M81 strain as a model, we studied the phenotypes of M81 strain mutants that are devoid of variable subsets of the BART-miRNA cluster. Our results showed that the BART miRNAs negatively regulate spontaneous lytic replication in EBV-infected B cells in vitro and in vivo. BART miRNAs regulate the expression of various viral and cellular proteins in EBV-immortalized B cells. For example, BALF5, BZLF1, LMP1, IPO7, and Dicer. However, the excision of the BART-miRNAs from the EBV genome has no influence on the transformation ability, infectivity, and proliferation of the infected B cells. We could also confirm that the BART miRNAs negatively regulation caspase3 expression, although their absence did not increase spontaneous or induced apoptosis. This is likely to be related to the ability of the BART miRNAs to down-regulate the expression of the key transforming viral protein LMP1 that is endowed with anti-apoptotic properties. We also tested whether previously suggested miRNA targets such as LMP2A, or EBV latent proteins, are modulated at the protein level, but there was no increased protein level shows compared to the wild type.

Whole-exome sequencing of primary and matched peritoneal metastasis of gastric adenocarcinoma- a preliminary report

Poster 26: Hao Liu,Fengping Li, Tingting Li, Yu Zhu, Haipeng Huang, Haipeng Zhou, Yanfeng Hu, Tingyu Mou, Tian Lin, Jiang Yu, Guoxin Li

Department of General Surgery, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China

Peritoneal metastasis occurs in more than half of patients with unresectable and recurrent gastric cancer through their clinical course and is associated with worse prognosis. In present study, the matched pair of normal gastric mucosa and peritoneal tissue and the matched pair of primary tumor tissue and peritoneal metastasis tissue were collected from one patient with gastric adenocarcinoma for whole-exome sequencing, aiming to discover the mutation spectrum of advanced gastric cancer with peritoneal metastasis. We found that C:G>A:T and G:C>T:A are two types of the highest frequent transversions in somatic mutations. Accordingly, we employed Sanger sequencing to identify 48 somatic mutations in primary site and 49 in peritoneal sits, respectively. In addition, twenty-five nonsynonymous somatic variations (SNVs) and 2 somatic insertion/deleteion(INDELs)were confirmed in primary tumor, while 30 nonsynonymous SNVs and 5 INDELS were verified in peritoneal metastasis. Approximately 59% of the somatic mutations were consistent between primary and metastatic sites. Four genes(BAI1, THSD1, ARID2 ,KIAA202 , C15orf57)verified in our study were also mutated above 5%in COSMIC database. We also identified 9 genes (ERBB4, ZNF721, NT5E, PDE10A, CA1, NUMB, NBN, ZFYVE16, NCAMI) that only mutated in metastasis. In conclusion, we founded that the majority of the somatic mutation in the primary site still remained in the metastasis,while a number of SNPs show up de novo at the second site.

Effect of Decitabine on colorectal cancer cell lines

Poster 27: Mohamed Refaat Mahdi (1, 2), Raouf Fekry Bedeer (2), Abd-El Hakiem Zohry Gabr (2), Huda Mohamed Eltahry (2), and Martin R. Berger (1)

(1) German Cancer Research Center (DKFZ), Toxicology and Chemotherapy Unit, Heidelberg, Germany

(2) Department of Human Anatomy and Embryology, Faculty of Medicine, Mansoura University, Egypt

Decitabine (5-aza-2'-deoxycytidine) is a demethylating agent. In the present study, we started from remarkable anti-cancer effects observed in colorectal cancer cells in vitro and then focused on the possible mechanism of action by investigating differences in mRNA expression by microarray technique between exposed and unexposed cancer cells.

Methods included exposing of different human and rat colorectal cell lines to low doses of decitabine followed by monitoring in vitro the effects on proliferation as well as colony formation, wound healing, and migration. In addition, the modulation in mRNA expression was examined by Illumina bead chip array in response to low concentrations of decitabine and candidate genes were selected by Ingenuity pathway analysis. Finally, the growth pattern of human colorectal cancer cells was established in vivo in an orthotopic liver metastasis model in nude rats.

A low dose of decitabine (0.25 µM) induced a change in morphology from spindle cell to rounded cell type, but did not cause significant anti-proliferative effects in human colorectal cancer cells from LS174T, SW620, or SW480 origin. The same dose, however, caused a more than 90% reduction in colony formation, as well as a remarkable inhibition of wound healing and migration. Interestingly, these inhibitory effects were not observed in rat CC531 cells.

