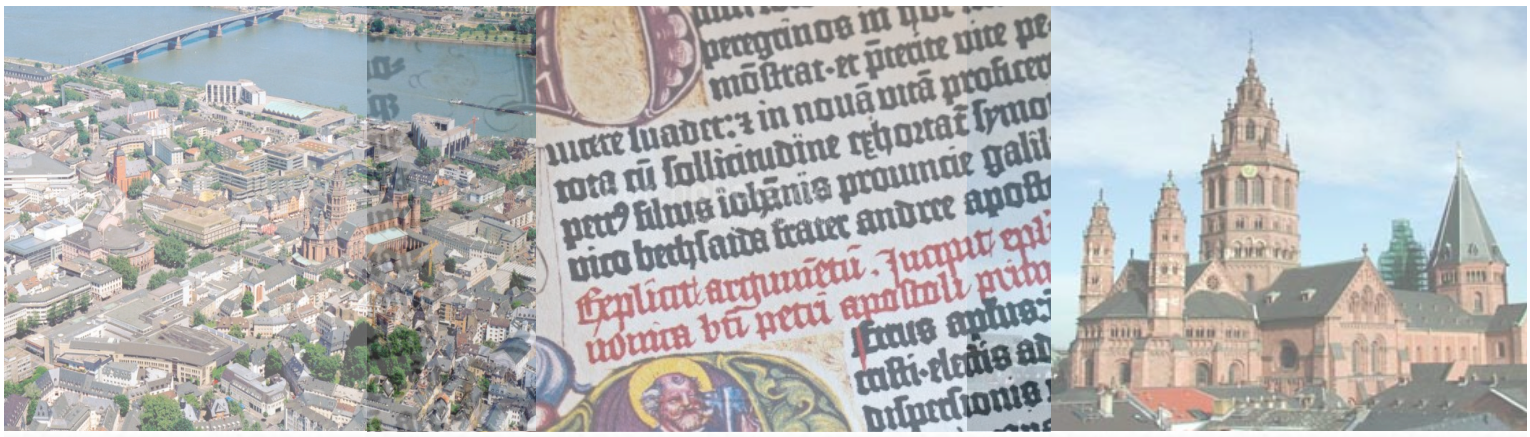


2nd SINO-GERMAN Symposium

on

DNA REPAIR AND HUMAN DISEASE

16. – 20. October 2011

University Medical Center, Mainz

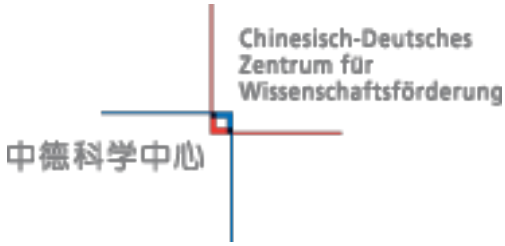
Venue: Favorite Park Hotel, Mainz

Organization:
Prof. Dr. Bernd Kaina, Mainz (kaina@uni-mainz.de)
Prof. Dr. Xingzhi (Xavier) Xu, Beijing (Xingzhi_Xu@mail.cnu.edu.cn)

Chinesisch-Deutsches Zentrum für Wissenschaftsförderung
中德科学中心
JG|U UNIVERSITÄTSmedizin

Sponsored by:

Sino-German Center for Science Promotion, NSFC & DFG



Organised and hosted by:

Johannes Gutenberg-University of Mainz

Foreword

Dear Colleagues,

It is our pleasure to welcome you to the 2nd Sino-German Symposium. The first symposium on DNA Repair and Human Disease was held in the Capital Normal University, Beijing in 2010. A number of leading German and Chinese scientists presented their work and exchanged ideas and concepts that could be the basis for collaborations. Now we are able to continue and strengthen the scientific exchange in the 2nd symposium. Twenty-five scientists from Germany, fifteen from China and outstanding guests from other countries were invited and immediately accepted the call to attend the workshop, indicating the importance of the event, which focuses on DNA base and double-strand break repair, issues of mutagenesis and its relationship with a number of important biological end points, including carcinogenesis and genomic instability.

The main goal of the symposium is to initiate collaborations between German and Chinese scientists and we hope the symposium will contribute to this goal.

The setting will be the historical city of Mainz and we hope that the lively social program will contribute to interactions. We hope that the meeting is enjoyable and fruitful for exploring new avenues in this exciting and topical research area. Our sincere thanks goes to the meeting sponsor, the Sino-German Center for Research Promotion, NSFC & DFG (http://www.sinogermanscience.org.cn/), and the local committee for their help in the organizational aspects of the meeting.

Welcome again to Mainz.

“Yes, as you can see, all the i’s are dotted and the t’s are crossed”
by Johannes Guttenberg, inventor of book printing, in the 1450s.

Bernd Kaina and Xingzhi Xu

Symposium Chair:

Prof. Dr. Bernd Kaina, Director of the Institute of Toxicology, University Medical Center, Mainz

Prof. Dr. Xingzhi (Xavier) Xu, Director of the Beijing Key Laboratory of DNA Damage Response, Capital Normal Univesity, Beijing

Scientific Organizing Committee:

Prof. Dr. Bernd Kaina, Institute of Toxicology, University Medical Center, Mainz

Prof. Dr. Markus Löbrich, Institute of Radiation Biology, Darmstadt, Germany

Prof. Dr. Xingzhi XU, Beijing Key Laboratory of DNA Damage Response, Capital Normal University, Beijing

Prof. Dr. Zhao-Qi Wang, Leibniz Institute for Age Research / Fritz Lipmann Institute, Jena

Local Organizing Committee:

Dr. Markus Christmann

Dr. Wynand P. Roos

Prof. Bernd Kaina

Secretary:

Dr. Christina Strauch

Ms. Brigitte Rudolph

Ms. Martina Schiffer

Symposium Venue:

The symposium venue is at the Favorite Parkhotel, Karl-Weiser Str.1, 55131 Mainz.

It is located within the scenic Volkspark in Mainz, to the east of the University Medical Center Mainz and near the Rhine River.

Speakers

Vilhelm A. Bohr, PhD, Laboratory of Molecular Gerontology at the National Institute on Aging and the National Institutes of Health, Baltimore
Junjie Chen, PhD, Professor, University of Texas M.D. Anderson Cancer Center, Houston
Grigory L. Dianov, PhD, Gray Institute of Radiation Oncology and Biology, University of Oxford
Ulrich Hübscher, PhD, Institute for Veterinary Biochemistry and Molecular Biology, University of Zürich-Irchel, Winterthurerstrasse 190, 8057 Zürich, Switzerland
Holger Bastians, PhD, Department of Molecular Oncology, Georg-August University, Göttingen
Alexander Bürkle, PhD, Professor, Molecular Toxicology Group, University of Konstanz
Jochen Dahm-Daphi, PhD, Professor, Institute of Radiobiology and Molecular Radiation Oncology, University Medical Center Hamburg-Eppendorf
Thilo Dörk, PhD, Department of Obstetrics and Gynaecology, Hannover Medial School
Steffen Emmert, PhD, Professor, Department of Dermatology, Venerology and Allergology, Georg-August-University Göttingen
Bernd Epe, PhD, Professor, Institute of Pharmacy and Biochemistry, University Medical Center, Mainz
Gerhard Fritz, PhD, Professor, Intitute of Toxicology, University of Düsseldorf
Frank Grosse, PhD, Professor, Leibniz Institute for Age Research, Fritz Lipman Institute, Jena
Andrea Hartwig, PhD, Professor, Institute of Food Chemistry and Toxicology, Institute of Applied Biosciences, University of Karlsruhe
George Iliakis, PhD, Professor, Institute of Medical Radiation Biology, University of Essen
Bernd Kaina, PhD, Professor, Institute of Toxicology, University Medical Center, Mainz
Caroline Kisker, PhD, Professor, Rudolf Virchow Center for Experimental Biomedicine, University of Würzburg
Oliver Krämer, PhD, Department of Biochemistry, University of Jena
Markus Löbrich, PhD, Professor Institute of Radiation Biology and DNA Repair, Darmstadt University of Technology
Christof Niehrs, PhD, Professor, Institute of Molecular Embryology, German Cancer Research Center Heidelberg
Odilia Popanda, PhD, Division of Epigenomics and Cancer Risk Factors, German Cancer Research Center Heidelberg
Detlev Schindler, PhD, Professor, Division of Somatic Cell Genetics/ Laboratory for Genomic Instability, Department of Human Genetics, University of Würzburg
Björn Schumacher, PhD, Cologne Excellence Cluster for Cellular Stress Responses in Aging Associated Diseases (CECAD) at the Institute for Genetics, University of Cologne
Tanja Schwerdtle, PhD, Professor, Institute of Food Chemistry, University of Münster
Jürgen Thomale, PhD, Professor, Institute for Cell Biology, University of Duisburg-Essen Medical School
Jörn Walter, PhD, Professor, FR 8.3 Biosciences, Laboratory of EpiGenetics, Saarland University
Zhao-Qi Wang, PhD, Professor, Leipniz Institute for Age Research, Fritz Lipman Institute, Jena
Lisa Wiesmüller, PhD, Professor, Department of Obstetrics and Gynaecology, University of Ulm
Haiying Hang, PhD, Professor, Chinese Academy of Sciences/ Institute of Biophysics, Beijing
Yuejin Hua, Zhejiang University/ College of Agriculture and Biotechnology, Hangzhou
Jun Huang, PhD, Professor, Zhejiang University/ Institute of Life Sciences, Hangzhou
Tao Jiang, PhD, Professor, Chinese Academy of Sciences/ Institute of Biophysics, National Laboratory of Biomacromolecules, Beijing

Cong Liu, PhD, Professor, Sichuan University/ Developmental and Stem Cell Institute, West China Women and Children's Hospital, Sichuan
Yulong Shen, PhD, Professor, Shandong University/ School of Life Sciences/ State Key Laboratory of Microbial Technology
Zhou Songyang, PhD, Professor, Sun Yat-sen University/ School of Life Sciences, Guangzhou
Weimin Tong, PhD, Associate Professor, Chinese Academy of Medical Sciences/ Institute of Basic Medical Sciences, Beijing
Wei Xiao, PhD, Professor, Capital Normal University/ College of Life Sciences, Beijing
Xingzhi Xu, PhD, Professor, Capital Normal University/ College of Life Sciences, Beijing
Yungui Yang, PhD, Professor, Chinese Academy of Sciences/ Beijing Institute of Genomics
Chuanmao Zhang, PhD, Professor, Peking University/ College of Life Sciences/ Department of Cell Biology and Genetics, Beijing
Guoliang Xu, PhD, Professor, Institute of Biochemistry and Cell Biology, CAS
Jinqiu Zhou, PhD, Professor, Shanghai Institutes for Biological Sciences/ Institute of Biochemistry and Cell Biology
Weiguo Zhu, PhD, Professor, Peking University Health Science Center/ Department of Biochemistry and Molecular Biology, Beijing

Brief Agenda

Dates	Time	Events	Location
Sunday, 16 October	14.00-18.00	Check-In, Registration	Favorite Park Hotel
	18.00	Warm Up/ Piano Concert Welcome Dinner	
Monday, 17 October	08.30	Welcoming Remarks	Favorite Lecture Hall
	09.00-10.30	Presentations	
	10.30-11.00	Break	
	11.00-12.30	Presentations	
	12.30-14.30	Lunch	Favorite Park Hotel
	14.30-16.30	Presentations	Favorite Lecture Hall
	16.30-17.00	Break	
	17.00-19.00	Presentations	
	20.00	Dinner	Favorite Park Hotel
Tuesday, 18 October	08.30-10.30	Presentations	Favorite Lecture Hall
	10.30-11.00	Break	
	11.00-13.00	Presentations	
	13.00-14.00	Lunch	
	14.00-15.30	Presentations	Favorite Park Hotel
	15.30-16.00	Break	Favorite Lecture Hall
	16.00-18.00	Presentations	
	18.00	Group Picture	
	18.30-22.30	Speakers' Dinner	Laubenheimer Höhe
Wednesday, 19 October	08.30-11.00	Presentations	Favorite Lecture Hall
	11.00-11.30	Break	
	11.30-13.30	Presentations	
	13.30-15.00	Lunch	
	15.00-17.00	Presentations	Favorite Park Hotel
	17.00-17.30	Break	Favorite Lecture Hall
	17.30-19.00	Presentations	
	19.00-20.00	Final Discussion	
	20.00	Dinner	Favorite Park Hotel
Thursday	All Day	Excursion (Chinese Delegates)	

Meeting Details and General Information

Arrival

For those arriving at Frankfurt Airport
We recommend that you take a taxicab from the airport to the Favorite Parkhotel in Mainz (running time 30 min. The price is approx. 45 Euros). Alternatively, you can also go by train (S-Bahn connecting Frankfurt with Mainz). The railway station at the airport is called "Regionalbahnhof" (5 min. walk through the airport) with an excellent connection to Mainz every 30 min (train number S8).

Upon arrival in Mainz main station, please take a taxi or bus numbers 62 or 63 and disembark at station "Volkspark". From there it is only 5 min walk.

Alternatively you can take bus numbers 60 (direction Ginsheim-Gustavsburg-Ginsheim Neckarstraße) or 61 (direction Mainz-Laubenheim Hans-Zöller-Straße) from Mainz main station and disembark at “Mainz Favorite Park Hotel”.

For those travelling by car
See directions at http://www.de.map24.com and http://www.favorite-mainz.de

Contact Details

Dr. Christina Strauch
Institute of Toxicology
University Medical Center
Obere Zahlbacher Str. 67
D-55131 Mainz
Germany
Email: strauch@uni-mainz.de
Tel. +49 (0)6131-17-9357 (9217)

Local organisers will be at the conference site from Sunday afternoon.

Electricity and Water

The electricity supply in Germany is 220 V.
Tap water in Mainz is drinkable.

Hotel

Accommodation for the official attendees are fully supported by the sponsor and has been arranged at the ***Favorite Park Hotel****,* Karl-Weiser-Strasse 1, Mainz, Tel. +49 (0)6131-80150 (www.favorite-mainz.de).

Rooms will not be available at the Favorite Parkhotel until 2 p.m. However, arriving earlier is no problem. You can leave your luggage in a special room and stay in one of the comfy seating areas or see the beautiful “Wintergarten” or the “Rose garden” outside in the park area next to the hotel.

Breakfast is in the “Sonnensalon” (1st floor), lunch and dinner are in the hotel restaurant.

Additional accommodation for external participants can be booked at the local youth hostel: ***Rhein-Main-Jugendherberge***, Otto-Brunfels-Schneise 4, Mainz, Tel. +49 (0)6131-85332 (http://www.diejugendherbergen.de/die-jugendherbergen-auf-einen-blick/mainz/portrait/); or at ***Hotel Stiftswingert,*** Am Stiftswingert 4, Mainz, Tel: +49 (0)6131-98264-0 (www.hotel-stiftswingert.de/).

Insurance

Please make sure that you have suitable coverage from insurance policies from your country in case of illness or accident during your stay in Germany. The Organizing Committee will not be liable for personal injuries sustained by loss or damage to property belonging to congress participants during the Conference and all tours.

Language

The official language of the Symposium is English.

Money and Foreign Exchange

Germany's currency is the EURO, which is available in 5, 10, 20, 50, 100, 200 and 500 EURO notes as well as 1, 2, 5, 10, 20 and 50 cent coins. Most major currencies can be exchanged at the airport and the banks. In Germany most major credit cards are accepted and withdrawing cash from your home account using a credit card is possible. If you need help with international banking, please contact the local banks in Mainz (opening hours of most banks: Monday to Friday 9.00 to 11.00 a.m. and 2.00 to 4.00 p.m.).

Presentations

All lectures should be PowerPoint presentations. Please bring your presentation on a USB pen drive and, if possible, CD backup. For those less experienced with Powerpoint, please make sure all writing is legible (at least 12, but preferably 14 point sans serif font such as Arial) and that there are no red on blue, black on blue or yellow on white combinations. Please avoid overhead presentation of your data, since this will require additional time to arrange.

Public Transport

The city of Mainz is served by an extensive public transport system consisting of a tram and bus service to all parts of the city. The historic town centre is a leisurely 20-minute walk from *Favorite Park Hotel* along the Rhine River.

For public transport maps and schedules, see http://www.mvg-mainz.de/zeiten-netz/routen-fahrplaene.html.

Registration

This Symposium is fully supported by the Sino-German Center for Science Promotion; therefore, there is no registration charge for invited speakers. Participants are welcome, for whom we charge a conference fee of €200 (exclusive evening meals and accommodation).

The registration desk is staffed from 16.00 hrs to 18.00 hrs on Sunday, 16 October.

At registration you will be asked to **submit your travel documents and invoices** for photocopying, unless you have organised your travel directly through the Sino-German-Center's contract travel agency, China Travel and Trading (Deutschland) GmbH.

Sight-seeing

To those of you who decide to stay one or a few days longer, we recommend the following tours and exhibitions:

- Gutenberg museum with the first items printed with movable letters including the Gutenberg Bibles

- City tours with the Gutenberg Express sightseeing train (http://www.gutenberg-express.de/)

- Eberbach's Monastery, the former Cistercian monastery, is one of the most important artistic monuments in Hesse built between the 12th and 14th centuries

- Eltville: The most beautiful Old Town of the "Rheingau"

- Johannisberg with castle is the cradle of the viniculture. The late vintage was discovered here. Admirable view over the Rhine valley where Goethe said: "Verweile doch, Du bist so schööön" (Abide here, you are so beauteous).

- Bingen: Marching to the Rochus chapel. Experience the magic view to the opposite side of the Rhine, to Rüdesheim

- Städel-Museum Frankfurt: Special exhibition "The Changing Face of Childhood"

- Trier: Exhibition of Constantin the Great, the first Christian Roman Emperor (306 – 337)

Telephone

Although most people will have a mobile phone, public telephones are abundant in Germany. Pre-paid cards for domestic calls are available from kiosks and most shops, and some telephones also accept coins. International calls can be dialled directly. Public telephones are situated throughout the city.

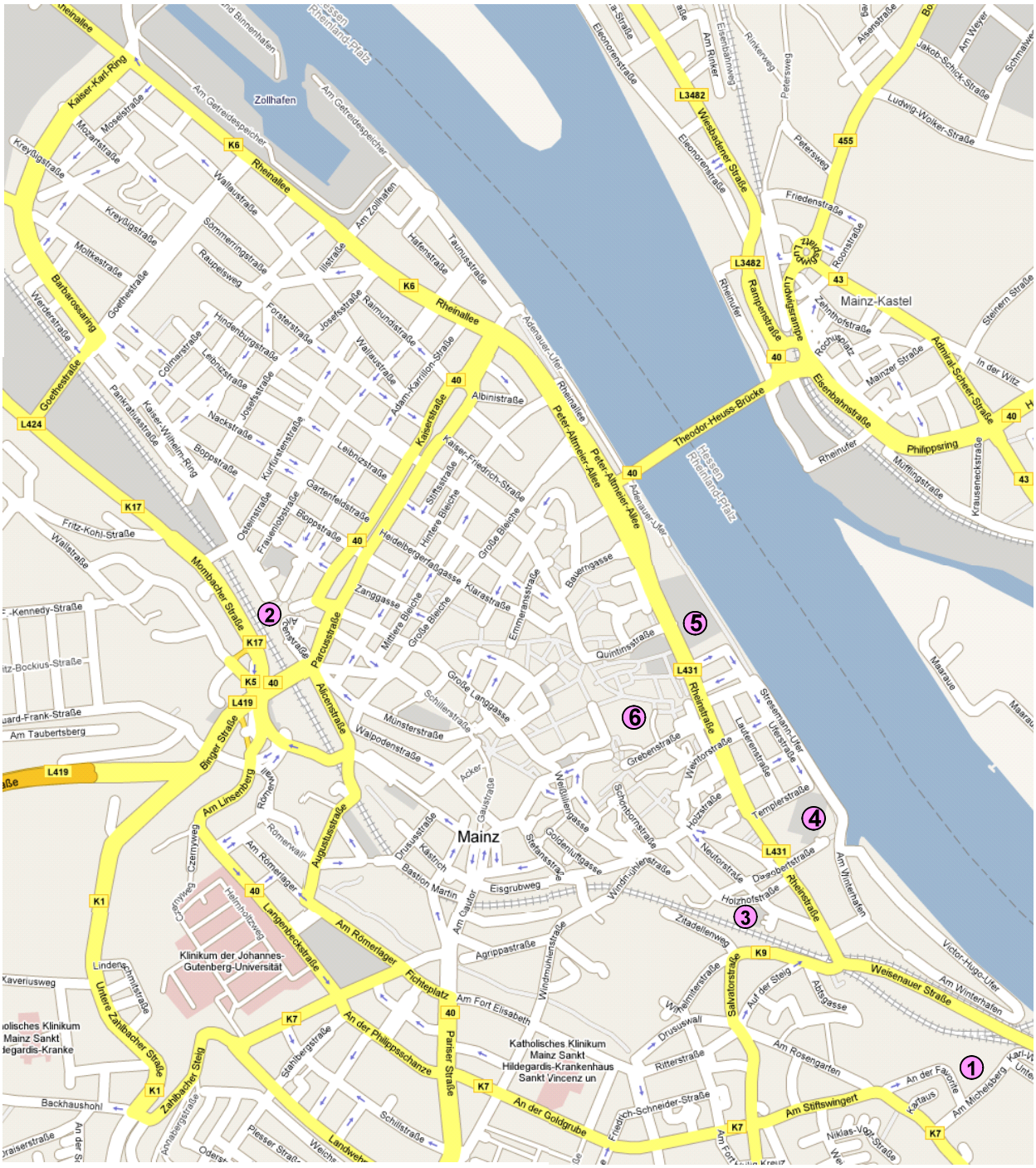
Tipping

Tipping is customary in Germany. The amount is flexible, for good service up to 10 % of the bill amount is usual.

Weather Conditions

Autumn clothing and sturdy footwear is recommended and it is advisable to carry an umbrella. The temperature in Mainz in October ranges from 8°C - 20°C and the weather can be quite changeable, with windy and rainy days to be expected. You should check www.magazine-germany.com/en/weather.html for current forecasts.

Map of Mainz



① Hotel Favorite
② Railwaystation „Mainz Hauptbahnhof“
③ Railwaystation „Mainz Süd“
④ Hotel Hyatt
⑤ Hotel Hilton, „Rheingoldhalle“, Cafè Rheinterassen
⑥ Gutenberg-Museum, Dom

Scientific Program

Sunday, 16th October

Favorite Park Hotel
Mainz
Karl-Weiser-Strasse 1
www.favorite-mainz.de

14.00-18.00 **Check-in, Registration**
18.00 **Warming up and Piano Concert by Susanne Lang (Basel)**
Welcome dinner

Monday, 17th October

Favorite Park Hotel, Lecture Hall

8.30 **Welcoming remarks**
Prof. Dr. Bernd Kaina (Mainz)
Prof. Dr. Xingzhi Xu (Beijing)
Prof. Dr. Ulrich Förstermann, Vice President of the University

Double-strand break repair

Chair: George Iliakis

9.00 – 9.30 **Markus Löbrich** (Darmstadt, Germany)
The limitations of radiation-induced cell cycle checkpoints

9.30 – 10.00 **Yun-Gui Yang** (Beijing, China)
Novel mechanisms of human holliday-junction resolvase GEN1 in maintaining genome stability

10.00 – 10.30 **Thilo Dörk-Bousset** (Hannover, Germany)
Role of ATM and Rad50 in DNA double strand break repair and human disease

10.30 – 11.00 Break

Chair: Wei Xiao

11.00 – 11.30 **Ekkehard Dikomey** (Hamburg, Germany)
Effect of EGFR on double-strand break repair

11.30 – 12.00 **Yulong Shen** (Jinan, China)
Functional studies on the archaeal RecA-like proteins RadC in the hyperthermophilic archaeon *Sulfolobus islandicus*

12.00 – 12.30 **Cong Liu** (Sichuan, China)
Requirement of genome integrity syste for embryonic neuronal development

12.30 – 14.30 **Lunch**

DSB Signaling

Chair: Alexander Bürkle

14.30 – 15.00 **Junjie Chen** (Houston, U.S.A.)
Beyond H2AX: What is next in DNA damage response?

15.00 – 15.30 **George Iliakis** (Essen, Germany)
Backup pathways of non-homologous end joining in genome maintenance and cancer formation

15.30 – 16.00 **Yuejin Hua** (Hangzhou, China)
DNA damage response in the extremely radioresistant bacterium Deinococcus radiodurans

16.00 – 16.30 **Xingzhi Xu** (Beijing, China)
Protein phosphatase PP6 in repair of DNA double-strand breaks

16.30 – 17.00 **Break**

Chair: Yun-Gui Yang

17.00 – 17.30 **Andrea Hartwig** (Karlsruhe, Germany)
Impact of toxic metal compounds and essential trace elements on the cellular response to DNA damage

17.30 – 18.00 **Tanja Schwerdtle** (Münster, Germany)
Disturbance of DNA repair and DNA damage-triggered signaling processes in metal species induced carcinogenicity and neurotoxicity

18.00 – 18.30 **Detlev Schindler** (Würzburg, Germany)
The role of the Fanconi anemia/breast cancer pathway in the caretaker network preserving genomic stability

18.30 – 19.00 **Lisa Wiesmüller** (Ulm, Germany)
Functional characterization of clinically relevant mutations in breast cancer susceptibility genes

20.00 **Dinner**

Tuesday, 18th October

Repair and Signaling

Chair: Zhao-Qi Wang

8.30 – 9.00 **Jochen Dahm-Daphi** (Marburg, Germany)
miR421-mediated ATM-depletion leads to a pronounced DNA repair defect: A novel mechanism for clinical tumor radiosensitivity

9.00 – 9.30 **Holger Bastians** (Göttingen, Germany)
A new role fort he *CHK2-BRCA1* tumor suppressor pathway required fort he maintenance of chromosomal stability in human matic cells

9.30 – 10.00 **Wei Xiao** (Beijing, China)
Unexpected mitotic functions of Rev3, the catalytic subunit of DNA polymerase zeta

10.00 – 10.30 **Gerhard Fritz** (Düsseldorf, Germany)
Impact of lipid-lowering drugs on DNA damage response and DNA repair

10.30 – 11.00 **Break**

Chair: Lisa Wiesmüller

11.00 – 11.30 **Vilhelm A. Bohr** (Baltimore, U.S.A.)
Premature aging proteins involved in nuclear and mitochondrial DNA repair

11.30 – 12.00 **Björn Schumacher** (Cologne, Germany)
DNA damage responses in development and aging

12.00 – 12.30 **Wei-Min Tong** (Beijing, China)
The role of DNA damage response in neurons

12.30 – 13.00 **Zhao-Qi Wang** (Jena, Germany)
Differential function of ATR and NBS1 in neuropathology

13.00 – 14.00 **Lunch**

Base excision repair and inflammation

Chair: Ekkehard Dikomey

14.00 – 14.30 **Grigory L. Dianov** (Oxford, U.K.)
Base excision repair targets for cancer therapy

14.30 – 15.00 **Ulrich Hübscher** (Zürich, Swizerland)
Oxygen as a friend and enemy: how to combat the mutational potential of 8-oxo-guanine

15.00 – 15.30 **Bernd Kaina** (Mainz, Germany)
The O6-alkylguanine response: implications for cancer formation and therapy

15.30 – 16.00 **Break**

Chair: Haiying Hang

16.00 – 16.30 **Alexander Bürkle** (Konstanz, Germany)
Poly(ADP-Ribosyl)ation and Ageing in Mammals

16.30 – 17.00 **Frank Grosse** (Jena, Germany)
Structural and functional characterization of the N-terminus of the human DNA helicase RecQL4

17.00 – 17.30 **Jun Huang** (Hangzhou, China)
hSWS-PASS1 is an evolutionarily conserved complex required for efficient homologous recombination repair

17.30 – 18.00 **Christof Niehrs** (Heidelberg, Germany)
Role of nucleotide excision repair in Gadd45 mediated DNA demethylation

18.00 **Gathering for group picture**

18.30 – 22.30 **Social event: Wine tasting excursion and Dinner**

Wednesday, 19th October

Chemotherapy and Regulation of DNA Repair

Chair: Markus Löbrich

8.30 – 9.00	**Stefan Jentsch** (München, Germany) Regulation of double-strand break repair and homology search
9.00 – 9.30	**Tao Jiang** (Beijing, China) Crystal structure of the rad9-hus1-rad1 cell cycle checkpoint complex
9.30 – 10.00	**Jürgen Thomale** (Essen, Germany) Formation and repair of Cisplatin-induced DNA damage: analyzing the hallmarks for drug resistance and side effects in vivo.
10.00 – 10.30	**Caroline Kisker** (Würzburg, Germany) Towards damage verification in nucleotide excision repair
10.30 – 11.00	**Oliver Krämer** (Jena, Germany) NFκB/p53 crosstalk — a new therapeutic target linked to replicational stress and DNA repair signaling
11.00 – 11.30	**Break**

Chair: Xingzhi Xu

11.30 – 12.00	**Chuanmao Zhang** (Beijing, China) Coordination of DNA replication initiation with centrosome replication, mitosis and RNA transcription
12.00 – 12.30	**Jin-Qiu Zhou** (Shanghai, China) The effect of G-quadruplex structure on telomere replication and recombination
12.30 – 13.00	**Steffen Emmert** (Göttingen, Germany) Cyclosporin A, but not everolimus, inhibits nucleotide excision repair via differential regulation of xeroderma pigmentosum proteins which is mediated by calcineurin: Implications for tumorigenesis under immunosuppression
13.00 – 13.30	**Zhou Songyang** (Guangzhou, China) The role of telomeric proteins in telomere maintenance and human diseases
13.30 – 15.00	**Lunch**

DNA Repair: Epigenetics and Immunology

Chair: Tanja Schwerdtle

15.00 – 15.30	**Bernd Epe** (Mainz, Germany) Influence of oxidative stress on DNA repair
15.30 – 16.00	**Guoliang Xu** (Shanghai, China) Oxidation of DNA methylcytosines in epigenetic regulation
16.00 – 16.30	**Odilia Popanda** (Heidelberg, Germany) An epigenetic screen of human DNA repair genes in head and neck squamous cell carcinoma: aberrant promoter methylation of NEIL1
16.30 – 17.00	**Jörn Walter** (Saarbrücken, Germany) Mechanisms of epigenetic reprogramming in the mouse zygote
17.00 – 17.30	**Coffee Break**
17.30 – 18.00	**Haiying Hang** (Beijing, China) The function of Rad9 in the antibody generation
18.00 – 18.30	**Weiguo Zhu** (Beijing, China) Class III HDAC and functions in cancer research
18.30 – 19.00	**Zhao Miaogen** (Sino-German Center, Bonn and Beijing) The Sino-German Center: Science support in China and Germany
19.00 – 20.00	**Summing up of the Meeting and Final Discussion**
20.00	**Dinner**

Thursday, 20th October

Excursion

Double-Strand Break Repair

Chairs: George Iliakis and Wei Xiao

Monday, 17 October
9.00-12.30

The limitations of radiation-induced cell cycle checkpoints

Markus Löbrich

Radiation Biology and DNA Repair, Darmstadt University of Technology
Schnittspahnstr. 13, D-64287 Darmstadt, Germany
(lobrich@bio.tu-darmstadt.de)

The DNA damage response pathways involve processes of double-strand break (DSB) repair and cell cycle checkpoint control to prevent or limit entry into S phase or mitosis in the presence of unrepaired damage. Checkpoints can function to permanently remove damaged cells from the actively proliferating population but can also halt the cell cycle temporarily to provide time for the repair of DSBs. Although efficient in their ability to limit genomic instability, checkpoints are not foolproof but carry inherent limitations. Recent work has demonstrated that the G1/S checkpoint is slowly activated and allows cells to enter S phase in the presence of unrepaired DSBs for about 4-6 h post irradiation. During this time only a slowing but not abolition of S-phase entry is observed. The G2/M checkpoint, in contrast, is quickly activated but only responds to a level of 10-20 DSBs such that cells with a low number of DSBs do not initiate the checkpoint or terminate arrest before repair is complete. At the time of release from the G2 checkpoint, cells have started but not completed DSB repair by homologous recombination (HR). Such cells are able to progress through mitosis but exhibit elevated damage levels in G1 due to de novo DNA breakage generated during mitosis. We suggest that HR-intermediates between sister chromatids combined with a negligent G2 checkpoint response leads to formation of additional DSBs in mitosis, an issue that may have hitherto unappreciated bearings for the impact of IR and other clastogens on genome integrity and cell survival. Here, I discuss the cellular consequences of the limitations in checkpoint control and DSB repair.

This work is supported by the DFG and BMBF/BMU.

References: Deckbar et al. (2007) J. Cell Biol. 176, 749-55; Krempler et al. (2007) Cell Cycle 6, 1682-86; Deckbar et al. (2010) Cancer Res. 70, 4412-21; Deckbar et al. (2011) Crit. Rev. Biochem. Mol. Biol. 46, 271-83.

Biography
Markus Löbrich obtained his Ph.D. in Biophysics in 1993 from the University of Gießen, Germany. He performed his postdoctoral training at the Lawrence Berkeley National Laboratory in Berkeley, CA, USA and continued his studies on double strand break repair as an Assistant Professor at the Physics Department of the University of Gießen and as an Associate Professor at the Medical Faculty of the Saarland University in Homburg. Since 2007, he is full Professor at the Biology Department of the Darmstadt University of Technology. His research program focuses on elucidating the mechanisms of double strand break repair and cell cycle checkpoint control after ionizing irradiation. Employing various cellular and molecular approaches, his primary research interest is centered on understanding the effects of low radiation doses.

Novel Mechanisms of Human Holliday-Junction Resolvase GEN1 in maintaining genome stability

Yun-Gui Yang

Genome Structure & Stability Group, Disease Genomics & Individualized Medicine Lab, Beijing Institute of Genomics, Chinese Academy of Sciences, No.7 Beitucheng West Road, Chaoyang District, Beijing 100029, PR China
ygyang@big.ac.cn

DNA double strand break (DSB) can be repaired by homologous recombination (HR) pathway. In HR, the resolution of four-way intermediates, also known as Holliday Junctions is necessary for chromosome segregation. Human GEN1 has been biochemically identified as Holliday junction resolvase. Since then, enumerous efforts have been invested to identify its in vivo biological functions. However, the deletion of yen1 has no obvious DNA repair defect, but the depletion of the budding yeast yen1 in conjunction with mus81 leads to MMS hyper-sensitivity. C. elegans gen-1 mutants are defective in IR-induced cell cycle arrest and apoptosis. In addition, cGEN-1 promotes DNA damage signaling independently of ATR, separable from its role in DNA repair. Moreover, the depletion of GEN1 using RNAi approaches leads to a mild sensitivity to DNA damage reagents MMC and CPT in human cells. Furthermore, depletion of MUS81 and GEN1, or SLX4 and GEN1, from Bloom's syndrome cells results in severe chromosome abnormalities. GEN1 can compensate for both MUS81 and SLX4 absence. Multicellular eukaryotes defective in GEN1 display differential cellular responses upon DNA damage with the context to DNA repair and recombination, suggesting GEN-1 may involve in other genome stability maintenance mechanisms besides its redundancy in DNA DSB repair processes in vivo. We will discuss novel mechanisms of hGEN1 in maintaining genome stability recently identified in our lab in this Nucleic Acid Enzymes and Enzymes in Human Disease symposium.

Keywords: Holliday-Junction Resolvase, GEN1, DSB repair, Genome stability

Biography
Dr Yun-Gui Yang is currently a professor of Beijing Institute of Genomics, Chinese Academy of Sciences (CAS). Dr Yang received his B.S. degree in Microbiology from FuDan University in 1995 and Ph.D. degree in Biochemistry and Molecular Biology from Shanghai Research Center of Biotechnology, CAS, in 2000. He performed his postdoctoral research in the laboratories of Dr Zhao-Qi Wang (International Agency for Research on Cancer-IARC, 2000-2003) and Dr Tomas Lindahl (Clare Hall Laboratories, Cancer Research UK London Research Institute 2005-2008). He had served as a staff scientist at IARC from 2003-2005. His main research interest is to dissect the DNA damage response and DNA repair in mammals and their critical roles in suppressing tumorigenesis, using experimental approaches including biochemistry, molecular/cell biology, system biology and mouse models.

Role of ATM and Rad50 in DNA double strand break repair and human disease

Thilo Dörk

Gynaecology Research Unit, Hannover Medical School, Hannover.
Carl-Neuberg-Str. 1, D-30625 Hannover, Germany
(doerk.thilo@hannover.de)

DNA double strand breaks are sensed and tethered by the Mre11-Rad50-Nibrin (NBN) complex and activate the ataxia-telangiectasia mutated (ATM) kinase which subsequently phosphorylates several hundreds of target proteins to trigger the cellular DNA damage response. I will briefly review the human disorders associated with mutations in these four genes and discuss how the genetic dissection of autosomal recessive syndromes has advanced our understanding of fundamental mechanisms in radiation biology. It has turned out that the MRN complex is not only upstream of ATM in the activation process but that all three members of the MRN complex are also ATM substrates and appear to play some role in the downstream regulation of cell cycle, apoptosis or DNA repair. For example, Rad50 has recently been found to become phosphorylated by ATM on Ser635 and to subsequently regulate S-phase progression through its paralog SMC1. Human Rad50 deficiency has been associated with a Nijmegen-Breakage Syndrome-like phenotype, and monoallelic mutations of *RAD50* - like those in *ATM* or *NBN* - predispose females to breast cancer. There are differences, however, between Ataxia-telangiectasia- and Nijmegen Breakage Syndrome-like disorders that may indicate some divergent roles of the underlying gene products and remain to be explained.