The effects in human CRC cells were correlated to concentration dependent increases in mRNA expression of a number of genes, including ALDH1A3, ANXA1, CDKN1A.

Ongoing experiments focus on following up the results observed at RNA level by determining respective protein concentrations. In addition, colorectal cancer cells with or without previous exposure to decitabine are being transplanted to rats for monitoring possible effects on growth in vivo.

In conclusion, low concentrations of the demethylating agent decitabine show a remarkable effect on certain malignancy characteristics of human colorectal cancer cells growing in vitro.

De novo DNA methyltransferase regulates melanoma growth via mTOR signaling

Poster 28: Goran Micevic (1), Viswanathan Muthusamy (2), William Damsky (1), Nicholas Theodosakis (1), Xiaoni Liu (1), Katrina Meeth (1), Emily Wingrove (1), Manjula Santhanakrishnan (2), Marcus Wolfram Bosenberg (1)

(1) Departments of Dermatology and Pathology, Yale School of Medicine, New Haven, Connecticut, 06520, USA

(2) Department of Chemistry, Yale University, New Haven, Connecticut, USA

The purpose of this study is to examine the role of DNA methyltransferases (DNMT) in melanoma formation and progression. The requirement of DNMT enzymes for melanoma formation is poorly understood, despite the prominent role aberrant DNA methylation plays in melanoma. In this study, we examined the effect of genetic inactivation of each DNMT enzyme in a mouse model of melanoma. We validated our key findings in a panel of human melanoma cell lines, and melanoma patient samples from The Cancer Genome Atlas (TCGA). We examined survival, proliferation, metastatic potential and performed thorough transcriptomic and functional signaling characterization. We uncovered that inactivation of DNMT3b resulted in a striking prolongation of survival in vivo ($p<0.0001$) and was associated with a dramatic decrease in Akt signaling activation. Analogously, CRISPR mediated inactivation of DNMT3 decreased colony formation ($p<0.01$) and growth of human melanoma xenografts ($p<0.001$) in nude mice. The decrease in colony formation and tumor growth could be rescued by activating mTORC2 signaling in vitro and in vivo. We uncovered that DNMT3 mechanistically regulates Akt activity by altering methylation of a specific microRNA family ($p<0.001$). Furthermore, we found that PI3K activation can upregulate DNMT3, that high expression is required for continued melanoma proliferation, and associated with poor patient 5-year overall survival. This study examined the requirement of DNMT enzymes for melanoma formation and uncovered a novel important role for DNMT3 in regulating and licensing chronic activation of the PI3K/Akt pathway, a key signaling pathway in melanoma. These findings have direct therapeutic implications, and suggest that developing DNMT3 specific inhibitors, to be used alone or together with PI3K signaling inhibitors, may lead to improved therapy and care of melanoma patients.

Cancer risk in Basel by municipality and district: A population-based cancer registry 1981-2010

Poster 29: Seyed Mohsen Mousavi(1)*, Ivka Avellina (1), Maria Caterina Cammarota(1), Désirée Lüscher(1), Jacqueline Minck (1)

Cancer Registration Office, Department of Projects and Services, Department of Health, Basel City, Switzerland

Basel cancer registry was established by Krebsliga in 1969. The cancer data has been registered in electronic form since 1981. We aimed to define cancer risks in Basel city and Basel country by municipality and district.

We used Basel cancer registry database from 1981 to 2010. Cancer data in this Database is coded by the International Classification of Disease for Oncology until 1998 (ICD-O-DA), 1998 to July-2003 (ICD-O), and from March-2003 (ICD-O-3). We calculated age-standardized incidence rates (ASRs per 100,000 person-years of population at risk). The European population was used for standardization. The ASRs were adjusted by age (5-year bands), period (5-year bands from 1981-2010) and sex. The confidence interval (95%CI) for the ASRs was calculated by making a Poisson approximation of the binomial variance of the age-specific incidence rate. The municipalities in Basel city are Basel, Riehen and Bettingen. There are five districts in Basel country: Arlesheim, Liestal, Sissach, Waldenburg and Laufen. The Laufen was not included.