References: Savitsky et al. (1995) Science 268:1749-53; Varon et al. (1998) Cell 93:467-76; Sandoval et al. (1999) Hum Mol Genet 8:69-79; Stewart et al. (1999) Cell 99:577-87; Waltes et al. (2009) Am J Hum Genet 84:605-16; Gatei et al. (2011) J Biol Chem, in press.

Biography
Dr. Dörk-Bousset obtained his Ph.D. in biochemistry in 1994 from the Leibniz University of Hannover, Germany. He completed his postdoctoral training at the Institute of Human Genetics at Hannover Medical School and obtained the qualification as a human geneticist (“Fachhumangenetiker”). From 2001 on he continued his studies on DNA double strand break repair syndromes at the Department of Obstetrics and Gynaecology and, since 2008, he heads the Gynaecology Research Unit at Hannover Medical School. His current research is aimed at elucidating the genetic basis of radiation sensitivity disorders and the genetic predispositions underlying common gynaecological malignancies.

Effect of EGFR on DNA double-strand break repair

Malte Kriegs, Ulla Kasten-Pisula, Jarob Saker, Laura Myllynen,
Ekkehard Dikomey

Laboratory of Radiobiology & Experimental Radiooncology,
University Medical Center Hamburg
Martinistr. 52, D-20246 Hamburg, Germany
(dikomey@uke.uni-hamburg.de)

The epidermal growth factor receptor (EGFR) regulates several highly important cellular signal pathways such as proliferation, differentiation, apoptosis as well as DNA repair. In tumors the *egfr* is often mutated or amplified, which is associated with poor prognosis and resistance to both chemo- and radiotherapy. It was shown by us for HNSCC cell lines that there is a huge variation in EGFR expression, which correlates with respective radiosensitivity. However, this correlation only exists for HNSCC with moderate (group A) but not for massive gen amplification (group B). For tumors with moderate gen amplification EGFR regulates DSB repair probably by controlling DNA PKcs, which is a key protein of NHEJ. In line with this stimulation or inhibition of EGFR was found to affect NHEJ as could be tested by using specific DSB repair substrates stably integrated into the genome. This regulation was independent of both p53 and KRas, which are often mutated in tumors. EGFR also affects homologous recombination as demonstrated via a specific repair substrate. These data suggest that EGFR may affect DSB repair by regulating specific DNA repair proteins but also by a more general mechanisms not known so far. Surprisingly inhibition of EGFR as achieved either by a specific antibody Cetuximab or by a specific inhibitor of the tyrosine kinase (erlotinib) does not strictly result in a depressed DSB repair and with that enhanced cellular radiosensitivity. For HNSCC cells this is found for only a minority of cell lines (1/10). This increased radiosensitivity appears to result from a depression of NHEJ as well as HR. For NHEJ this is due to a block of MAPK signaling. Direct inhibition of MAPK also results in a depressed DSB repair. In tumors with wt p53 radiosensitisation may also result from an elevated radiation-induced permanent G1 arrest and also from an enhanced premature senescence. These data demonstrate that although EGFR inhibition is already used in the clinics to treat cancer both as a monotherapy as well as in combination with radiotherapy, more information is needed especially to identify patients which actually will benefit from this new therapeutic approach.

Work was supported by DFG Di457 as well as BMBF.

References: Toulany et al. (2006) Clin Cancer Res 12, 4119-4126; Baumann et al. (2007) Radiother & Oncol 83, 238-248; Kriegs et al. (2010), DNA Repair 9, 889-897; Kasten-Pisula et al. (2011) Int J Radiat Oncol Biol Phys 4, 1181-1188; Myllynen et al. (2011), Radiother & Oncol epub.; Kriegs et al. (2011) Oncogene, subm.;

Biography
Dr. Dikomey obtained his Ph.D. in radiobiology in 1976 from the University Hamburg, Germany. He completed his postdoctoral training at the Institute of Biophysics and Radiobiology in Hamburg and continued his studies on DNA repair in respect to hyperthermic radiosensitisation as well as individual radiosensitivity. In 1996 he finished his habilitation on DNA repair and in 2002 he was elected as a professor of radiobiology at the Laboratory of Radiobiology & Experimental Radiooncology at the University of Hamburg. His research program is aimed at elucidating the mechanisms of DSB repair, biological targeting, genomic instability, individual radiosensitivity and tumor radiosensitivity. As part of translational research program, his group is involved in several studies on the mechanism of cell kill and radioresistance of HNSCC and genomic instability especially in breast cancer cells.

Functional studies on the archaeal RecA-like proteins RadC in the hyperthermophilic archaeon *Sulfolobus islandicus*

Yulong Shen

School of Life Sciences, Shandong University, Jinan,
Shanda Nan Rd. 27, 250100 Jinan, China
(yulgshen@sdu.edu.cn)

AAA+ ATPase superfamily is a group of proteins with diverse cellular functions, among which RecA family proteins play essential roles in DNA repair and maintenance of the genome stability. In higher eukaryotes, mutations of Rad51C, one of the parologs of Rad51 recombinase are found to be associated with deseases Fanconi amonia and breast or ovarian cancers. In archaea, a novel group RecA family proteins RadC, named after RadA and RadB, is found to be ubiquitas with one to four homologous in each archaeon. In the model organism of *Sulfolobus islandicus*, there are two RadC proteins RadC1 and RadC2 that are conserved in the genus. It has been reported that the RadC1 possesses DNA-binding and ssDNA-dependent ATPase activities. However, detailed characterization of the these proteins is limited and the physilogical roles of the proteins in the cell remain a mystery.

In this research, RadC1 and RadC2 from *Sulfolobus tokodaii* and *S. islandicus*, were studied. It was revealed that RadC was more similar to the N terminal of KaiC, a circadian rhythm protein of cyanobacteria than to the recombinases of archaea (RadA) or bacteria (RecA). RadC1 could form hexamer induced by ATP, ADP, or ATPγS. In the presence of substoichiometric ATP, StoRadC1 formed dimer and tetramer. Besides, RadC1 oligomer induced by ADP is looser than those induced by ATP and ATPγS by cross linking analysis. And the DNA-binding activity of RadC1 was significantly stimulated by ATP and ADP rather than ATPγS.

To analyze *in vivo* function of RadC1 and RadC2, *radC1* or *radC2* deletion strains (Δ*radC1*, Δ*radC2*) and the double deletion strain (Δ*radC1*Δ*radC2)* were constructed. The phenotypic analysis revealed that none of the two genes is essential for cell survial and their deletion does not apparently affect the growth of the strains. However, Δ*radC1* and Δ*radC1*Δ*radC2* showed increassed sensitivity to DNA damaging agents HU, MMS, and cisplatin. However, Δ*radC2* did not show any increased sensitivitive to any of the chemicals. Strangely, Δ*radC1* and Δ*radC1*Δ*radC2* did not show any increased sensitivity to high UV treatment but showed increased sensitivity to UV treatment at lower dosage. The results implicated that RadC1 rather than RadC2 is involved in HU MMS, cisplatin, and UV induced DNA repair pathways. Using shuttle vector pSeSD, we over-expressed His-tagged RadA, RadC1, and RadC2 *in vivo*. We also expressed His-RadC1 *in situ*, and putative binding proteins have been identified. Phenotypes and putative associated proteins of these strains will be presented .

Biography

Dr. Shen obtained his Ph.D. degree in molecular and cell biology in 1996 from University of Birmingham, UK. He completed his postdoctoral training at the Department of Citrus, Institute of Fruit Tree Sciences in Okitsu, Japan. In 1999 he began to study on DNA replication and repair in the hyperthermophilic archaea at the Bioinformatics Research Center, Institute of Advanced Science and Technology in Tsukuba, Japan. In 2003, he was appointed as a professor of Microbiology at the School of Life Sciences, Shandong University in Jinan, China. His research program is aimed at unraveling DNA damage and Repair mechanism in the hyperthermophilic crenarchaeon *Sulfolobus*. The research group is involved in studies on the characterization of proteins involved in homologous recombination repair and maintenance of genome integrity. The recently established genetic tools of *Sulfolobus* are currently being applied in order to analyze the *in vivo* functions of DNA repair genes and reveal the network of DNA damage signaling and DNA Repair in *Sulfolobus*.

Requirement of genome integrity system for embryonic neuronal development

Cong Liu, Penny Jeggo, Tony Carr
Sichuan University, Chengdu, 610041, PR China
Email: Congliu72@gmail.com

DNA damage, including double strand breaks (DSBs), is highly toxic to developing brains. Proper brain development relies on efficient detecting and repair pathways to maintain the viability of neural (stem) cells. Impairment of these genome surveillance pathways leads to defective neural development such as primary microcephaly. By taking advantage of a mutant mouse harbouring a hypomorphic form of DNA ligase IV (Lig4) that is essential for DSB repair, we found that post-mitotic compartment of intermediately zone/cortical plate (IZ/CP) is hypersensitive to persistent double strand breaks whereas proliferating ventricular zone (VZ) is predisposed to apoptosis following acute IR-induced damage. Via combined analysis of DNA breakage, apoptosis, and cell-cycle checkpoint control in tissues, we propose a novel model in which microcephaly in LIG4 syndrome arises from sensitive apoptotic induction from persisting DSBs in the IZ, which arise from high endogenous breakage in the VZ/SVZ and transit of damaged cells to the IZ. In addition, we invented a conditional knock-in strategy to generate an mutant form of 9-1-1 protein under the control of a cre-lox system that leads to the perturbation of replicative checkpoint and consequently causes deformed brain. These observations provide further insights into the roles of DNA integrity system in proper development of the central nervous system.

Biography
Professor Cong Liu principle investigator of Developmental and Stem Cell Institute, West China Second University hospital, Sichuan University. He obtained his PhD from Sussex University and completed the postdoctoral training with Prof Tony Carr and Penny Jeggo in the MRC/Genome Damage and Stability Centre. Once being a genetist, he worked on fission yeast to investigate the molecular mechanism of checkpoint activation and cell cycle progression. Later he joined the European Framework Project to study genome instability diseases by establishing mouse models defective in DNA structural metabolism. By using various model systems (ie. transgenic mice and yeast) and clinical materials, his lab of genome stability in Sichuan University studies the maintenance of genomic integrity and relevant human diseases resulting from disturbing of the signaling network of DNA damage responses. Their work has shed lights on the pivotal roles of DNA repair in neural development, tumorigenesis and radio-/chemo-therapy.

DSB Signaling

Chairs: Alexander Bürkle and Yun-Gui Yang

Monday, 17 October
14.30-19.00

Beyond H2AX: What is next in DNA damage response?

Junjie Chen

Department of Experimental Radiation Oncology, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030, USA
(jchen8@mdanderson.org)

The ability to sense DNA damage and activate responsive pathways that coordinate cell cycle progression and DNA repair is critically important for the maintenance of genomic stability and tumor suppression. ATM and ATR are two key upstream regulators involved in DNA damage response. They phosphorylate many substrates including H2AX and control multiple aspects of the DNA damage responsive pathways. A recent development in the field is the discovery of ubiquitination-dependent signaling transduction pathway initiated by RNF8, which works hand in hand with ATM/ATR-dependent phosphorylation events and contributes to the accumulation of many DNA damage and repair proteins near DNA damage sites. We are now investigating how protein phosphorylation and protein ubiquitination are regulated at sites of DNA damage and how these post-translation modifications participate in DNA repair.

Although the well-studied H2AX/MDC1/RNF8 pathway contributes significantly to DNA damage signaling and DNA repair, we and others have demonstrated the existence of H2AX-independent pathway or pathways that are also involved in DNA damage repair. We are studying the regulation of homologous recombination (HR) repair pathways in mammalian cells. In particular, we identified several new components involved in HR repair. These new data will be presented at the meeting.

Biography
Junjie Chen received B.S. degree from Fudan University in China in 1988 and Ph.D. degree from the University of Vermont in 1993. After finished postdoctoral trainings with Drs. Anindya Dutta and David Livingston at Harvard Medical School, he joined Mayo Clinic in 1999 and became a tenured associate professor in 2003. He moved to Yale University in 2006 as a full professor at the Department of Therapeutic Radiology. In 2009, he moved to the University of Texas MD Anderson Cancer Center and serves as the professor and chair of the Department of experimental Radiation Oncology. His group demonstrated that the BRCA1 C-terminal (BRCT) motifs are phospho-protein binding domains. This phospho-peptide binding activity of BRCT domains is not only important for BRCA1 functions, but also critical for many other BRCT domain-containing proteins involved in DNA damage and/or other cellular pathways. In addition, his group discovered several key DNA damage checkpoint proteins and demonstrated that the proper DNA damage response depends not only on protein kinases but also on a group of mediator proteins that facilitate DNA damage signal transduction.

Backup Pathways of Non-Homologous End Joining in Genome Maintenance and Cancer Formation

George Iliakis

Institute of Medical Radiation Biology, University of Duisburg-Essen, Medical School, Hufelandstr. 55, D-45122 Essen, Germany
(georg.iliakis@uk-essen.de)

In higher eukaryotes DNA double strand breaks (DSBs) are repaired by homologous recombination (HRR) or non-homologous end joining (NHEJ). Whereas HRR depends on genes of the Rad52 epistasis group and requires extensive homology with an undamaged DNA segment, NHEJ in its classical form, utilizes the DNA-PKcs/Ku complex for signaling and regulation and the DNA ligase IV/XRCC4/XLF complex for end ligation. This form of DSB repair is very efficient, requires no homology in the vicinity of the DSB, or elsewhere in the genome, and is assisted by various nucleases and end-modifying activities that generate ligatable ends. This pathway of NHEJ is dominant over HRR in cells of higher eukaryotes and we have proposed to term it D-NHEJ to indicate its requirement for DNA-PK. The parameters determining selection between HRR and D-NHEJ remain unknown. To contribute to the elucidation of this question, we initiated over ten years ago genetic experiments designed to quantify possible modulation of HRR under conditions compromising D-NHEJ. To our surprise we observed that cells defective in D-NHEJ repair the majority of DSBs not via HRR, as we had expected, but using an alternative pathway of NHEJ which is normally suppressed by D-NHEJ and which was functioning under these conditions as a backup (B-NHEJ). B-NHEJ shows strong dependence on growth state as well as throughout the cell cycle. The function of alternative pathways operating as backup is now widely demonstrated not only for genomic stability but also for class switch and V(D)J recombination in B and T cells. B-NHEJ seems to be error-prone and is therefore directly implicated in carcinogenesis. I will review genetic and biochemical experiments indicating that B-NHEJ utilizes DNA ligase III and possibly also PARP-1. In addition, I will outline the functions of other proteins, directly or indirectly implicated in B-NHEJ. Finally, I will briefly review the daily expanding literature demonstrating a role for B-NHEJ under a variety of conditions and for different cellular activities. Work supported by grants from ESA-AO-08-IBER (BMWWi-50WB0929) and the BMBF (02NUK001B and 02NUK005C).

References: DiBiase et al Cancer Res. 60, 1245-1253, 2000. Wang et al, Oncogene 20, 2212-2224, 2001, Wang et al Nucleic Acids Res 31, 5377-5388, 2003. Iliakis et al Cytogenet Genome Res 104, 14-20, 2004. Perrault et al, J. Cell Biochem 92, 781-794, 2004. Wang et al, Cancer Res 65, 4020-4030, 2005. Wang et al Nucleic Acids Res 43, 6170-6182, 2006. Windhofer et al Int J Radiat Oncol Biol Phys 68, 1462-1470, 2007. Iliakis et al in: Chromosomal Aberrations, Obe and Vijayalaxmi (Eds), Springer-Verlag Berlin, 67-85, 2007. Wu et al, DNA Repair 7, 329-338, 2008. Rosidi et al, Nucleic Acids Res 36, 1610-1623, 2008. Wu et al, Radiat Res 170, 512-520, 2008. Iliakis, Radiother Oncol 92, 389-397, 2010. Singh et al, Int J Radit Oncol Biol Phys 79, 540-548, 2011.

Biography
Dr. Iliakis obtained his Ph.D. in Biophysics, 1978, from the University of Frankfurt/Main, Germany. He continued with postdoctoral training till 1983 at the Institute of Biophysics, University of Frankfurt/Main studying repair of DNA DSBs in mammalian cells after exposure to ionizing radiation. In 1983, he moved to the USA joining first the Research Division of the Cleveland Clinic Foundation, Cleveland, OH, and then the Department of Radiation Oncology, Thomas Jefferson University, Philadelphia, PA, where he eventually became Professor and Director of the Division of Experimental Radiation Oncology. In 2001, he returned to Germany as Professor and Director of the Institute of Medical Radiation Biology, University of Duisburg-Essen, Medical School. His research interests focus on the elucidation and regulation of pathways involved in the repair of DNA DSBs and their connection to checkpoint response.

DNA Damage Response Mechanisms of *Deinococcus radiodurans*

Yuejin Hua

Institute of Nuclear-Agricultural Sciences, Zhejiang University,
Kaixuan Road 268, Hangzhou, 310029, China
(yjhua@zju.edu.cn)

The extremely radioresistant bacterium *Deinococcus radiodurans* can survive acute gamma radiation at a dose of 20 kGy and the super radioresistance benefits from the rapid DNA damage response, efficient DNA repair ability and strong antioxidant system. The organism can mobilize one-third of its genome to survive lethal radiation damage with the rapid and efficient DNA damage response system. However, the details of the mechanism are poorly known. To get valuable clues, we investigated global response of *D. radiodurans* after radiation damage, including transcription profiles, proteomes, and phosphoproteomes. Our proteomic assay revealed that expression of over 60 proteins were enhanced after radiation, 25% of which are the proteins involved in DNA metabolism, including DNA repair proteins RecA, PprA, SSB. Four proteins involved in stress response, posttranslational modification ion metabolism, and nucleotide metabolism were evidenced their involvements in post-irradiation recovery by investigating their knockout mutants' survival ability with radiation stress. These results suggest that the expressional induction of radiation responsive genes is an important competent in DNA damage response. The microarray assay showed that the process of DNA repair was induced in *D. radiodurans* in order, i.e. genes involved in base excision repair, nucleotide excision repair, and single-strand annealing were induced immediately after ionizing radiation, and genes for recombination repair, including *recA*, *recD* and *recQ* were activated later. Especially, *recD* and *recQ* were specifically induced at low dose irradiation, and this phenomenon informed us that these two genes would play a role in anti-oxidation. Some genes such as ddrA and ssb were activated during the whole repair phase. Furthermore, many anti-oxidative genes were induced to scavenge reactive oxygen species directly, other associated systems also changed their expression patterns during the recovery time, such as iron metabolism systems, intracellular mutagenic precursors sanitize systems. These characteristics indicate that there is a powerful and orderly recovery process in *D. radiodurans*. We found that the phosphoproteomic alteration happened in *D. radiodurans* after radiation. We identified 101 phosphosites in 76 proteins under normal growth conditions and 98 phosphosites in 58 proteins after radiation, which were confirmed by western blotting. Interestingly, only 10 proteins were identified in the both situations, indicating that phosphorylation is involved in the cellular response to radiation. Taken together, *D. radiodurans* processes a comprehensive DNA damage response mechanism to program the process of DNA repair and cellular survival after radiation damage.

References: Cox and Battista (2005) *Nat Rev Microbiol*. 8, 882-892; Zhang *et al*. (2005) *Proteomics*, 5, 138–143; Chen et al. (2007) *PNS*, 17, 529-536; Wang *et al*. (2008) *Mol. Microbiol.* 67,1211-1222; Lu *et al*. (2009) *Mol. Cell. Proteomics*, 8, 481-494; Lu and Hua, unpublished; Wang and Hua, unpublished.

Biography
Dr. Hua obtained his Ph.D. in Human Biology in 1994 from the Medical School of Philipps-University Marburg, Germany. He completed his post-doctoral research in 1999 in the National Institute of Agricultural and Biological Resources, Japan as a special researcher of Japanese Science and Technology Agency (1995-1999).In 1999, he was elected as a professor of Biophysics at College of Agriculture and Biotechnology of Zhejiang University, China. In 2005, he was elected as a Changjiang Endowed Professor. His research group aims at elucidating the mechanisms of DNA damage response and repair, regulation of DNA damage response and repair genes. Besides, his group also works on mechanisms of antioxidantion and explores natural antioxidant materials.

Protein phosphatase PP6 in repair of DNA double-strand breaks

Xingzhi (Xavier) Xu

Beijing Key Laboratory of DNA Damage Response, College of Life Sciences, Capital Normal University, Beijing 100048, China
(Xingzhi_Xu@mail.cnu.edu.cn)

Reversible protein phosphorylation controls most, if not all, cellular activities, including repair of DNA double-strand breaks (DSBs). DSBs are among the most lethal lesions associated with genome stability which, when destabilized, predisposes organs to cancers. DSBs are primarily fixed either with little fidelity by non-homologous end joining (NHEJ) repair or with high fidelity by homology-directed repair (HDR). The phosphorylated form of H2AX on serine 139 (g-H2AX) is a marker of DSBs. In this study, we explored if the protein phosphatase PP6 is involved in DSB repair by depletion of its expression in human cancer cell lines, and determined PP6 expression in human breast cancer tissues by immunohistochemistry staining. We found that bacterially-produced PP6c (the catalytic subunit of PP6)-containing heterotrimeric combinations exhibit phosphatase activity against g-H2AX in the in vitro phosphatase assays. Depletion of PP6c or PP6R2 led to persistent high levels of g-H2AX after DNA damage and defects both in HDR and NHEJ. Chromatin immunoprecipitation assays demonstrated that PP6c was recruited to the region adjacent to the DSB sites. Expression of PP6c, PP6R2, and PP6R3 in human breast tumors was significantly lower than those in benign breast diseases. Taken together, our results suggest that g-H2AX is a physiological substrate of PP6, and PP6 is required for HDR and NHEJ and its expression may harbor a protective role during the development of breast cancer. Molecular mechanisms of involvement of PP6 in DSB repair is under investigation.

Biography
Dr. Xingzhi Xu obtained his bachelor of medicine in 1992 from the Shanghai Medical University, China and his PhD in 1999 from the University of South Carolina School of Medicine, USA. He completed his postdoctoral training on DNA damage response (DDR) with Dr. David Stern at the Yale Medical School in 2004 and continued his study on DDR at the City of Hope National Medical Center/Beckman Research Institute as a Beckman Fellow till 2006. He became a professor and a PI at the Capital Normal University, Beijing in 2006 and director of the Beijing Key Laboratory of DNA Damage Responses in 2011. His research focuses on molecular mechanisms regulating the reversible phosphorylation mediated by protein kinases and phosphatases in DDR, and Serine/Threonine protein phosphatases in particular.

Impact of toxic metal compounds and essential trace elements on the cellular response to DNA damage

Andrea Hartwig, Claudia Keil and Sarah F. Risnes

Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Karlsruhe,
Kaiserstr. 12, D – 76131 Karlsruhe, Germany
(Andrea.Hartwig@kit.edu)

Interactions with different DNA repair processes such as nucleotide excision repair (NER) and base excision repair (BER) have been identified as major mechanisms in metal-induced genotoxicity. One example is cadmium, which has been shown to disturb nucleotide excision repair, base excision repair and mismatch repair. For example, water soluble and particulate compounds inhibit the removal of bulky DNA adducts induced by benzo[a]pyrene diolepoxide, UVC-induced photoproducts as well as oxidative DNA base modifications recognized by the bacterial formamidopyrimidine DNA glycosylase (Fpg). Particularly sensitive targets appear to be proteins with zinc binding structures, present in DNA repair proteins such as XPA, PARP-1 as well as in the tumor suppressor protein p53. With respect to the latter, water soluble as well as particulate cadmium compounds provoke an unfolding of the “wild type” conformation into a so-called “mutant” form, leading to diminished expression of DNA repair proteins, which may – in addition to the inhibition of specific DNA repair proteins - explain for example the disturbance of NER. Cadmium also inhibits poly(ADP-ribosyl)ation, and detailed investigations suggest a direct interaction with PARP-1, presumably by inactivation of thiol groups. Finally, the unfolding of p53 diminishes apoptosis induced by sodium selenite and thus provokes resistance towards DNA-damaging agents. Particularly the combination of these multiple mechanisms may give rise to a high degree of genomic instability in cadmium-adapted cells, relevant not only for tumor initiation, but also for later steps in tumor development. Nevertheless, interactions with zinc binding proteins are not restricted to toxic metal compounds but may also occur in case of essential trace elements on conditions of celluar overload; examples are copper and reducible selenium species. Altogether, intact cellular zinc homeostasis ensures genomic stability but may be disturbed by toxic metal ions and certain micronutrient overload.

The work was supported by the Deutsche Forschungsgemeinschaft (DFG).

Selected references: Hartwig, 2010, Biometals 23, 951 – 960; Schwerdtle et al., 2010, Chem. Res. Toxicol. 23, 432 -442; Hamann et al., 2009, Mutation Research 669, 120 -130; Schwerdtle et al., 2007, DNA Repair, 6, 61 – 70.

Biography
Andrea Hartwig received her Diploma in Chemistry and her Habilitation in Biochemistry in Bremen. Since 2010 she is Chair of Food Chemistry and Toxicology at the Karlsruhe Institute of Technology (KIT). The main research area focuses on the impact of carcinogenic metal compounds, essential trace elements and bioactive food ingredients on genomic stability, with special emphasis on DNA damage induction and effects on DNA repair, gene expression, cell cycle control and tumor suppressor functions. Special attention is given to interactions with zinc binding proteins, including their redox regulation. Further research focuses on the toxicology of nanomaterials. Besides laboratory research, she is actively involved in risk assessment concerning exposures at workplaces and via food. She is chair of the “Deutsche Gesellschaft für DNA-Reparatur” (DGDR) and President of the German MAK Commission.

Disturbance of DNA repair and DNA damage-triggered signalling processes in metal species induced carcinogenicity and neurotoxicity

Tanja Schwerdtle, Julia Bornhorst, Franziska Ebert

Institute of Food Chemistry, University of Muenster, Muenster,
Corrensstrasse 45, D-48149 Muenster, Germany
(tanja.schwerdtle@uni-muenster.de)

Although inorganic arsenic (iAs) induced carcinogenicity as well as manganese (Mn) and mercury (Hg) species induced neurotoxicity are well documented, so far modes of actions are not understood.
Concerning arsenic, at exposure relevant concentrations inorganic arsenic and its metabolites are neither directly DNA reactive nor mutagenic. Thus, more likely epigenetic and indirect genotoxic effects, among others a modulation of the cellular DNA damage response and DNA repair, are important molecular mechanisms contributing to its carcinogenicity. Our *in vitro* data demonstrate a strong NER and BER inhibition, with arsenite and its metabolites affecting both common and different molecular key players. Thus, trivalent methylated and sulphur-containing arsenicals especially disturb NER damage recognition. Cellular activity of the BER 8-oxoguanine DNA glycosylase was most sensitively affected by a pentavalent dimethylated metabolite, the cellular level of LIGIIIα by arsenite and the cellular amount of XRCC1 by a pentavalent monomethylated metabolite, with significant effects starting at ≥ 3.2 µM cellular arsenic. Additionally a sulphur-containing metabolite strongly disturbed cell cycle progression.
Regarding manganese our data indicate that after Mn(II) overexposure, manganese easily reaches the brain in the first line via the blood-CSF-barrier. As mechanistic hint for its neurotoxic mode of action we observed a strong inhibition of H_2O_2-stimulated poly(ADP-ribosyl)ation at low micromolar, exposure relevant Mn concentrations in human astrocytes, which was neither due to a downregulation of *PARP-1* nor a misregulation of the cellular energy related nucleotides.
Very recently in human astrocytes we also found an inhibition of H_2O_2 stimulated poly(ADP-ribosyl)ation by low nano- to micromolar concentrations of inorganic $HgCl_2$ and thiomersal, which is still used as a preservative in many vaccines throughout the world. These effects might be due to the affinity of Hg species towards thiol groups. Moreover, thiomersal additionally strongly affected cell cycle progression.
In summary our data point out that after mixed arsenic species exposure, a realistic scenario after oral inorganic arsenic intake and subsequent metabolism in humans, most likely NER and BER will be affected by different mechanisms and therefore very effectively, which might facilitate the carcinogenic process of inorganic arsenic. With respect to manganese and mercury the observed inhibition of H_2O_2 stimulated poly(ADP-ribosyl)ation gives notice to a disturbance of the response to DNA damage in brain cells, which has been shown before to result in neurological diseases.

References: Nollen et al. (2009), Mol Nutr. Food Res, 53, 572-82; Bornhorst et al. (2010), J Environ Monit 12, 2062-9; Janzen et al. (2011), Metallomics, 3, 847-52; Ebert et al. (2011) Mutat Res, in press; Bartel et al (2011), J. Toxicol, in press.

Biography
Dr. Schwerdtle obtained her Ph.D. in Food Chemistry in 2002 from the University of Karlsruhe, Germany. She completed her postdoctoral training at the Institute of Food Chemistry at the Technical University in Berlin, and worked as guest scientist in the team of Dr. Dianov at the Medical Research Council Harwell (UK) as well as in the team of Dr. Mullenders at the Leiden University Medical Center (The Netherlands). Since 2006 she is a board member of the German Society for Research on DNA Repair. In 2008 she was elected as full professor at the Institute of Food Chemistry at the University of Muenster (Germany). Her research program is aimed at elucidating the molecular mechanisms of metal species induced carcinogenicity and neurotoxicity, thereby especially focusing on the impact of metal species on DNA repair and DNA damage response pathways.

The role of the Fanconi anemia/breast cancer pathway in the caretaker network preserving genomic stability

Detlev Schindler

Department of Human Genetics, University of Wuerzburg,
Biozentrum, Am Hubland, D-97074 Wuerzburg, Germany
(schindler@biozentrum.uni-wuerzburg.de)

Fanconi anemia (FA) is a DNA repair disorder characterized by profound sensitivity to agents that produce DNA interstrand cross-links (ICL). To date, 15 complementation groups (FA-A, B, C, D1, D2, E, F, G, I, J, L, M, N, O and P) have emerged. They result from biallelic mutations in each one of 15 corresponding genes [*FANCA, B, C, D1 (BRCA2), D2, E, F, G, I, J (BACH1/BRIP1), L, M, N (PALB2), P (SLX4/BTCD12)*, and *O (RAD51C)*]. It is believed that all of the 15 FA gene products cooperate in a common pathway and that cellular resistance to DNA interstrand cross-linking agents requires all 15 FA proteins. The current concept of the FA/BRCA pathway features a conserved DNA-remodeling complex, including FANCM, FAAP24 and the histone-fold heterodimer MHF1 and MHF2, which scans replicating DNA. Once it encounters a fork-stalling lesion, it stimulates assembly of the FA nuclear core complex (FANCA, -B, -C, -E, -F, -G, and -L), a large multi-subunit ubiquitin E3 ligase. A major function of the FA core complex is to mono-ubiquitylate its two substrates, FANCD2 and FANCI, a reversible regulatory modification, and to recruit them to chromatin. Multiple phosphorylation events also contribute to the pathway activation. FANCD2 and FANCI form a protein complex called the ID complex that assembles at the sites of DNA damage, where it subsequently colocalizes with downstream effector FA proteins. Removal of the ICL is achieved by opening of the DNA (FANCJ) and by the coordinated action of structure-specific endonucleases (FANCP). The gap is filled in by DNA polymerases of low specificity to result in translesion synthesis. Resorting to the correct nucleotide at that position requires the involvement of the other chromosome by the proteins involved in the process of homologous recombination, including FANCD1, FANCN and FANCO. Monoallelic mutations of many of the downstream FA proteins predispose to breast and ovarian cancer. Identification of FA genes and discovery of their functional interconnections paved the way to our yet incomplete understanding of the mechanisms of the FA/BRCA pathway in the maintenance of genomic integrity.

Biography
Detlev Schindler is director of the Division of Somatic Cell Genetics and head of the Laboratory for Genomic Instability at the Dept. of Human Genetics within the Biocenter of the University of Wuerzburg, Germany. After graduation as a physician from the University of Hannover School of Medicine he became a medical fellow and later intern at the Dept. of Pediatrics at the University of Wuerzburg from where he earned his MD. Detlev Schindler underwent full Pediatric and Human Genetics training and received board certifications in both disciplines. During 1987 and 1997 he spent several research terms at the Dept. of Genetics and Genome Sciences of the Mount Sinai School of Medicine, NYU, where he also served as an invited Visiting Professor after he had become lecturer at the University of Wuerzburg in 1988. In 2003 he evolved as a Full Professor of Human Genetics. His main research interests include biochemical genetics and metabolic diseases as well as genomic instability, DNA repair and related disorders. During the last years his group was involved in the identification of several disease-causing genes and the elucidation of their pathogenic mechanisms at the molecular level. He authored and co-authored more than 150 publications in peer-reviewed journals. His work received several national and international awards.

Functional Characterization of Clinically Relevant Mutations in Breast Cancer Susceptibility Genes

Marlen Keimling[1], Meta Volcic[1], Andreea Csernok[1], Britta Wieland[2], Thilo Dörk[2], and Lisa Wiesmüller[1]

[1] Department of Obstetrics and Gynecology, Ulm University, Prittwitzstr. 43, D-89075 Ulm, Germany (lisa.wiesmueller @uni-ulm.de)
[2] Gynecology Research Unit, Hannover Medical School, Germany

Breast cancer occurs at the highest frequency and is the second leading cause of cancer mortality in western women. Already eleven high to moderate penetrance breast cancer susceptibility genes have been identified as components of DNA double strand break repair (DSBR). ATM is known to exert a broad influence on DNA-double strand break repair (DSBR): Dysfunction in patients causes chromosomal radiosensitivity, and damage-induced ATM triggers a cascade of posttranslational modification events that target all DSBR mechanisms. However, the precise functions of ATM in DSBR have remained enigmatic, since previous studies yielded conflicting data regarding both the pathway of homologous recombination (HR) and non-homologous end joining (NHEJ). To clarify this issue, we explored the effect of clinically relevant *ATM* mutations. For this purpose, we characterized DSBR between mutated *EGFP* genes and ATM kinase signalling in 11 lymphoblastoid cell lines (LCLs) derived from Ataxia telangiectasia (AT) and breast cancer patients with defined versus 3 control LCLs without *ATM* mutations. Our study revealed that the DSBR phenotype in AT cells is not uniform, but appears to depend on the mutation causing up to 32-fold increased or up to 3-fold decreased activities in particular pathways. Comparison with a further 10 LCLs mutated in downstream breast cancer susceptibility gene products (BRCA1, BRCA2, Nibrin, Rad50, Chk2) showed that the most diametrically opposed DSBR patterns in AT cells phenocopied *NBN/RAD50* or *BRCA1* mutations. Importantly, re-expressing wild-type ATM reversed these defects by 2.3- to 3.5-fold. Our data suggest that ATM stimulates repair proteins like Nibrin, which execute HR, single-strand annealing (SSA), and NHEJ. Concomitantly ATM minimizes error-prone repair (SSA, NHEJ) through activation of surveillance factors like BRCA1. Since the outcome of the individual defect can be diametrically opposed, distinguishing repair patterns in patients with *ATM* mutations may also be relevant regarding therapeutic responses. Work was supported by the Deutsche Krebshilfe, the BMBF, and the DFG.

References: Walsh and King (2007) Cancer Cell 11, 103-5; O'Connor et al. (2007) Oncogene 26, 7816-28; Keimling et al. (2008) Int. J. Cancer 123, 730-6; Keimling et al., FASEB J, epub ahead of print (2011)

Biography

Lisa Wiesmüller, Head of the Department of Gynaecological Oncology at the Department of Obstetrics and Gynecology, Ulm University, did her doctorate at the Max Planck Institute of Biochemistry, Martinsried, a fellowship at the Institut Pasteur, Paris, and a postdoc at the Max Planck Institute of Medical Research, Heidelberg. In the 1990s her group started working on the role of p53 in DNA double strand break repair at the Heinrich Pette Institute in Hamburg. At that time, this was a completely new concept. During the course of her mechanistic studies on p53 (summarized in Gatz and Wiesmüller, 2006. Cell Death Differ) and meanwhile also on many other DNA repair factors (Plo et al., 2008. Blood; Siehler et al., 2009. DNA Repair; Uhl et al., 2010. DNA Repair Volcic et al., 2011. Nucleic Acids Res), she developed new DNA repair assays with promising applications. Thus, her group applies siRNA-based screening to identify new breast cancer risk genes in cells from corresponding animal models or genome destabilizing factors causally involved in therapy-induced leukemia. Most interestingly, she established new functional marker systems to detect increased breast/ovarian cancer risk and to predict therapeutic responsiveness of breast/ovarian cancer patients, for which she received the Innovation Award 2009 in Germany.

Repair and Signaling

Chairs: Zhao-Qi Wang and Lisa Wiesmüller

Tuesday, 18 October
8.30-13.00

miR421-mediated ATM-depletion leads to a pronounced DNA repair defect: A novel mechanism for clinical tumor radiosensitivity

J. Dahm-Daphi[1,2,], , U. Kasten-Pisula[2], T. Rieckmann[2], S. Köcher[1,2], K. Borgmann[2], M. Baumann[5], M. Krause[5], C. Petersen[3], T. Dörk[4], E. Dikomey[2], and Wael Y. Mansour[1,2,*]

[(1)]Institute of Radiobiology and Molecular Radiation Oncology, Philipps-University Marburg,.[(2)]Laboratory of Radiobiology&Experimental Radiooncology and [(3)]Department of Radiotherapy & Radiooncology, University Medical Center Hamburg–Eppendorf, .[(4)]Clinics of Gynecology and Obstetrics, and [(6)]Clinics of Radiation Oncology,Hannover Medical School.[(5)]Department of Radiation Oncology and Centre for Radiation Research in Oncology, , Technical University Dresden, Germany.