We observed 21,140 male and 19,366 female cancer cases in Basel, 2,628 and 2,491 cases in Riehen and Bettingen, 14,560 and 12,177 cases in Arlesheim, 4,449 and 3,690 cases in Liestal, 2,398 and 1,849 cases in Sissach, and 1,178 and 944 cases in Waldenburg, respectively. An increase in the cancer rate was seen among all Basel residents from 1981-1985 to 2006-2010: Basel (male: ASR from 627.6 to 692.4; female: 377.0 to 491.0), Riehen and Bettingen (male: 486.7 to 611.2, female: 358.7 to 453.8), Arlesheim (male: 535.8 to 608.6 , female: 357.8 to 467.3), Liestal (male: 511.1 to 528.8, female: 325.4 to 446.6), Sissach (male: 488.3 to 481.2, female: 300.7 to 384.8), and Waldenburg (male: 464.3 to 547.4 , female: 358.4 to 396.9).

Our study shows an increased rate of 26% in Riehen and Bettingen males and 37% in Liestal females. Implementing the cancer control program is a high priority in Basel department of health.

Laser machine for generating high density arrays of biologically active compounds

Poster 30: Alexander Nesterov-Müller, Felix F. Loeffler, Tobias C. Foertsch, Roman Popov, Daniela Althuon, Barbara Ridder, Clemens von Bojnicic-Kninski, Laura K. Weber, Andrea Fischer, Juliane Greifenstein, F. Ralf Bischoff, Michael A. R. Meier, Stefan Bräse, Annie K. Powell, Teodor Silviu Balaban, Frank Breitling, Alexander Nesterov-Mueller

Institute of Microstruture Technology, Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany

We present a novel machine for the cost-efficient combinatorial synthesis of high-density arrays of natural and synthetic compounds which could be screened for their biological activity in high density array format. Such machine will accelerate the development of anticancer therapeutics by significant reduction of candidate molecules on the earthly development stage. In an automated laser-based transfer process, nanometer thin spots with chemical reagents embedded in a polymer matrix are rapidly generated on a synthesis slides. Coupling of the reagents occurs in a separate heating step, where the matrix becomes viscous and building blocks diffuse and couple to the synthesis slide surface. Repetition of such procedure leads to the synthesis of a large amount of combinatorially composed molecules in spatially separated microcompartments with a density of >17,000 spots per cm2.

CYP3A5 mediates basal and acquired therapy resistance in different subtypes of pancreatic ductal adenocarcinoma

Poster 31: Elisa Noll, Christian Eisen (1), (2), (*), Albrecht Stenzinger (3), (4), (16), Elisa Espinet (1), (2), (16), Alexander Muckenhuber (3), Corinna Klein (1), Vanessa Vogel (1), Bernd Klaus (5), Wiebke Nadler (1), (2), Christoph Rösli (1), Christian Lutz (6), Michael Kulke (6), Jan Engelhardt (1), (2), Franziska M Zickgraf (1), (2), Octavio Espinosa (7), Matthias Schlesner (7), Xiaoqi Jiang (8), Annette Kopp-Schneider (8), Peter Neuhaus (9), Marcus Bahra (9), Bruno V Sinn (10), Roland Eils (7) , (11), (12), Nathalia A Giese (13), Thilo Hackert (13), Oliver Strobel (13), Jens Werner (13), Markus W Büchler (13), (14), Wilko Weichert (3), (4), (14), Andreas Trumpp (1), (2), (14), (*) & Martin R Sprick (1), (2), (14), (*)

1) Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM), Heidelberg, Germany. (2) Division of Stem Cells and Cancer, German Cancer Research Center (DKFZ), Heidelberg, Germany. (3) Department of Pathology, University of Heidelberg, Heidelberg, Germany. (4) National Center for Tumor Diseases (NCT), Heidelberg, Germany. (5) Genome Biology Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany. 6Heidelberg Pharma GmbH, Ladenburg, Germany. (7) Division of Theoretical Bioinformatics, German Cancer Research Center (DKFZ), Heidelberg, Germany. (8) Division of Biostatistics, German Cancer Research Center, Heidelberg, Germany. (9) Department of General, Visceral and Transplantation Surgery, Charité-Universitätsmedizin Berlin, Berlin, Germany. (10) Institute of Pathology, Charité-Universitätsmedizin Berlin, Berlin, Germany. (11) Institute of Pharmacy and Molecular Biotechnology, Bioquant, University of Heidelberg, Heidelberg, Germany. (12) Heidelberg Center for Personalized Oncology (DKFZ-HIPO), German Cancer Research Center, Heidelberg, Germany. (13) Department of General and Visceral Surgery, University Hospital Heidelberg, Heidelberg, Germany. (14) German Cancer Consortium (DKTK), Heidelberg, Germany. (*) These authors contributed equally to this work.