Head and neck tumors are generally characterized by a moderate radiosensitivity. Here, we report on a tumor case (SKX, SCC oral cavity, T4, N2, M0,) with remarkable radiosensitivity that responded extremely well to a standard radiotherapy protocol without further need for surgery. Both, isolated SKX cells and xenografts showed a pronounced repair defect with a high number of residual γ-H2AX foci 24h after IR. No classical endjoining defect was observed as protein expression of KU70, KU80, DNA-PKcs, LIGIV, XRCC4, Artemis and RAD51 as well as DNA-PK activity were normal. Importantly, we found an ATM defect as SKX cells express no ATM protein and show impaired ATM damage signalling (absence of p-ATM, p-SMC1, p-Chk2, p-Kap1) after IR. Sequencing of the 66 exons of ATM gene revealed no somatic or splicing mutations in SKX cells. We found moderately (~2x) reduced ATM mRNA levels that could be reverted by anti-methylating 5'-aza-2'-deoxycytidine treatment, however, without raising ATM protein levels. Instead, we demonstrate a post-transcriptional regulation of ATM in SKX cells via 6-fold enhanced miR421 level compared to reference FaDu cells which targets the 3'-UTR of ATM mRNA. Transfection of anti-miR421 inhibitor or expression of microRNA insensitive ATM-cDNA strikingly recovered ATM expression and also reverted the hyper-radiosensitivity and the DSB repair defect of SKX cells. Together, this is the first report about ATM regulation via microRNA in a human malignancy that leads to clinically manifest tumor radiosensitivity. Our findings shed new light on the putative role of ATM dysfunction in carcinogenesis and tumour therapy in general.

Biography

Dr. Jochen Dahm-Daphi studied medicine and was further trained in internal medicine. He received his M.D. in 1992 from the University of Hamburg, Germany. Since then, he worked in the field of DNA repair in human and mammalian systems at the Institute of Radiobiology, Hamburg. In 1994 -95 he completed his postdoctoral training at the Lawrence Berkeley National Laboratory, Berkeley California. In 1996 he started a new Experimental Oncology Lab at the Department of Radiotherapy, University of Hamburg. In 2002 he completed his "Habilitation" thesis on "Radiation-induced DNA Damage, DNA Repair and Biological effects". In 2009 he became Associate Professor in Hamburg and in 2011 elected Professor for Radiobiology at the University of Marburg, Germany. His research program is aimed at elucidating the mechanisms of DNA double-strand break repair, the genetics of DNA recombination in cross-talk with the cell cycle, DNA repair in human tumours and translational research towards combined clinical radio- and chemotherapy.

A new role for the *CHK2-BRCA1* tumor suppressor pathway required for the maintenance of chromosomal stability in human somatic cells

Ailine Stolz, Norman Ertych and Holger Bastians

Department of Molecular Oncology, University Medical Center Göttingen,
Grisebachstrasse 8, D-37077 Göttingen, Germany
(holger.bastians@uni-goettingen.de)

Chromosomal instability, which is defined as the perpetual gain or loss of whole chromosomes during mitotic cell division, is a major characteristic of human cancer and can contribute to tumorigenesis and tumor progression. However, the molecular basis underlying this highly penetrant phenotype is largely unknown, but it is conceivable that defects in mitotic progression and chromosome segregation can directly contribute to the chromosomal instability phenotype. We have identified the tumor suppressor genes *CHK2* and *BRCA1* as genes required for the maintenance of chromosomal stability in human somatic cells. Both genes have been previously implicated as important tumor suppressor genes that are involved in the cellular response to DNA damage and in regulating DNA repair. In addition to that, we showed that a Chk2 mediated phosphorylation of Brca1 is required for the proper and timely assembly of mitotic spindles in the absence of DNA damage. Loss of *CHK2* or *BRCA1* or inhibition of its Chk2 mediated phosphorylation inevitably results in the transient formation of abnormal spindles that facilitate the establishment of faulty microtubule-kinetochore attachments associated with the generation of lagging chromosomes. Importantly, expression of the *CHK2* tumor suppressor gene is lost at very high frequency in aneuploid human lung adenocarcinomas that are also typically induced in knockout mice exhibiting chromosomal instability. Thus, these results suggest novel roles for Chk2 and Brca1 in the absence of DNA damage that might contribute to their tumor suppressor functions.

References: Stolz et al. (2010) Nature Cell Biology 12: 492 – 499; Stolz et al. (2011). Clinical Cancer Research, 17; 401 – 405.

Biography
Dr. Bastians obtained his PhD in molecular and cell biology in 1996 from the German Cancer Research Center (DKFZ) in Heidelberg, Germany, where he worked on cell cycle regulated protein phosphatases. After completion of the Ph.D. thesis he went to the Harvard Medical School in Boston, USA, and joined the laboratory of Prof. Joan Ruderman as a postdoctoral researcher focussing on mitotic signaling pathways. After returning to Germany, he became an independent junior research group leader at the Institute for Molecular Biology and Tumor Research (IMT) at the University in Marburg, Germany. In 2008, Dr. Bastians was honoured with a Heisenberg fellowship from the Deutsche Forschungsgemeinschaft and in 2011 he was elected as a Heisenberg Professor for Cellular Oncology at the University Medical Center in Göttingen, Germany. His work is focussing on the molecular mechanisms of chromosomal instability and therapy responses in human cancer cells. In particular, he is interested in investigating the functions of components of the DNA damage response machinery including DNA repair proteins like Brca1. These proteins were shown to have additional functions during mitosis required for the maintenance of chromosomal stability.

Unexpected Mitotic Functions of Rev3, the Catalytic Subunit of DNA Polymerase zeta

Wei Xiao
College of Life Sciences, Capital Normal University, Beijing, China 100048
Department of Microbiology and Immunology, University of Saskatchewan, Saskatoon, SK Canada S7N 5E5 (wei.xiao@usask.ca)

Translesion synthesis (TLS) is a cellular process by which specialized DNA polymerases synthesize DNA across otherwise replication-blocking lesions. Apart from their roles in TLS, some TLS polymerases also play additional roles such as in somatic hypermutation and nucleotide excision repair. Polζ, the only known B-family TLS polymerase, consists of two subunits: the catalytic subunit Rev3 and a regulatory subunit Rev7. Rev3 in mammals is twice the size of its yeast counterpart and its inactivation causes embryonic lethality, while transgenic mice lacking other known TLS polymerases are viable. The reason for an essential role of mammalian *Rev3* in embryonic development is currently unclear primarily due to experimental limitations like lack of specific antibody and difficulty in ectopically expressing *REV3* because of its extremely large size. On the other hand, Rev7, which is also known as mitotic arrest deficient-like 2 (MAD2L2), appears to play multiple roles in addition to TLS. To understand whether such functions are part of Polζ as a protein complex or are specific to Rev7 we developed specific antibodies and carried out a parallel study in human HCT116 and HeLa cells using synthetic and lentiviral siRNA systems. It was found that cellular Rev3 and Rev7 levels increase during metaphase perhaps largely du e to increased transcript levels. Surprisingly, the two proteins localize independently of each other in mitotic cells. Rev3 localizes to the chromatin whereas Rev7 is found around the spindle structure. The ablation of Rev3 but not Rev7 resulted in a significant increase in anaphase bridges and double-strand chromosomal breaks. On the other hand Rev7 depletion caused an increase in lagging chromosomes but no such increase was observed in Rev3-depleted cells. Interestingly, ablation of Rev1, which is also required for TLS, did not result in the above mitotic abnormalities. These observations demonstrate that Rev3 and Rev7 work independently of each other during metaphase and the resulting chromosomal breaks may act as a trigger for cell death during embryonic development.

Biography
Dr. Wei Xiao**,** Professor and Dean, College of Life Sciences, Capital Normal University, China and Professor, Department of Microbiology and Immunology, University of Saskatchewan, Canada. He obtained his B.Sc. (1982) from Nanjing Agricultural University, China, M.Sc. (1984) from the University of Toronto, and Ph.D. (1988) from the University of Saskatchewan. Following a PDF training with Dr. L. Samson at Harvard University, he became an Assistant Professor at the University of Saskatchewan in 1992, was promoted to Associate (1995) and Full Professor (2000), and served as the Department Head (2003-2008). He was recruited to CNU in 2010 while maintaining a joint faculty status at the University of Saskatchewan. The primary research interests in his laboratory are unconventional ubiquitination and its roles in DNA-damage responses, mutagenesis, carcinogenesis and signal transduction in eukaryotes, from yeasts to human.

1. Andersen, P.L., Xu, F., Ziola, B., McGregor, W.G. and Xiao, W. (2011) Sequential assembly of translesion DNA polymerases at UV-induced DNA damage sites. *Mol. Biol. Cell*. 22: 2373-2383.
2. Pastushok, L., Hanna, M. and Xiao, W. (2010) Constitutive fusion of ubiquitin to PCNA provides DNA damage tolerance independent of translesion polymerase activities. *Nucleic Acids Res.* 38: 5047-5058.
3. Fu, Y., Zhu, Y. Zhang, K., Yeung, M., Durocher, D. and Xiao, W. (2008) Rad6-Rad18 mediates a eukaryotic SOS response by ubiquitinating the 9-1-1 checkpoint clamp. *Cell* 133: 601-611.

Impact of lipid-lowering drugs on DNA damage response and DNA repair

Gerhard Fritz

Institute of Toxicology, Heinrich-Heine University, Universitätsstrasse 1, D-40225 Düsseldorf, Germany
(fritz@uni-duesseldorf.de)

The lipid lowering drug lovastatin (Lova) exhibits pleiotropic effects beyond its cholesterol lowering activity. With respect to tumor cells, statins increase anticancer drug-induced cell death *in vitro* and *in vivo*[3]. Regarding non-malignant cells we found that lovastatin affects the DNA damage response (DDR) of human endothelial cells (HUVEC) to the anticancer drug doxorubicin (Doxo)[1] and ionizing radiation (IR)[2] in an agent specific manner. Moreover, the statin protects HUVEC from the toxicity of the aforementioned agents. In order to investigate the effect of Lova on Doxo- and IR-induced normal tissue damage *in vivo*, Balb/c mice were exposed to IR (total body irradiation) or Doxo with or without Lova cotreatment. Acute toxicity was analyzed after a high single dose of IR (6 Gy) or Doxo (10 mg/kg). Subchronic toxicity was analyzed 3 weeks after administration of multiple low doses of IR or Doxo. In liver, Lova cotreatment did not impact early DNA damage formation following IR exposure. Yet, the statin attenuated proinflammatory and profibrotic gene expression. Assaying subchronic radiation toxicity we found that the mRNA expression of proinflammatory and profibrotic marker genes was reduced by Lova in a tissue specific manner. Regarding Doxo, Lova co-treated animals showed less initial DNA damage as well as attenuated acute inflammatory and fibrotic responses. Animals treated with Doxo for an extended period of time showed clear signs of liver and heart toxicity. Lova reduced liver and heart damage and lowered the expression of fibrosis markers. As analyzed by real-time RT-PCR using a semi-customized PCR-array, we found that Lovastatin increases the expression of a variety of DNA repair genes in heart and liver. In combination with Doxo, Lova was able to mitigate Doxo-stimulated expression of a subset of DNA repair genes. In conclusion, Lova protects normal tissue from acute and subacute damage provoked by Doxo and IR *in vivo*. We hypothesize that the protective effect of lovastatin is at least partially due to inhibition of DNA damage induction, resulting in an attenuated DDR, and acceleration of DNA repair. With respect to the clinic, the data indicate that Lova might attenuate side effects of Doxo- and IR-based anticancer therapy.

References: [1]Damrot et al (2006) British J. Pharmacology 149, 988-97; [2]Nübel et al. (2006) *Clin Cancer Res* 12, 933-39; [3]Fritz G. (2009) Curr Cancer Drug Targets 9, 626-38.

Biography
Dr. Fritz obtained his Ph.D. in genetics in 1991 from the Universtity of Karlsruhe (Germany), where he worked on the regulation and biological function of the repair factor MGMT. He completed his postdoctoral training at the Institute of Pharmacology and Toxicology at the Medical Faculty in Homburg/Saar (Germany), where he was focusing on regulatory aspects of small GTPases of the Rho (Ras-homologous) family. In 1994 he moved to the Institute of Toxicology at the University of Mainz (Germany) where he stayed as post-doc and assistant professor until 2005. From 2005-2010 he was associate professor at the Institute of Pharmacology and Toxicology in Giessen (Germany) (2005-2006) and Institute of Toxicology in Mainz (Germany) (2006-2010). In 2011 he became the head of the Institute of Toxicology of the Heinrich Heine University Düsseldorf (Germany). His research programme aims at elucidating mechanisms of the cellular response to genotoxic stress (focusing on both DNA damage-dependent and DNA damage-independent mechanims), DNA repair, the involvement of Rho GTPases in tumor progression and cell death following exposure to radiation as well as chemical genotoxins and anticancer drugs. For his research he is using both *in vitro* and *in vivo* model systems (i.e. wild-type and transgenic mice strains).

Premature aging proteins involved in nuclear and mitochondrial DNA repair

Vilhelm A. Bohr
Laboratory of Molecular Gerontology, National Institute on Aging, NIH
251 Bayview Blvd., Baltimore, MD 21042

An increasing number of human premature aging proteins seem to play roles in DNA repair. We have studied Werner syndrome (WS) protein and other RecQ helicases and characterized interactions with major DNA double strand break repair (DSBR) and base excision repair (BER) proteins. We find that WRN protein plays several roles in BER, via its substrate specificity and via its protein interactions. Oxidative damage also accumulates in WS cells. We now find that another RecQ helicase, the Rothmund Thomson protein (RTS, RecQ 4) is also involved in DNA repair, including BER. This protein interacts with a number of BER proteins and RTS cells are deficient in both nuclear and mitochondrial DNA repair. In our ongoing studies on mitochondrial DNA repair, we are pursuing its precise role in mitochondrial DNA maintenance. Another important premature aging protein is Cockaynes syndrome protein group B (CSB). This protein also participates in BER at different levels, and a plays a role in both nuclear and mitochondrial BER. These studies will be discussed further.

Biography
Dr. Bohr obtained his MD from the University of Copenhagen, Denmark, in 1978. He received his PhD from Unievrsity of Copenhagen in 1986. He was an intern at the University of Copenhagen in Medicine, later obtained postdoctoral fellowships at the University of Copenhagen and Stanford University. He became an investigator at the National Cnacer Insitute, NIH, Washington in 1986 and the Department chair at the National Institute on Aging, NIH, Baltimore in 1992, where he has remained since. He is also a professor at the University of Aarhus and University of Copenhagen in Denmark

DNA Damage Responses in Developmental and Aging

Michael M. Müller[1], Maria A. Ermolaeva[1], Laia Castells-Roca[1], Peter Frommolt[1,2], Sebastian Greiss[1,3], Jennifer I. Schneider[1]& Björn Schumacher[1]

[1]Cologne Excellence Cluster for Cellular Stress Responses in Aging Associated Diseases (CECAD), Institute for Genetics, University of Cologne, Zülpicher Str. 47a, 50674 Cologne, Germany
[2]Cologne Center for Genomics, University of Cologne, Weyertal 115b, 50931 Cologne, Germany
[3]present address: MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 0QH, United Kingdom
(bjoern.schumacher@uni-koeln.de)

Congenital defects in genome maintenance systems cause complex disease phenotypes characterized by developmental failure, cancer susceptibility and premature aging. In contrast to well-characterized cellular DNA damage checkpoint mechanisms, it remains poorly understood how DNA damage responses function during organismal development and maintain functionality of tissues when DNA damage gradually accumulates with aging. Here we report that transcription-coupled repair defects that in human Cockayne syndrome patients lead to developmental growth defects and progeria, specifically impair somatic development upon UV damage in *C. elegans*. Using transcriptome analysis we identified a network of insulin/insulin-like growth factor signalling (IIS) genes responding to UV-induced DNA damage during development and demonstrate that the IIS effector transcription factor DAF-16/FoxO is activated in response to DNA damage. We show that DAF-16 activation alleviates DNA damage induced developmental arrest and promotes developmental growth and enhances somatic tissue functionality even in the absence of functional DNA repair. Our findings suggest that DNA damage induced DAF-16 regulates growth promoting target genes in a distinct mode that contrasts the previously established starvation induced DAF-16 activity. We propose that IIS mediates developmental DNA damage responses and that regulation of DAF-16 activity enables developmental progression and prolongs tissue functioning when DNA damage persists.

Work is supported by DFG (CECAD and SFB829), ERC (Starting grant 260383), Marie Curie (European Reintegration Grant 239330), the German-Israeli Foundation (GIF, YIG 2213) and the Deutsche Krebshilfe (109453)

References: Garinis et al. (2009) Nat Cell Biol. 2009 May;11(5):604-15, Schumacher (2009) Bioessays Dec;31(12):1347-56. Garinis & Schumacher (2009) Cell Cycle Jul 20;8(14), Schumacher et al. (2009) Mol Cell Endocrinol. Feb 5;299(1):112-7, Schumacher et al. (2008) PLoS Genet. Aug 15;4(8):e1000161, Greiss et al. (2008) BMC Genomics 2008 Jul 15;9(1):334, Schumacher et al. (2008) Trends Genet. 2008 Feb;24(2):77-85, Schumacher B et al. (2005) Cell 11 February; 120: 357-368, Schumacher et al. (2005) Cell Death Differ. Feb;12(2):153-61.

Biography
Dr. Schumacher studied Biology at the University of Konstanz and the Stony Brook University, New York. During his PhD, initially at the Cold Spring Harbor Laboratories, NY, then at the Max Planck Institute for Biochemistry, Martinsried, he identified an ancestral p53 pathway in *C. elegans* and uncovered novel regulatory mechanisms of p53 mediated apoptosis in response to DNA damage. As EMBO and Marie Curie postdoctoral fellow at the Erasmus Medical Centre, Rotterdam, Dr. Schumacher identified mechanistic links between stochastic accumulation of DNA damage during aging and longevity assurance pathways. Since 2009 he heads an independent junior group at the CECAD excellence cluster for aging research at the University of Cologne where he uses the *C. elegans* and mammalian models to investigate DNA damage responses in Development, Aging and Cancer. He was awarded the Innovation Prize of the State of Northrhine-Westphalia in 2009 and in 2010 received the ERC Independent Starting Researcher Grant.

The Role of DNA Damage Response in Neuron

Wei-Min Tong

Department of Pathology and Center for Experimental Animal Research
Institute of Basic Medical Sciences (IBMS), Chinese Academy of Medical Sciences (CAMS)
& Peking Union Medical College (PUMC) 100005 Beijing CHINA
(tongwm@ibms.pumc.edu.cn)

Genomic integrity plays an important role in normal development and formation of the central nervous system. Nijmegen breakage syndrome (NBS) is an autosomal recessive genetic disease with characteristic chromosomal breakage and microcephaly. Upon DNA damage, the product of NBS (known as Nbn in mice) gene, Nbs1, is believed to be involved in intracellular signaling pathways and as a central player in DNA damage checkpoint activation and the control of cell cycle. To study the role of Nbs1 in microcephaly formation, we have specifically deleted Nbn in the mouse central nervous system (CNS). These mice displayed ataxia, microcephaly as a result of altered cell proliferation and apoptosis. Additional deletion of p53 substantially rescues the ataxia and microcephaly phenotype in Nbn-deficient mice. In the present study, we show that Nbs1-deficient neurons display differential response to DNA damage, such as cell cycle arrest or apoptosis. Constitutive endogenous DNA damage increased expression of p53 proapoptotic target genes like Bax, Noxa, Puma specifically in the Nbn-CNS-del developing cerebella rather than neocortex. Thus, Nbs1-deficient cerebellar degeneration is likely generated by selective induction of p53 proapoptotic target genes in Nbs1-deficient mice.

References: Frappart et al. (2005) Nat. Medicine 11: 538-544; Yang et al. (2006) DNA Repair 5: 885-893; Shull et al. (2009) Genes Dev. 23:171-180; Mckinnon (2009) Nat. Rev. Neurosci. 10:100-112

Biography
Dr. Tong obtained his Medical diploma in 1986 from the China Medical University and Pathology license in 1991 from the Norman Bethune Medical University of Medical Sciences, China. After graduating with a Ph.D. in 1996 from the University of Vienna, Austria, he continued his post-doctoral training in the International Agency for Research on Cancer, World Health Organization (IARC/WHO, Lyon, France) to focus on basic mechanisms of defects in DNA strand breaking sensing molecules, such as PARP-1 and NBS1 in development and tumorigenesis. Particularly, combined with molecular pathology and transgenic techniques (conventional and Cre/lox-P approach), mouse models for human Li-Fraumeni syndrome, medulloblastomas, multiple endocrine neoplasia type I (MEN1), and Nijmegen breakage syndrome (NBS) were generated and the role of DNA damage response in neuronal degeneration and tumorigenesis was investigated. He was a Staff Scientist in 2000, and then Scientist in 2003 in the IARC/WHO. Since end of 2007, he is a Professor of Pathology at the IBMS, CAMS/PUMC, and Director of Center for Experimental Animal Research at IBMS/CAMS.

Differential function of ATR and NBS1 in neuropathology

Zhong-Wei Zhou[1], Ralph Gruber[1], Christopher Bruhn[1], Haizhen Lu[1] and Zhao-Qi Wang[1,2]

1. Leibniz Institute for Age Research – Fritz Lipmann Institute (FLI), Jena, Germany.
2. Faculty of Biology and Pharmacy, Friedrish-Schiller-University Jena, Jena, Germany.

NBS1, ATM and ATR are major DNA damage response components, which control DNA damage signalling, DNA repair, cell cycle checkpoints and apoptosis. Mutations of NBS1 and ATR cause human genomic instability syndrome NBS and ATR-Seckel, respectively, both of which feature neurodevelopmental defects. We previously reported that neuron-specific deletion of Nbs1 resulted in ataxia and a neuronal attrition that is Atm-p53 dependent. Nbs1 deletion dramatically reduced the amount of granule neurons whereas the amount of Purkinje cells was not significantly affected. To investigate whether Nbs1-deletion mediated ataxia is specifically due to defects in granule cells or Purkinje cells, we generated Purkinje cell-specific Nbs1 knockout mice (*Nbs1*-PCdel). These mice developed and behaved normally during an observation period of 2 years. The morphology and numbers of Purkinje cells were apparently normal in young and aged *Nbs1*-PCdel mice, despite both Mre11 and Rad50 are exclusively mislocated in the cytoplasm of *Nbs1*-PCdel mutant Purkinje cells.
To investigate the interaction of ATR and NBS1 in preventing neuropathology, we knocked out Nbs1 or Atr in the mouse CNS. We found that disruption of each of these two genes resulted in neurodevelopmental defects characterized by proliferation defects and increased apoptosis in embryonic brains. Deletion of Nbs1 and Atr together caused dramatic proliferation defects in neuroprogenitors. Whereas most apoptosis in Nbs1-deleted cortex is restricted to the highly proliferating progenitors, Atr knockout induced apoptosis in both proliferating and non-proliferating neural cells. Consistently, an inducible deletion of Atr or Nbs1-Atr, but not of Nbs1, triggers a cell death pathway in differentiated neurons. Altogether, we identify a distinct function of Nbs1 and Atr in neurogenesis, namely a specific function of Nbs1 in proliferating neuroprogenitors and of Atr in both proliferating and non-dividing neural cells.

Base Excision Repair and Inflammation

Chairs: Ekkehard Dikomey and Haiying Hang

Tuesday, 18 October
14.00-18.00

Base excision repair targets for cancer therapy

Grigory L Dianov

Gray Institute for Radiation Oncology and Biology Unit, University of Oxford,
Old Road Campus Research Building, Roosevelt Drive, Oxford OX3 7DQ, UK
(grigory.dianov@rob.ox.ac.uk)

Base excision repair (BER) is a frontline DNA repair system that is responsible for maintaining genome integrity, thus preventing many human diseases, including premature aging and cancer, by repairing DNA base lesions and single strand breaks caused by endogenous and exogenous mutagens. It is also the principal DNA repair system in cancer cells that counteracts the killing effect of the major cancer treatments e.g. chemotherapy (alkylating agents) and ionizing radiation (about 80 % of DNA damage induced by ionizing radiation are base lesions). Changes in BER capacity most probably are responsible for many cases of cancer treatment efficiency since many cancers have alternated expression of BER proteins. Although BER enzymes are well studied the mechanisms involved in BER coordination and regulation of BER capacity are not clear. This knowledge gap is impeding the finding of new cancer therapy targets and the development of novel treatment strategies.

We have lately pioneered new studies on the regulation of BER protein levels and activity by posttranslational modifications, including ubiquitylation, deubiquitylation and phosphorylation. These studies included identification of the enzymes involved in regulation of the key BER proteins: DNA polymerase β, XRCC1, DNA ligase III and AP-endonuclease. The results of these studies allowed us to identify the major proteins involved in BER regulation as well as to formulate novel general principles of BER regulation and evaluate their potential as new targets for cancer therapy.

References: Parsons et al. (2008) Mol Cell, 29, 477-487; Parsons et al. (2009) EMBO J. 28, 3207-3215; Parsons et al. (2011) Mol Cell, 41, 609-615.

Biography

Dr. Dianov is a Senior Scientist and Head of the Biochemistry Group at the Gray Institute for Radiation, Oncology and Biology at the University of Oxford. He obtained his PhD and Doctor of Sciences degrees from the Institute of Cytology and Genetics (Siberian Department of Russian Academy of Sciences). He continued to work at the Institute of Cytology and Genetics as a Professor and Head of the Laboratory of Molecular Mechanisms of Mutagenesis until 1990. From 1990 to 1993 Dr. Dianov worked as a Senior Fellow at the Imperial Cancer Research Fund, (London) with Dr. Tomas Lindahl and in 1993 he moved to the United States where he worked as a visiting Professor with Dr. Errol Friedberg (Southwestern Medical Center, Dallas) and later as a Senior Fellow with Dr. Vilhelm Bohr (National Institute on Aging, NIH). In 2000 Dr. Dianov moved back to England to take his present position as Head of the Biochemistry Group. Dr. Dianov is interested in the biochemistry and molecular biology of the mechanisms protecting human cells from environmental stress damaging genomic DNA. He is also involved in research into the molecular mechanisms of DNA repair and their role in protecting cells from cancer. This includes uncovering molecular events leading to the development of cancer and identifying new molecular targets for cancer treatment.

Oxygen as a friend and enemy: how to combat the mutational potential of 8-oxo-guanine

Enni Markkanen[1], Barbara van Loon[1], Elena Ferrari[1], Jason L. Parsons[2], Grigory L. Dianov[2] and Ulrich Hübscher[1]

[1]Institute for Veterinary Biochemistry and Molecular Biology, University of Zürich-Irchel, Winterthurerstrasse 190, 8057 Zürich, Switzerland
[2]Biochemistry Group, Gray Institute for Radiation Oncology & Biology, Department of Oncology, University of Oxford, Old Road Campus Research Building, Roosevelt Drive, Oxford OX3 7DQ, UK

The maintenance of genetic stability is of crucial importance for any form of life. Prior to cell division in each mammalian cell, the process of DNA replication must faithfully duplicate the three billion bases with an absolute minimum of mistakes. Various environmental and endogenous agents, such as reactive oxygen species (ROS), can modify the structural properties of DNA bases and thus damage the DNA. Upon exposure of cells to oxidative stress, an often generated and highly mutagenic DNA damage is 7,8-dihydro-8-oxo-guanine (8-oxo-G). The estimated steady-state level of 8-oxo-G lesions is about 10^3 per cell/per day in normal tissues and up to 10^5 lesions per cell/per day in cancer tissue (reviewed in ref. (*1*)). The presence of 8-oxo-G on the replicating strand leads to frequent (10-75%) misincorporations of adenine opposite the lesion (formation of A:8-oxo-G mispairs), subsequently resulting in C:G to A:T transversion mutations. These mutations are among the most predominant somatic mutations in lung, breast, ovarian, gastric and colorectal cancers. Thus, in order to reduce the mutational burden of ROS, human cells have evolved base excision repair (BER) pathways ensuring (i) the correct and efficient repair of A:8-oxo-G mispairs and (ii) the removal of 8-oxo-G lesions from the genome. Very recently we showed that MutY glycosylase homologue (MutYH) and DNA polymerase (Pol) l play a crucial role in the accurate repair of A:8-oxo-G mispairs (*2-3*). The correct repair of the abundant and highly miscoding oxidative DNA lesion 8-oxo-G is performed by an accurate repair pathway that is coordinated by the MutY glycosylase homologue (MutYH) and Pol λ in mammalian cells (*4*). It is a mystery how the levels of Pol λ are controlled, how phosphorylation promotes its stability and how the engagement of Pol λ in active repair complexes is coordinated. The E3 ligase Mule mediates degradation of Pol λ and the control of the Pol λ levels by Mule has functional consequences for the ability of mammalian cells to deal with 8-oxo-G lesions. Phosphorylation of Pol λ by Cdk2/CyclinA counteracts its Mule-mediated degradation by promoting recruitment of Pol λ to chromatin into active 8-oxo-G repair complexes through an increase in Pol λ's affinity to chromatin-bound MutYH. Finally, pol λ not engaged in repair on chromatin is subject for degradation.

Own references: (1) B. van Loon, E. Markkanen, U. Hubscher (2009 Oxygen as a friend and enemy: How to combat the mutational potential of 8-oxo-guanine. *DNA Repair (Amst)*, **9**, 604. (2) G. Maga *et al.*, Replication protein A and proliferating cell nuclear antigen coordinate DNA polymerase selection in 8-oxo-guanine repair. *Proc Natl Acad Sci U S A* **105**, 20689. (3) G. Maga *et al.*, 8-oxo-guanine bypass by human DNA polymerases in the presence of auxiliary proteins. *Nature* **447**, 606. (4) B. van Loon, U. Hubscher, An 8-oxo-guanine repair pathway coordinated by MUTYH glycosylase and DNA polymerase lambda. *Proc Natl Acad Sci U S A* **106**, 18201.

Biography

Dr. Hübscher obtained his DVM in 1976 from the University of Zurich. After a two year postgraduate training in Biomedical Sciences in Switzerland, he worked as a postdoctoral fellow form 1978-1980 in the Department of Biochemistry at the Stanford Medical School with Arthur Kornberg and from 1980-1801 at the Medical Research Council at Mill Hill, with Robin Holliday and Jeffrey Banks. Since 1981 he has his own research group and is interested in enzymology of DNA replication and DNA repair. 1989 he became Associate Professor and in 1998 Full Professor and Director of the Institute of Veterinary Biochemistry and Molecular Biology. The last few years he devoted to regulatory aspects in DNA repair of oxidation damage.

The O6-Alkylguanine Response: Implications for Cancer Formation and Therapy

Bernd Kaina

Department of Toxicology, University Medical Center, Mainz,
Obere Zahlbacher Str. 67, D-55131 Mainz, Germany
(kaina@uni-mainz.de)

First line therapy in the treatment of gliomas and malignant melanomas are alkylating agents. These agents induce a dozen different DNA lesions, some of them have been identified to be carcinogenic, genotoxic and cytotoxic. A critical DNA adduct is O^6-methylguanine (O^6MeG). This damage causes mutations and is responsible for most of the carcinogenic effects of alkylating agents. At the same time O^6MeG is a highly powerful cytotoxic lesion giving rise to apoptosis, necrosis and autophagy induction. The damage is repaired by the suicide enzyme alkyltransferase (MGMT), which is a very important defense mechanism and marker of alkylating drug resistance, notably in malignant glioma. For malignant melanoma the situation is less clear; very likely other mechanisms of alkylation drug resistance come into play as well, such as mismatch repair and regulators of apoptosis. O^6MeG is a potent trigger of apoptosis. We have studied in detail how apoptosis is induced following O^6MeG and executed in glioma and melanoma cells. We have shown that O^6MeG triggered cell death is executed via the death receptor and the mitochondrial damage pathway, involving DNA double-strand breaks (DSB). The major pathway of repairing O^6MeG and O^6-chloroethylguanine (induced by the anticancer drugs ACNU and CCNU) triggered DSB is homologous recombination (HR). Therefore, players involved in DSB recognition and HR are potential targets for therapy, such as NBS-1, ATM, Rad51, XRCC2 and XRCC3. In glioma, the efficiency of O^6MeG to trigger the p53 dependent death receptor pathway is higher than the p53 independent endogenous mitochondrial pathway, which explains why p53 wt glioma cells are more sensitive to temozolomide than p53 mutated cells. Interestingly, p53 wt glioma cells are more resistant than p53 mutant glioma cells to chloroethylating agents (CCNU, ACNU), which are also applied in glioma therapy. This indicates that p53 has a dual role: one is the up-regulation of the death receptor thus sensitizing to methylating agents, the other is the upregulation of DNA repair genes such as *ddb2* thus protecting against O^6chloroethylguanine-induced apoptosis (and necrosis). Melanoma cells display a high intrinsic level of resistance to temozolomide, which is due to silencing of caspase-8 and induction of DNA repair. Strategies aimed at abrogating intrinsic drug resistance will be discussed, including interferon and valproic acid co-treatment that reactivate the apoptotic pathway. Work was supported by DFG KA724 and Deutsche Krebshilfe.

References: Batista et al. (2007) Cancer Res. 67, 11886-95; Naumann et al. (2009) Br. J. Cancer, 100, 322-33; Quiros et al. (2010) Cell Cycle, 9, 168-78; Roos et al. (2011) Cancer Res. 71, 4150-60; Christmann et al., BBA-Rev Cancer, in press (2011)

Biography
Dr. Kaina obtained his Ph.D. in genetics in 1976 from the Universtity of Halle, Germany. He completed his postdoctoral training at the Institute of Genetics in Gatersleben and continued his studies on DNA repair at the Department of Molecular Biology in Leiden, Netherlands, at the German Cancer Research Center in Heidelberg and, as a Heisenberg fellow, at the Department of Genetics of the Research Center in Karlsruhe, Germany. In 1993 he was elected a professor of toxicology at the Institute of Toxicology of the University of Mainz. His research program is aimed at elucidating the mechanisms of DNA repair, regulation of repair genes, DNA damage signaling, genotoxicity, cancer formation and death of cells upon exposure to radiation and chemical genotoxins and anticancer drugs. As part of translational research program, his group is involved in studies on the mechanism of cell kill and resistance of glioma and melanoma cell upon treatment with alkylating anticancer drugs. He is also interested in Traditional Chinese medicine (TCM) and studied the response of cells to artesunate and other TCM drugs.

Poly(ADP-Ribosyl)ation and Ageing in Mammals

Alexander Bürkle

Chair of Molecular Toxicology, Dept of Biology,University of Konstanz,
D-78457 Konstanz, Germany
(alexander.buerkle@uni-konstanz.de)

Poly(ADP-ribosyl)ation is a posttranslational modification is involved in many cellular functions, including DNA repair and maintenance of genomic stability, transcription, inflammation, and cell death. Poly(ADP-ribosyl)ation has also been implicated in cellular and organismal ageing and maximum poly(ADP-ribosyl)ation capacity in mononuclear blood cells is correlated with mammalian life span. Recently we have shown that the difference between a long-lived and a short-lived species tested (*i.e.* man and rat) is directly mirrored by the enzymatic parameters of recombinant poly(ADP-ribose) polymerase-1 (PARP-1) from the two species [1] Beneke et al., MAD 2010 in press]. Due to its versatile role, PARP-1 is currently discussed both as a longevity factor and as an aging-promoting factor. We therefore generated a mouse model with ectopic integration of full-length *hPARP-1* [2]. *hPARP-1* mice exhibit impaired survival rates, reduced hair growth, and premature development of several inflammation and age-associated pathologies, such as adiposity, kyphosis, nephropathy, dermatitis, pneumonitis, cardiomyopathy, hepatitis, and anemia. Moreover, mutant male mice display impaired glucose tolerance, yet no manifest diabetes. Overall tumor burden was comparable in wild-type and *hPARP-1* mice, but tumor spectrum was shifted in mutant mice, showing lower incidence of sarcomas and increased incidence of carcinomas. Furthermore, DNA repair was delayed in splenocytes of *hPARP-1* mice, and gene expression of pro-inflammatory cytokines was dysregulated [3]. Viewed together, our results reveal that *hPARP-1* mice show impaired DNA repair as well as a continuous low-level increase in pro-inflammatory stimuli, which both may be causative for development of chronic diseases and impaired survival.

This work was supported by the the DFG (International Research Training Group 1331) and the “Studienstiftung des Deutschen Volkes”.

References:

1. Beneke et al., Mech. Ageing Dev 2010;. 131, 366-369
2. Mangerich et al. Transgenic Res 2009;18, 261-79
3. Mangerich et al., Mech Ageing Dev 2010; 131, 389-404.

Biography

Alexander Bürkle studied Medicine at the *Albert-Ludwigs-Universität,* Freiburg, Germany, supported by a fellowship of the ‚Studienstiftung des Deutschen Volkes‘. In 1982 completed his MD thesis in Virology under the supervision of Prof. Harald zur Hausen. After his compulsory military service as captain in the medical corps, he joined the German Cancer Research Centre (DKFZ), Heidelberg, Germany and worked as a postdoc with Harald zur Hausen and later as junior group leader at the Institute of Applied Tumor Virology. In parallel he completed his habilitation at the *Ruprecht-Karls-Universität* Heidelberg in Toxicology and Chemotherapy. In 2000 he was appointed as a Senior Lecturer at the University of Newcastle upon Tyne, UK, an in 2002 as Full Professor and Chair of Molecular Toxicology at the University of Konstanz, Germany. Since then he has been serving as Head of the Department of Biology (3 yrs), Treasurer and Member of the Executive Board of the International Union of Toxicology (IUTOX), Member of various other Executive Boards and Editorial Boards, and Coordinator of the ongoing European Project (FP7) MARK-AGE. His scientific interest is focused on mechanisms of DNA repair, especially poly(ADP-ribosyl)ation, and ageing/cancer.