Pancreatic ductal adenocarcinoma (PDAC) is a very aggressive disease with poor prognosis. Treatment with gemcitabine, the FOLFIRINOX scheme or nab-paclitaxel offer only a modest increase in overall survival. For a number of other carcinomas, tumor subtypes have been uncovered that allow the use of targeted therapies. Although subtypes of PDAC were described, this malignancy is clinically still treated as a single disease. We established patient-derived models representing the full spectrum of previously identified quasi-mesenchymal (QM-PDA), classical and exocrine-like PDAC subtypes, and identified two markers—HNF1A and KRT81—that enable stratification of tumors into different subtypes by immunohistochemistry. Patients bearing tumors of these subtypes show significant differences in overall survival and their tumors differ in drug sensitivity, with the exocrine-like subtype being resistant to tyrosine kinase inhibitors and paclitaxel. The xenobiotic enzyme,

cytochrome P450 3A5 (CYP3A5), metabolizes these compounds in tumors of the exocrine-like subtype, and pharmacological or short hairpin RNA (shRNA)-mediated CYP3A5 inhibition sensitizes tumor cells to these drugs. Additionally, retrospective analysis of a large patient cohort confirmed that CYP3A5 is predominantly found in those patient tumors classified as exocrine-like. Whereas the hepatocyte nuclear factor 4, alpha (HNF4A) controls basal expression of CYP3A5, drug-induced CYP3A5 upregulation is mediated by the nuclear receptor NR1I2. Interfering with these regulatory mechanisms may provide an alternative approach to suppress the CYP3A5 pathway. CYP3A5 also contributes to acquired drug resistance in QM-PDA and classical PDAC, and is highly expressed in several additional malignancies. These findings designate CYP3A5 as predictor of therapy response and as a tumor cell-autonomous detoxification mechanism that must be overcome to prevent drug resistance.

Loss of DNMT3A function prevents reporter gene inactivation in leukemic cell lines and mES cells

Poster 32: Desiree Melanie Schneider (1,2); Jessica Beckert (1,2); Sophia Ehrenfeld (1,2); Robin Khan (1); Jan Mitschke (1,2); Khalid Shoumariyeh (1); Pia Veratti (1,2); Cornelius Miething (1,2)

(1) Department of Medicine I, Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Germany

(2) DKFZ Heidelberg, DKTK, Freiburg, Germany

More than 70% of de novo AML cases harbor at least one amino acid change in a DNA methylation-related gene or epigenetic modifier. Up to 30% of NK-AML cases show a somatic mutation in DNA-methyltransferase 3 alpha (DNMT3A). These mutations are mostly heterozygous and more than half of the DNMT3A mutations alter R882 in its catalytic domain (mostly R882H). This mutation has been shown to dominantly inhibit DNMT3Awt by blocking its homo-tetramerization. The importance of DNMT3A for the differentiation of hematopoietic stem cells (HSCs) is demonstrated by the fact that DNMT3A-KO HSCs show increased self-renewal and reduced differentiation. DNMT3A mutations are assumed to arise early in AML development, most probably in HSCs, so that a clonally expanded pool of pre-leukemic cells arises from which AML evolves.

As DNMT3A is highly conserved, we explored the effect of DNMT3A mutation in leukemogenesis via its knock-down and over-expression in human AML cell lines and murine embryonic stem (mES) cells. The knock-down was achieved using a two colored, antibiotic selectable and tet-inducible retroviral shRNA expression vector system. mES cells carrying a homing cassette were targeted via recombination-mediated cassette exchange to contain DNMT3A, GFP and the hygromycin resistance gene. To analyze the role of DNMT3A in tumor maintenance, bone marrow transplantation experiments with FLt3-ITD and inducible DNMT3Awt or R882H and a shRNA targeting mDNMT3A are ongoing.

Analyzing the DNMT3A-inducible cell lines, we found that over-expression of DNMT3Awt increases the inactivation of retroviral reporter constructs via promoter methylation, both in the leukemia cell lines HL60 and K562 as well as in mES cells. In contrast, the R882H mutation reduced the effect of DNMT3A over-expression in HL60 and mES cells, but less so in K562 cells. DNMT3A knock-down was significantly selected against in the different leukemic cell lines, suggesting residual functionality of DNMT3A is required.