Structural and functional characterization of the N-terminus of the human DNA helicase RecQL4

Oliver Ohlenschläger[1], Anja Kuhnert[3], Annerose Schneider[2], Sebastian Haumann[1], Peter Bellstedt[1], Heidi Keller[2], Hans Peter Saluz[3], Frank Hänel[3], Matthias Görlach[1], Helmut Pospiech[2] and Frank Grosse[2]

[1]Research group Biomolecular NMR Spectroscopy and [2]Research group Biochemistry, Leibniz Institute for Age Research – Fritz Lipmann Institute, Beutenbergstr. 11, D–07745 Jena, Germany, [3]Dept. of Cell and Molecular Biology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Beutenbergstr. 11a, D–07745 Jena, Germany

The human RecQL4 helicase is involved in the maintenance of genome integrity and in DNA replication. Mutations in the human RecQL4 gene cause the Rothmund–Thomson, RAPADILINO, and Baller–Gerold syndromes, syndromes displaying heterogeneous clinical profile that include physical and mental abnormalities, some features of premature ageing as well as an increased incidence of cancer, in particular osteosarcomas. Mouse models and experiments in humans and Xenopus have proven the N–terminal part of RecQL4 to be vital for cell growth. We have identified the first 54 amino acids of RecQL4 (RecQL4_N54) as the minimum interaction region with the human repair and checkpoint protein TopBP1. The solution structure of RecQL4_N54 was determined by heteronuclear liquid–state NMR spectroscopy (PDB 2KMU; backbone r.m.s.d. 0.73 Å). Despite low sequence homology, the well-defined structure carries an overall helical fold similar to homeodomain DNA–binding proteins but lacks their archetypical, minor groove binding N-terminal extension. Sequence comparisons indicate that this N-terminal homeodomain-like fold is a common hallmark of metazoan RecQL4 and yeast Sld2 DNA replication initiation factors. RecQL4_N54 binds Y-shaped DNA with preference over double-stranded DNA without apparent sequence specificity. Chemical shift perturbation as monitored by [^{1}H,^{15}N]- and [^{1}H,^{13}C]-HSQC spectra upon titration with Y-shaped and dsDNA shows a major contribution of helix α3 to DNA binding, and additional arginine side chain interactions for the Y-shaped DNA.

hSWS1-PASS1 is an evolutionarily conserved complex required for efficient homologous recombination repair

Jun Huang
Life Sciences Institute, Zhejiang University, Hangzhou, Zhejiang 310058, China
(jhuang@zju.edu.cn)

The Shu complex in yeast plays an important role in homologous recombination pathway, which is critical for the maintenance of genomic integrity. The identification of human SWS1 (hSWS1) as the homologue of budding yeast Shu2 implicated that the Shu complex is evolutionarily conserved. However, the human counterparts of other components in this complex have not yet been identified and characterized. Here we describe the characterization of a novel human component of this complex, PASS1 (Partner and stabilizer of human SWS1). We show that hSWS1 and PASS1 form a stable complex *in vivo* and *in vitro*. hSWS1 and PASS1 are mutually interdependent for their stability. Moreover, PASS1 interacts with RAD51 and RAD51 paralogs and depletion of PASS1 causes defects in homologous recombination repair. Thus, our results suggest that the human Shu complex (hSWS1-PASS1) has an evolutionarily conserved function in homologous recombination.

Biography
Dr. Huang is a professor at Life Sciences Institute, Zhejiang University, Hangzhou, China. Dr. Huang obtained his B.S. from Nankai University in 2001 and his Ph.D. from Peking University in 2006. He was a postdoctoral fellow at Yale University during 2006-2009 and then in MD Anderson Cancer Center during 2009-2011. His primary research interests lie in understanding the molecular mechanisms underlying genomic instability and tumorigenesis.

Role of nucleotide excision repair in Gadd45 mediated DNA demethylation

Christof Niehrs
Division of Molecular Embryology, Deutsches Krebsforschungszentrum, Heidelberg, and Institute of Molecular Biology, Mainz, Germany.

DNA methylation is an epigenetic modification that is essential for gene silencing and genome stability in many organisms. Although methyltransferases that promote DNA methylation are well characterized, the molecular mechanism underlying active DNA demethylation is poorly understood and controversial. We previously showed that Gadd45a (growth arrest and DNA-damage-inducible protein 45 alpha), a nuclear protein involved in maintenance of genomic stability, DNA repair and suppression of cell growth, has a key role in active DNA demethylation (Barreto et al., Nature 2007). I will present data from unscheduled DNA synthesis assays, pharmacological inhibition, protein interaction and siRNA experiments that active demethylation by Gadd45 occurs by nucleotide excision repair (NER). We suggest that NER is not limited to maintain genomic integrity in the face of induced lesions, but also plays a role in epigenetic gene regulation.

References

1. Barreto G, Schafer A, Marhold J, Stach D, Swaminathan SK, Handa V, Doderlein G, Maltry N, Wu W, Lyko F, Niehrs C. (2007). Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. Nature 445(7128):671-5
2. Schaefer A, Schomacher L, Barreto G, Doederlein G, Niehrs C. (2010) Gemcitabine functions epigenetically by inhibiting repair mediated DNA demethylation. PLoS One 5 (11), e14060
3. Sytnikova YA, Kubarenko AV, Schaefer A, Weber AN, Niehrs C (2011) Gadd45a is an RNA binding protein and is localized in nuclear speckles. PLoS One 6(1), e14500

Biography

Christof Niehrs studied biochemistry at the Freie Universität Berlin. He did his PhD degree at the European Molecular Biology Laboratory (EMBL) in Heidelberg in 1990 working on protein tyrosine sulfation. He then joined Dr. E. M. De Robertis' laboratory at the University of California in Los Angeles as a postdoctoral fellow from working on cell-fate determination by homeobox genes in Xenopus laevis. In 1994 he was appointed Head of the Division of Molecular Embryology at the German Cancer Research Center (DKFZ) in Heidelberg where he became the Chair of Molecular Embryology in 2000. He has received several scientific awards and distinctions. In 2010 he became Founding and Scientific Director of Institute of Molecular Biology in Mainz, Germany.

Christof Niehrs' field of research is developmental biology. His work has contributed to solve fundamental problems in early embryogenesis and growth factor signalling. In particular, he has contributed solving the molecular mechanism of the Spemann organizer function. Other major topics include Wnt signalling and DNA demethylation.

Chemotherapy and Regulation of DNA Repair

Chairs: Markus Löbrich and Xingzhi Xu

Wednesday, 19 October
8.30-13.30

Crystal structure of the rad9-hus1-rad1 cell cycle checkpoint complex

Min Xu, Lin Bai, Yong Gong, Wei Xie, Haiying Hang, Tao Jiang*.
National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing 100101. China E-mail: tjiang@ibp.ac.cn

Cellular DNA lesions are efficiently countered by DNA repair in conjunction with delays in cell-cycle progression. Previous studies have demonstrated that Rad9, Hus1, and Rad1 form a heterotrimeric complex (the 9-1-1 complex) that plays dual roles in cell cycle checkpoint activation and DNA repair in eukaryotic cells. Here we report the crystal structure of the human 9-1-1 complex at 3.2Å resolution. The crystal structure, together with biochemical assays, reveals that the interdomain connecting (IDC) loops of Rad9, Hus1, and Rad1 are largely divergent, and further crystallographic study indicates that a peptide derived from Fen1 binds tightly to the IDC loop of Rad1. Structural and biochemical analysis reveal that the Rad17-RFC2-5 clamp loader loads the 9-1-1 clamp onto DNA via the interaction between Rad17 and Rad1 by opening the Rad1-Rad9 interface. Moreover, structural comparison with PCNA reveals other unique structural features of the 9-1-1 complex (the II1- II2 loop in Rad9 and the I2- I7 loop in Rad1) that are proposed to contribute to DNA damage recognition. Taken together, we provide new insights into DNA damage sensing by the 9-1-1 complex.

Biography
Born in 1968, Dr. Jiang gained his Ph.D. in Biophysics in 1998 from the Institute of Biophysics, Chinese academy of science, China. In 2004, he was appointed professor of structural biology in the Institute of Biophysics. Recent years, his group concentrates efforts on structural studies of biomacromolecules including (1) DNA repair proteins which involved in processes that minimize cell killing, mutations, replication errors, persistence of DNA damage and genomic instability; (2) Neurotrophin receptors which affect essentially all biological aspects of vertebrate neurons, including the survival, differentiation, growth and apoptosis of neurons; (3) Membrane proteins, in particular the transporters. The goal of his group is to use X-ray crystallography to determine the atomic resolution structures of these proteins, in conjugation with functional studies to understand their molecular mechanisms.

Formation and repair of cisplatin-induced DNA damage: analyzing the hallmarks for drug resistance and side effects *in vivo*

Jürgen Thomale

Institute for Cell Biology (Cancer Research)
University of Duisburg-Essen Medical School
Hufelandstr. 55, D-45122 Essen, Germany
(juergen.thomale@uni-essen.de)

Successful cancer chemotherapy with DNA-reactive drugs such as cisplatin is hampered by two major obstacles, the development of non-responsiveness in tumor cells and the accumulating toxicity in non-malignant, physiological cells. The molecular mechanisms underlying both problems in the clinical setting are still poorly understood, This is partly due to the lack of appropriate analytical tools for the drug-induced DNA damage and to the investigation of uninformative cell culture systems.
We have employed a panel of transgenic mouse models and DNA damage-specific immuno-analytical methods to uncover such mechanisms for the clinically important and widely used group of platinum anticancer drugs.
Thereby, we have identified in the inner ear and in the kidney (the two tissues suffering most from the systemic toxicity of cisplatin) a small number of specific “target cells” which accumulate excessive levels of drug-induced platination products in their nuclear DNA. The causing mechanism was identified as aberrant import of cisplatin into these cells by physiological membrane transporters. Measurements of DNA damage kinetics in repair-proficient and -deficient mice clearly linked the levels of cisplatin-DNA intrastrand cross inks to the induction of autophagic cell death and to the functional loss of the inner ear and the kidney. The *in vivo* screening for potential inhibitors of the transporter-mediated uptake of cisplatin disclosed a compound which, when given in combination with cisplatin, prevented the excess formation of DNA damage in the critical cells and averted the nephro- and ototoxicity of the drug without affecting its antineoplastic activity for primary tumors in mice.
With respect to drug resistance in primary tumor cells *in situ* we have exploited a *Ki-ras*^{mut}-driven mouse model for human non-small cell lung cancer (NSCLC). Like in the clinical situation the tumors frequently forfeit their initial responsiveness to cisplatin after several courses of treatment. When analysed for cisplatin-induced DNA damage, most cells of resistant tumors depicted strongly reduced adduct levels in contrast to still unaltered high burden in neighbouring normal lung tissue. Data from *in vivo* adduct kinetics suggest augmented cellular DNA repair capacity to cause the drug-resistant phenotype of NSCLC tumors in mice. To translate these findings into the clinical context we have started to measure adduct kinetics in circulating tumor cells (CTC) isolated from the peripheral blood of NSCLC patients under cisplatin therapy. If we can confirm a similar mechanism CTC analyses could be used as a reliable early biomarker for the onset of cisplatin resistance and as a screening tool for resistance modifiers.

Biography
Dr. Thomale graduated in microbiology and biochemistry from the University of Göttingen and did his Ph.D. at the Max-Plank-Institute for Experimental Medicine, Göttingen in 1977. He did two post doc terms at the Dept. of Pediatric Oncology, University of Hamburg and at the DFG Institute for Mutation Research (Freiburg). In 1985 he joined the group of Prof. Manfred Rajewsky at the Institute of Cell Biology (Cancer Research), University Essen and became a group leader at the West German Cancer Center. Since then his research was focused on the mechanisms of DNA damage and repair and their role in carcinogenesis and in cancer chemotherapy. In 1987 he was a Visiting Scientist of the Max Planck-Society at the Institute of Cell Biology, Academia Sinica, in Shanghai.

Towards damage verification in nucleotide excision repair

Jochen Kuper, Stefanie Wolski, Gudrun Michels, Caroline Kisker

Rudolf Virchow Center for Experimental Biomedicine, University of Würzburg, Würzburg, Germany

DNA damage recognition by the nucleotide excision repair pathway requires an initial step identifying helical distortions in the DNA and a proofreading step verifying the presence of a lesion. This proofreading step is accomplished in eukaryotes by the TFIIH complex. The critical damage recognition component of TFIIH is the XPD protein, a 5'-3' DNA helicase that unwinds DNA and verifies the damage. We solved the three dimensional structure of the XPD protein from *Thermoplasma acidophilum* protein which reveals how its structural framework is combined with additional elements for strand separation and DNA scanning. The protein consists of two RecA-like helicase domains complemented by a 4Fe4S cluster domain and an additional α-helical domain. The first RecA domain together with the helical and 4Fe4S-cluster-containing domains form a central ring with a diameter sufficient in size to allow passage of single stranded DNA. Domain 4 could link ATP-hydrolysis to pore size dynamics thus providing a dynamic model how the pore size can vary upon the expected movement of the two RecA domains during ATP hydrolysis. We pursued extensive mutagenesis studies and were able to verify our previously proposed DNA binding model via a detailed biochemical characterization of the generated variants. We also identified a regulatory hot spot comprised of three amino acid residues regulating helicase activity in a positive and negative way. In addition we have been able to deduce the polarity of the translocated ssDNA strand with respect to the helicase scaffold by co-cristallization with a short single stranded DNA fragment. This has important mechanistic implications for the translocation mechanism of XPD and SF2B heliacses in general.
Based on our results we suggest a model how DNA is bound to the XPD protein and where the potential area of damage verification is located. The structure also provides a framework to explain why some of the mutations in the human XPD gene lead to one of three severe diseases, xeroderma pigmentosum, Cockayne Syndrome, and trichothiodystrophy. Recent data indicate that the 4Fe4S cluster does not only fulfil a structural role, but plays a decisive role in the presence of DNA.

Coordination of DNA replication initiation with centrosome replication, mitosis and RNA transcription

Shijiao Huang, Fei Lu, Qing Jiang and Chuanmao Zhang

The MOE Key Laboratory of Cell Proliferation and Differentiation and the State Key Laboratory of Bio-membrane and Membrane Biotechnology, College of Life Sciences, Peking University, Beijing 100871, China (zhangcm@pku.edu.cn)

DNA replication occurs once and only once per cell cycle to ensure genomic stability, which is strictly controlled by regulating DNA replication initiation. DNA replication initiation is licensed at the end of mitosis by a mechanism of Pre-Replication Complex (Pre-RC) assembly. Recently, more and more evidences show that many Pre-PC proteins function in other cell events, including centrosome duplication, mitosis and RNA transcription, to coordinate proper cell cycle progression.

Pre-RC is assembled by sequential recruitment of ORCs, Cdc6, Cdt1 and MCMs to the DNA replication origins. It is reported that ORC1, ORC2 and MCM5 localize to centrosomes and prevent centrosome reduplication. Geminin is an inhibitor of Cdt1. Through targeting Cdt1 and interfering with Cdt1-MCM interaction, geminin prevents the recruitment of MCM2-7 by Cdt1 to the chromatin during S phase and G2 phase. It has also been reported that knockdown of geminin resulted in centrosome over-duplication in addition to the re-replication of the genome, suggesting geminin might function as a licensing inhibitor of the centrosome duplication. However, the mechanism of centrosome duplication regulated by geminin is unknown. We further found that geminin is localized to centrosomes and this localization is dynein/dynactin-dependent and mediated by actin-related protein 1 (Arp1). Depletion of geminin by siRNA leads to centrosome over-duplication, similar to the defective phenomenon in hydroxyurea-arrested S phase cells, while over-expression of exogenous geminin could rescue the defective centrosome over-duplication induced by hydroxyurea. Our study provides a clue for understanding of the mechanism of centrosome duplication regulated by geminin.

DNA replication licensing proteins are also reported to function in mitosis to coordinate DNA replication and cell division to ensure genome stability. Lack of DNA replication licensing proteins ORC1, ORC2, ORC6 and geminin all result in aberrant mitosis including chromosomes misalignment, multipolar spindle assembly, cytokinesis failure and multinucleated cell formation. We further showed that depletion of Cdc6 leads to aberrant multipolar spindles, over-amplified centrosomes, and multi-nucleated cells, indicating that Cdc6 might coordinate DNA replication and mitosis, although the mechanism of this coordination is still under investigation.

More interestingly, we found that DNA replication correlates with RNA transcription to control the cell proliferation. It has been reported that some DNA replication proteins MCMs are involved in RNA polymerase (Pol) II transcription. In our present work, we found that Cdc6 activates rDNA transcription in the nucleolus by influencing RNA polymerase (Pol) I recruitment and histone methylation state at the promoter of rRNA genes. These results may reveal a mechanism that correlates DNA replication and rDNA transcription by Cdc6.

In summary, our studies together with other previous reports provide the understanding that DNA replication licensing proteins may coordinately regulate the DNA replication initiation, the centrosome duplication, the mitosis and the RNA transcription to ensure the proper cell growth and segregation.

The effect of G-quadruplex structure on telomere replication and recombination

Jin-Qiu Zhou

The State Key Laboratory of Molecular Biology, Shanghai Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai, 200031, China
(jqzhou@sibs.ac.cn)

Telomeres are the protein-DNA complexes at the ends of linear chromosomes in eukaryotes. They are essential for maintaining the genome integrity and play important roles in cellular aging and cancer. The guanine (G)-rich strand of telomeric DNA, which is usually elongated by the reverse transcriptase telomerase, can form a higher order structure, known as a G-quadruplex (G4) in vitro and in vivo. The functional importance of the G4 in telomere maintenance is not well-understood. We discovered that Saccharomyces cerevisiae telomerase subunit Est1 possesses the ability to convert single-stranded telomeric G-rich DNA into G4. This activity of Est1 is required for both telomere replication and protection. Therefore, in addition to telomerase recruitment, Est1p plays roles in both telomerase activation and telomere protection.

References: Zhang et al (2010) *Nat. Struct. Mol. Biol.* 17: 202-209; Tong et al (2011) *Mol. Cell. Biol.* 31(6): 1263-1274

Biography
Dr. Jin-Qiu Zhou obtained his Ph.D. in biochemistry and molecular biology in 1997 from the Universtity of Miami School of Medicine, USA. He received his postdoctoral training at the Department of Molecular Biology, Princeton University, USA from 1998. In 2001, he was appointed as a Principal Investigator in the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (CAS). From 2002 to 2007, he was the Leader of Max Planck Junior Research Group in Institute of Biochemistry and Cell Biology, CAS. His research program is aimed at understanding the processes that eukaryotic cells faithfully maintain their chromosomes to avoid chromosomal instability. Currently, most of his work has been focused on the structure, function and replication of telomeres mainly using the baker's yeast as a model. His group uses a combination of genetic, biochemical and cell biological approaches to address (1) how telomere length and structure are maintained, and the biological relevance of telomere regulation to genome stability and cellular ageing; (2) how a cell establishes the boundary between the telomere silent chromatin and adjacent active chromatin to prevent the telomeric hetrochromatin spreading; (3) what epigenetic codes and information are employed when telomere replication or DNA double-strand break occurs; (4) what is the molecular basis for telomere-length-dependent or -independent cellular aging.