DNA methylation array analysis identifies breast cancer associated methylation changes in peripheral blood DNA

Poster 33: Qiuqiong Tang(1,2), Tim Holland-Letz(3), Alla Slynko(3), Katarina Cuk(1,2), Frederik Marme(1), Sarah Schott(1), Jörg Heil(4), Bin Qu(5), Michael Golatta(4), Melanie Bewerunge-Hudler(6), Christian Sutter(7), Harald Surowy(1,2), Barbara Wappenschmidt(8), Rita Schmutzler(8), Markus Hoth(5), Peter Bugert(9), Claus R.Bartram(7), Christof Sohn(1), Andreas Schneeweiss(1,10), Rongxi Yang(1,2) and Barbara Burwinkel(1,2)

(1) Molecular Biology of Breast Cancer, Department of Gynecology and Obstetrics, University of Heidelberg, Heidelberg, Germany

(2) Division of Molecular Epidemiology (C080), German Cancer Research Center (DKFZ), Heidelberg, Germany

(3) Division of Biostatistics (C060), German Cancer Research Center (DKFZ), Heidelberg, Germany

(4) Department of Gynecology and Obstetrics, University Women's Clinic, Heidelberg, Germany

(5) Department of Biophysics, Center for Integrated Physiology and Molecular Medicine (CIPMM), Saarland University, Homburg (Saar), Germany

(6) Genomics and Proteomics Core Facility, German Cancer Research Center (DKFZ), Heidelberg, Germany

(7) Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany

(8) Centre of Familial Breast and Ovarian Cancer, Department of Gynaecology and Obstetrics and Centre for Integrated Oncology (CIO), Center for Molecular Medicine Cologne (CMMC), University hospital of Cologne, Cologne, Germany

(9) Institute of Transfusion Medicine and Immunology, Medical Faculty Mannheim, University of Heidelberg, German Red Cross Blood Service Baden - Württemberg - Hessen, Mannheim, Germany

(10) National Centre for Tumor Diseases, Heidelberg, Germany

DNA methylation changes in peripheral blood DNA have been shown to be associated with solid tumors. We sought to identify methylation alterations in whole blood DNA that are associated with breast cancer (BC). We performed genome-wide DNA methylation profiling on blood DNA from BC cases and healthy controls applying Infinium HumanMethylation450K BeadChips. Promising CpG sites were selected and validated in three independent larger sample cohorts via MassARRAY EpiTyper assays. CpG sites located in three genes (cg06418238 in RPTOR, cg00736299 in MGRN1 and cg27466532 in RAPSN), which showed significant hypomethylation in BC patients compared to healthy controls in the discovery cohort

($p < 1.00 \times 10\text{-}6$) were selected and successfully validated in three independent cohorts (validation I, n =211; validation II, n=378; validation III, n=520). The observed methylation differences are likely not cell-type specific, as the differences were only seen in whole blood, but not in specific sub cell-types of leucocytes. Moreover, we observed in quartile analysis that women in the lower methylation quartile of these three loci had higher ORs than women in the higher quartiles. The AUC of three loci combined was 0.79 (95%CI 0.73-0.85) in validation cohort I, 0.60 (95%CI 0.54-0.66) in validation II and 0.62 (95%CI 0.57-0.67) in validation III, respectively. Our study suggests that hypomethylation of CpG sites in RPTOR, MGRN1 and RAPSN in blood is strongly associated with BC and might serve as blood-based marker supplements for BC if these could be verified in prospective studies.

Pre-diagnosis Insulin-like growth factors and pancreatic cancer survival

Poster 34: Adetunji T. Toriola (1), Mark Ziegler (1),Yize Li (1), Yinjiao Ma (1), Rachael Stolzenberg-Solomon (2)

(1) Division of Surgery, Department of Public Health Sciences, Washington University School of Medicine, Saint Louis, MO, USA

(2) Division of Cancer Epidemiology & Genetics, Nutritional Epidemiology Branch, National Institutes of Health, National Cancer Institute, Rockville, MD, USA

Background: Pre-diagnosis obesity is associated with worse pancreatic cancer survival. Obesity is associated with dysregulation in circulating insulin-like growth factor (IGF) levels but no study has evaluated the associations of pre-diagnosis IGF levels with pancreatic cancer survival. We hypothesize that pre-diagnostic circulating IGF levels (IGF-I, IGF-II), and IGF binding protein 3 (IGFBP-3) will be associated with pancreatic cancer survival.

Methods: We evaluated our hypothesis in 178 participants enrolled in the intervention arm of the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial who developed exocrine pancreatic cancer during follow-up. IGF biomarkers were assayed in blood samples donated prior to cancer diagnosis. Cox proportional hazards regression model, adjusting for stage, age, body mass index, history of diabetes, and smoking status, was used to investigate the associations of IGF biomarkers with pancreatic cancer survival. Because IGF biomarker levels differed by gender, we evaluated associations separately among males (N=116), and females (N=62).

Results: 96% of cases died during follow-up. Median survival was 172 days. Elevated IGF-II, and IGF-II and IGFBP-3 concentrations were inversely associated with pancreatic cancer survival among males, but not among females. Men within the highest tertiles of IGF-II (HR=0.40, 95%CI 0.23-0.71, p-trend <0.01), and IGFBP-3 (HR=0.59, 95%CI 0.35-0.97, p-trend=0.10) had better survival compared to men within the lowest tertiles. The corresponding HRs for women were: IGF-II (1.60, 95%CI 0.79-3.23, p-trend=0.73); IGFBP-3 (1.58, 95%CI 0.76-3.27, p-trend=0.72). There were no statistically significant associations between IGF-I concentrations, IGF-I/IGFBP-3 and pancreatic cancer survival.

Conclusion: Our findings indicate that associations of pre-diagnostic IGF-II and IGFBP-3 with pancreatic cancer survival may be gender dependent, with higher levels associated with better survival in men.

Development of a therapeutic cancer vaccine based on p16INK4a

Poster 35: Katharina Urban (1), Rafael Carretero (2), Elena-Sophie Prigge (1), Wolfram Osen (3), Günter J. Hämmerling (4), Miriam Reuschenbach (1), Magnus von Knebel Doeberitz (1)

(1) Department of Applied Tumor Biology, Institute of Pathology, University of Heidelberg, and Clinical Cooperation Unit Applied Tumor Biology, German Cancer Research Center (DKFZ), Heidelberg, Germany

(2) DKFZ/BHC Joint Immunotherapy Lab, German Cancer Research Center (DKFZ), Heidelberg, Germany

(3) Department of Translational Immunology, German Cancer Research Center (DKFZ), Heidelberg, Germany

(4) Tumorimmunology Program, German Cancer Research Center (DKFZ), Heidelberg, Germany

For the development of therapeutic cancer vaccines, tumor-specific antigens need to be identified. The cyclin-dependent kinase inhibitor p16INK4ais strongly overexpressed in human papillomavirus (HPV)-induced cancers, whereas it is barely detectable in normal tissue. Therefore, it is an established surrogate marker for high risk HPV infections.

Co-staining of p16INK4a and the proliferation marker Ki-67 showed that the majority of p16INK4a-overexpressing cells are proliferative. This co-expression has also been detected in non-HPV-related tumor entities, suggesting p16INK4a as a broad tumor antigen that is not only specific for HPV-associated cancers.

In a phase I/IIa trail to test safety and immunogenicity of a p16INK4a peptide vaccine in patients with p16INK4a-overexpressing, HPV-associated cancers we could show the induction of a humoral and cellular immune response against p16INK4a.

Presently we are establishing a p16INK4a-specific tumor mouse model to analyse the effect of a p16INK4a-based vaccine on tumor growth and its potential to be combined with current immunomodulators as TLR ligands and checkpoint inhibitors.

The possibility to generate an effective tumor response against p16INK4a could lead to a new immunotherapeutic treatmentfor tumors overexpressing p16INK4a.

Network analysis reveals cancer-driven pathways from integrative epigenetic data

Poster 36: Qi Wang (1), Roland Eils (1,2), Carl Herrmann (1,2)

(1) Division of Molecular Genetics, German Cancer Research Center, Heidelberg, Germany

(2) Institute for Pharmacy and Molecular Biotechnologies, University Heidelberg, Germany

Cancer is a complex disease which involved many different types of alterations in both coding and non-coding parts of the genome. In order to understand the effects of these alterations, and uncover the pathways behind them, we used a network approach by integrating epigenetic data in analyzing several diseases. By inferring significant altered subnetworks from enriching differentially methylated/histone modified nodes, we have not only found several diseases related pathways, but also common enhancers involved in these pathways. With the rapidly increasing amount of available pigenetic data in the public domain, we believe such approach will be very useful in understanding the regulatory landscape of the diseases.

Preclinical test systems for the evaluation of nanocarriers in precision medicine.

Poster 37: Rainer Wittig (1), Clemens K. Weiss (2), Rainer Will (3), Mika Lindén (4), Katharina Landfester (5)

(1) Biology Group, Institute for Laser Technologies in Medicine & Metrology (ILM) at Ulm University, Ulm, Germany

(2) Bingen University of Applied Science, Bingen, Germany

(3) Genomics & Proteomics Core Facility, DKFZ, Heidelberg, Germany

(4) Institute of Inorganic Chemistry II (Synthesis and Characterization of Inorganic Materials), Ulm University, Ulm, Germany

(5) Physical Chemistry of Polymers, Max Planck Institute for Polymer Research, Mainz, Germany

Nanocarriers represent promising and versatile tools for future biomedicine. Their flexibility with regard to loading and surface design offers a broad range of applications. Nanocarrier-related parameters such as surface functionalization, chemical composition, size and shape, as well as environment / application-related surface adsorption (the “corona”) govern cellular uptake, cargo release as well as organ accumulation in vivo. However, our knowledge about relationships between design and function is incomplete, which is a major reason why only few nanocarriers entered clinical trials yet.

We aim at an optimization nanocarriers for biomedical applications and use advanced cell culture methodology as well as the chorioallantoic membrane in vivo model (CAM) for their evaluation. Molecular targeting of mesoporous silica nanocarriers (MSN) via surface bound ligands was recently found to elicit preferential uptake into cancer cells expressing a corresponding receptor, as demonstrated in vitro by flow cytometry, as well as in vivo by superior therapeutic efficacy on receptor-positive vs. receptor negative CAM-xenografts. Isogenic cancer cell lines established at DKFZ and engineered for doxycycline-regulated gene expression were employed for preliminary investigations on the specificity of enzymatic cleavage of organic nanocapsules in vitro. In future studies, we intend to combine those in vitro and in vivo approaches to setup a preclinical evaluation pipeline for nanocarrier-mediated precision medicine in cancer.

Comparison and combination of blood DNA methylation changes at smoking-associated genes and at lung cancer related genes in prediction of lung cancer mortality

Poster 38: Yan Zhang (1), Lutz P Breitling (1), Yesilda Balavarca (2), Bernd Holleczek (3), Ben Schöttker (1), Hermann Brenner(1,2,4)

(1) Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

(2) Division of Preventive Oncology, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Im Neuenheimer Feld 460, D-69120 Heidelberg, Germany

(3) Saarland Cancer Registry, Saarbrücken, Germany

(4) German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

Background Epigenome-wide association studies have established methylation patterns related to smoking, the major risk factor of lung cancer (LC), which are distinct from methylation profiles disclosed in LC patients. The current study simultaneously investigated the associations of 151 smoking-associated CpGs (smoCpGs) and 3806 LC-related CpGs (caCpGs) with LC mortality in baseline blood samples of 1565 older adults in a population-based case-cohort study.

Results During a median follow-up of 13.8 years, 60 participants who had a first diagnosis of LC died from LC. The time between blood sample collection and LC diagnosis ranged from 3 months to 11.8 years [median: 5.8 years]. Hypomethylation at 77 smoCpGs and 121 caCpGs, and hypermethylation at 4 smoCpGs and 66 caCpGs were found to be associated with LC mortality. The associations were much stronger for smoCpGs than for caCpGs. Hazard ratios (95% CI) were 7.82 (2.91-21.00) and 2.27 (0.75-6.85), respectively, for participants in highest quartile of Score-I (based on 81 smoCpGs) and Score-II (based on 187 caCpGs), compared to participants in the corresponding lower three quartiles. Score-I outperformed Score-II, with an optimism-corrected C-index of 0.87 vs. 0.77.

Conclusions Although methylation changes of both smoking-associated and LC-related genes are prospectively associated with LC mortality, only smoking-associated methylation markers predict LC mortality with high accuracy, and may thus serve as promising candidates to identify high risk populations for LC screening.

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