Cyclosporin A, but not everolimus, inhibits nucleotide excision repair via differential regulation of xeroderma pigmentosum proteins which is mediated by calcineurin: Implications for tumorigenesis under immunosuppression

Steffen Emmert

Department of Dermatology, Venereology and Allergology,
University Medical Center Göttingen,
Robert-Koch-Str. 40, D-37075 Göttingen, Germany
(semmert@gwdg.de)

Unlike other immunosuppressive drugs including everolimus, cyclosporin A causes a dramatic increase of UV-induced skin cancer, a feature that is reminiscent of xeroderma pigmentosum, where defective nucleotide excision repair (NER) of UV-induced DNA damage results in cutaneous carcinogenesis. The molecular basis of the clinically very important differential activities of cyclosporin A and everolimus is still unclear. We measured post-UV cell survival of cyclosporin A- and everolimus-treated human fibroblasts and lymphoblasts using MTT. The cellular NER capacity was assessed by host cell reactivation (HCR). Using an ELISA and specific antibodies, cyclobutane pyrimidine and pyrimidine-6,4-pyrimidone photoproduct removal from the cellular genome was measured. The effect of calcineurin on NER was investigated using a calcineurin A expression vector and specific RNAi. Cyclosporin A led to a dose dependent decrease in post-UV cell survival, inhibited NER and blocked photoproduct removal. In contrast, none of these effects where seen in everolimus-treated cells. Overexpression of calcineurin A resulted in increased NER and complemented the Cyclosporin A-induced reduction of NER. Downregulation of calcineurin using RNAi inhibited NER comparable to cyclosporin A-treatment. We further investigated the molecular mechanisms of CsA-induced NER reduction by measuring the xeroderma pigmentosum (XP) protein expression of all known XP genes (XPA-XPG). Western blot analyses revealed that XPA and XPG protein expression were reduced in the cytosol of GM00637 fibroblasts exposed to 0.1 µM or 0.5 µM CsA, respectively. Calcineurin knockdown led to the same results suggesting the involvement of calcineurin-dependent signalling in XPA and XPG protein regulation. CsA-induced reduction of NER could be complemented by overexpression of either XPA or XPG protein as assessed by host cell reactivation (HCR) and transfection of XPA or XPG cDNA-containing plasmids. Likewise, XPA-deficient fibroblasts stably corrected with XPA (XP2OS-pCAH19WS) did not retain the inhibitory effect of CsA on NER. In contrast, CsA reduced NER in XPC-deficient fibroblasts (XP4PA-SV-EB) complemented with XPC. We conclude that cyclosporin A, but not everolimus, leads to an increased skin cancer risk via a calcineurin signalling-dependent impairment of NER. Our data indicate that the CsA-induced inhibition of NER is a result of the downregulation of XPA and XPG protein in a calcineurin-dependent manner.

Work was predominately supported by the DFG and the Deutsche Krebshilfe.

References:
Eggermont AM. et al. (2008) Lancet, 372,117-126; Schoof N. et al. (2009) Genes and Immunity, 10, 586-590; Böckmann L et al. (2009) Pharmacogenet. Pharmacogenomics, 19, 760-769; Haenssle H. et al. (2010) Arch Dermatol. 146, 257-264; Thoms KM. et al. (2011) Exp Dermatol. 20, 232-236.

Biography
Prof. Emmert obtained his M.D. in 1996 from the University of Würzburg, Germany, and then joined the Department of Dermatology in Göttingen as an intern and resident. From 1997 until 2000 he was a research fellow at the National Cancer Institute, NIH, Bethesda, MD, USA, and continued research in UV-induced (skin-) cancer, DNA repair, and nucleotide-excision-repair-defective syndromes like xeroderma pigmentosum since then. Prof. Emmert is board certified in Dermatology and Venereology (2002), Allergology (2003), Phlebology (2005), and Medicamentous Tumor Therapy (2007). He was appointed as University-Professor for Dermatology and Venereology at the Department in Göttingen in 2005 and holds the position of an executive senior physician. Prof. Emmert performs clinical studies mainly in the field of Dermato-Oncology. In his basic research laboratory he aims to elucidate DNA repair functions, tumor risk factors, and cancer treatment resistance factors based on genetic model diseases like xeroderma pigmentosum and tries to translate these basic findings into clinical application. He is also interested in natural products like herbs that can reduce DNA damage formation or enhance DNA repair in the skin.

NFκB/p53 crosstalk – a new therapeutic target linked to replicational stress and DNA repair signaling

Oliver H. Krämer

Center for Molecular Biomedicine, Friedrich-Schiller-University Jena, Institute of Biochemistry, Hans-Knöll-Str. 2, D-07745 Jena, Germany
(Oliver.Kraemer@uni-jena.de)

The transcription factors p53 and NF-κB determine cellular fate and are involved in the pathogenesis of most -if not all- cancers. The crosstalk between p53 and NF-κB becomes increasingly appreciated as an important mechanism operative during stress, tumorigenesis, metastasis, and immunological surveillance. A prominent example is replicational stress which activates NF-κB p65 and triggers its nuclear interaction with p53. Remarkably, both proteins are required for increased NF-κB activity during S-phase checkpoint activation involving the kinases ATM and CHK1. The pro-inflammatory cytokine TNF-α also triggers the formation of a transcriptionally active nuclear p65-p53-complex. Hence, p53 is unexpectedly necessary for NF-κB-dependent gene expression induced by atypical and classical stimuli. Data from gain- and loss-of function approaches furthermore argue that a p53 hot-spot mutant, which is frequently found in tumors, constitutively evokes anti-apoptotic NF-κB. These observations suggest possible explanations why p53 mutations rather than its complete loss arise in tumors of various origins. In addition, the p53-NF-κB signaling module represents a target for novel intervention strategies using established compounds and powerful combination therapies.

This work is supported by Deutsche Krebshilfe.

References: Schneider et al. (2010) Oncogene 29(19): p. 2795-806; Schneider and Krämer (2011) BBA-Rev Cancer, 1815(1):90-103

Biography
Dr. Krämer studied Biology and Pharmacology at the Ruprecht-Karls-University in Heidelberg, Germany, and at the University of Adelaide, Australia. He obtained his Ph.D. in Biochemistry in 2004 from the Johann-Wolfgang-Goethe-University of Frankfurt/Main, Germany. He completed his postdoctoral training at the Georg-Speyer-Haus Frankfurt/Main, Germany and at the Institute of Biochemistry, Friedrich-Schiller-University Jena, Germany. In 2010 he became associate professor and group leader at the Center for Molecular Biomedicine, Friedrich-Schiller-University Jena, Germany. His research program elucidates the crosstalk between transcription factors and how posttranslational modifications alter protein functions. His group specifically analyzes proteins relevant for tumorigenesis and focuses on their acetylation, phosphorylation, sumoylation and ubiquitylation. As part of a translational research program, he is involved in studies on the cellular and biochemical effects of histone deacetylase inhibitors alone and in combination with kinase inhibitors, cytokines and genotoxic drugs.

The role of telomeric proteins in telomere maintenance and human diseases

Dong Yang, Yi Zhang and Zhou Songyang
Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas 77030. School of Life Sciences, SYSU, Guangzhou, P.R. China

In mammalian cells, the telomeres are bound by the core telomeric proteins TRF1, TRF2, RAP1, TIN2, TPP1, and POT1. These six telomeric proteins form large protein complexes and recruit different signaling molecules for telomere length control and the protection of telomeres from being recognized as DNA double-stranded breaks. Among the six core telomeric proteins, TIN2 interacts with several core telomeric proteins and plays a major role in telomere protein complex formation. Our previous study has demonstrated that TIN2-TPP1 interaction is critical for telomeric protein complex formation, and spatial regulation of TIN2 and TPP1 localization plays an important role in telomere maintenance. Mutation of TIN2 has also been implicated in human disease Dyskeratosis congenita. However, the molecular mechanism that TIN2 mutation causes Dyskeratosis congenita is largely unknown. To understand role of TIN2 in telomere protection and Dyskeratosis congenita, we expressed TIN2 miss-sense mutants in human cells and found that these mutants induced telomere shortening as in Dyskeratosis congenita. We further investigated the effects of TIN2 mutants on telomere end protection and their interaction with other telomeric proteins. Our findings indicate that TIN2 mutants are defective for telomere length control that may lead to Dyskeratosis congenita. In addition to its nuclear localization, we found an unexpected role of TIN2 in the mitochondria. The mitochondrial localization of TIN2 is mediated by its N-terminal targeting sequences. TIN2-TPP1 interaction mediates nuclear localization and inhibits mitochondrial localization of TIN2. Overexpression of mitochondrial TIN2 led to mitochondrial structural change and dysfunction. In addition, TIN2 regulates ATP synthesis. Our findings indicate that TIN2 is a multi-function protein, linking telomere regulation to metabolic control.

Reference: Xin et al.(2007), *Nature*, 45, 559-62. Chen et al. (2007), *Mol. Cell Biol.* 27: 5898-909. Kim et al. (2009), *Nature Struc. Mol. Biol.* 16: 372-9. Yang et al. (2011), *J Biol Chem*. 286:23022-30.

Biography

Dr. Songyang obtained his Ph.D.in Molecular Physiology in 1995 from Tufts University, USA. He did his postdoctoral training in Harvard Medical School and Massachusetts Institute of Technology. In 1998, he started his own laboratory at Baylor College of Medicine, USA. In 2008, he was elected as an endowed Professor of Biochemistry and Molecular Biology at Baylor College of Medicine. In 2009, he became the Dean of School of Life Sciences, SUN-YAT SEN University, P.R. China. Dr. Songyang is interested in the molecular mechanisms that regulate cell survival, genome stability, stem cell pluripotency, and cancer initiation through proteomic and functional genomic approaches. Dr. Songyang's major research areas include telomeres and telomerase, DNA damage repair signaling, and embryonic stem cell self-renewal and differentiation. Dr. Songyang has also developed several technology platforms to study protein-protein interactions and signaling pathways.

DNA Repair: Epigenetics and Immunology

Chair: Tanja Schwerdtle

Wednesday, 19 October
15.00-19.30

Influence of oxidative stress on DNA repair

Bernd Epe

Institute of Pharmacy and Biochemistry, University of Mainz,
Staudingerweg 5, D-55099 Mainz, Germany
(epe@uni-mainz.de)

Oxidative DNA base modifications such as 8-oxo-7,8-dihydroguanine (8-oxoG) are endogenously generated in apparently all types of cells and continuously removed by special repair glycosylases, e.g. OGG1 in the case of 8-oxoG. We have previously shown that a deficiency of OGG1 not only causes the expected repair retardation of the substrate lesions in cultured cells, but also increases the basal (steady-state) levels of 8-oxoG in mice, increases the spontaneous mutation frequencies (observed in a transgenic *lacI* locus) and initiates carcinogenesis. The OGG1 activity and its cellular regulation therefore appear to be of high relevance for the integrity of the genome. Surprisingly, we found that the base excision repair initiated by OGG1 is vulnerable to oxidative stress. This is particularly pronounced for a rather frequent variant of the human enzyme, OGG1-326Cys. The repair retardation observed in human lymphocytes that are homozygous for this variant can be prevented if the cells are supplemented with the antioxidant dithiothreitol during the repair incubation. The Ogg1-326Cys cells also have elevated spontaneous levels of micronuclei, suggesting a link between a defective removal of oxidative base modification and the generation of chromosomal damage. Interestingly, treatment of mice with the polyphenol resveratrol, a constituent of red wine, gives rise to a significant reduction of the basal 8-oxoG levels in the livers of the animals, associated with an (adaptive?) upregulation of several proteins of the antioxidant response (SOD1, SOD2, GPX1, HO1). The decrease of the endogenous DNA damage levels, however, is not caused by improved OGG1 activity, but rather by a lower generation of the oxidative DNA modifications. In cultured AS52 cells, resveratrol is a rather potent inhibitor of the repair of different types of DNA damage (8-oxoG, pyrimidine dimers and single-strand breaks). The effect appears to be mediated by a compaction of the chromatin structure, which is observed under the same conditions. The underlying mechanisms are presently under investigation.

The work is supported by the Deutsche Forschungsgemeinschaft (EP11/11-1 and EP11/8-2).

References

Osterod et al (2002) Oncogene 21, 8232-39; Trapp et al. (2007) Oncogene 26, 4044-48; Trapp et al (2007) Cancer Res. 67, 5156-61; Khobta et al. (2010) Nucleic Acids Res. 38, 4285-95; Fusser et al. (2011) Carcinogenesis, 32, 80-85.

Biography

Bernd Epe obtained his Ph.D. in Organic Chemstry at the University of Kiel in 1977. He was post-doc at the Max-Planck-Institute for Molecular Genetics, Berlin and habilitated at the Institute of Pharmacology and Toxicology of the University of Würzburg in 1989. Since 1994, he is full Professor of Pharmacology and Toxicology at the Institute of Pharmacy and Biochemistry of the University of Mainz. His research is focussed on oxidative DNA damage in mammalian cells, its repair and its consequences for mutagenesis and carcinogenesis.

Oxidation of DNA Methylcytosines in Epigenetic Regulation

Fan Guo, Qinyan Cui, Runrui Zhang, Tianpeng Gu and Guoliang Xu

Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, 320 Yueyang Rd, Shanghai, China
(glxu@sibs.ac.cn)

The mammalian genomic DNA is methylated at 5% of all cytosines. Genomic methylation patterns vary among different cell-types. Sperm and oocytes have distinctive DNA methylation states that are adjusted by reprogramming upon fertilization in the early embryo. The paternal genome from sperm is thought to undergo active demethylation in fertilized eggs before the first mitosis. The mechanism and biological significance of this paternal epigenome remodeling are unclear. We find that hydroxylation of 5-methycytosines (5mC) occurs specifically on the paternal genome in mouse zygotes, which coincides with the loss of 5mC. Tet3, a member of the recently characterized dioxygenase family, is enriched in the paternal pronucleus. In zygotes lacking Tet3, hydroxylation of 5mC in the paternal genome fails to occur while the 5mC level remains constant. Therefore, decrease of 5mC in the paternal genome in developing zygotes is likely caused by its conversion to 5hmC that requires Tet3. Moreover, female mice lacking Tet3 in the germ line show significantly reduced fertility. Therefore, paternal epigenome remodeling associated with Tet3-mediated hydroxylation is important for embryonic development. Besides the role of DNA oxidation in zygotes, hydroxylation appears to be implicated in germ cell specification as well as in neurogenesis. The mechanism of DNA oxidation in connection with demethylation and transcriptional activation will also be discussed.

References: Gu et al. (2011) The role of Tet3 DNA dioxygenase in epigenetic reprogramming by oocytes. Nature, DOI 10.1038/nature10443, in press; He et al., (2011) Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. Science 333, 1303-1307.

Biography
Dr. Guoliang Xu obtained his Ph.D. in molecular genetics in 1993 from the Max-Planck Institute for Molecular Genetics, Germany. He completed his postdoctoral training at the Dept Genetics and Development, Columbia University and continued his studies on DNA methylation as a Principal Investigator at the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China. His group conducts studies on the mechanism of DNA methylation and demethylation. Recently his group has shifted their focus on DNA oxidation that is mediated by dioxygenases.

An epigenetic screen of human DNA repair genes in head and neck squamous cell carcinoma: aberrant promoter methylation of NEIL1

Odilia Popanda, Jittiporn Chaisaingmongkol, Rolf Warta, Gerhard Dyckhoff, Christel Herold-Mende, Christoph Plass, Peter Schmezer

Division of Epigenomics and Cancer Risk Factors, German Cancer Research Center (DKFZ), Department of Otorhinolaryngology, Head and Neck Surgery, University of Heidelberg (o.popanda@dkfz.de)

Aberrant DNA methylation within the promoter region of different DNA repair genes such as *MGMT*, *MLH1*, or *MSH2* is known to play a critical role in the development and progression of various cancer types including head and neck squamous cell carcinomas (HNSCC). A systematic analysis of known human repair genes for promoter methylation is however missing although 98% of the repair genes contained CpG islands in their promoter region, a finding which highlights DNA methylation as a possible regulatory mechanism of repair gene expression.
We therefore generated quantitative promoter methylation profiles of 160 human DNA repair and repair-related genes using bisulfite conversion of DNA and MassARRAY technology* in a set of DNAs from fresh frozen HNSCC (n=20) and normal mucosa (n=5) samples. Methylation readout was obtained for 146 genes, and 15 genes showed more than 20% differences in methylation level between tumor and normal tissues. Differences manifested either as hypermethylation or hypomethylation in the tumor. Correlation analyses of promoter methylation and mRNA expression (measured by quantitative RT-PCR) identified the DNA glycosylase NEIL1 as the most prominent candidate which was therefore selected for a more detailed characterization. For further verification, tumor-specific *NEIL1* promoter hypermethylation was determined in additional tissue samples (HNSCC, n=135; normal mucosa, n=38), HNSCC cell lines and in primary human skin keratinocytes. The methylation level was significantly elevated in HNSCC versus noncancerous tissue or cells ($p<0.0001$) thus supporting our screening results. The difference in *NEIL1* methylation was confirmed in laser-microdissected tumor and non-tumor tissues (n=6 pairs). Finally, immunohistological staining of NEIL1 showed lower protein expression in tumor tissues. In addition, we investigated the functional impact of *NEIL1* promoter methylation. Demethylating experiments using 5-aza-2'-deoxycytidine (5-Aza-dC) or DNMT1 knockdown demonstrated the re-expression of NEIL1 in HNSCC cell lines with initially hypermethlyated promoter regions and low NEIL1 expression.
In conclusion, our results suggest that DNA methylation at the identified CpG units might contribute to down-regulate *NEIL1* expression and might thus play a role in modulating treatment outcome in HNSCC.

*References: Goeppert et al., Hepatology 2010, 52(6), 2023-2033; Kuhmann et al., Radiotherapy and Oncology 2011, in press.

Biography
After a diploma in chemistry, Dr. Popanda obtained in 1985 a PhD in biochemistry at the University of Heidelberg, Germany. She was working as a postdoc and senior scientist at the DKFZ (German Research Center) in Heidelberg in the Divisions of "Interaction of Carcinogens with Biological Macromolecules" and "Toxicolgy and Cancer Risk Factors". Since 2007, she is a senior scientist in the Division of "Epigenomics and Cancer Risk Factors" at the DKFZ. The special aim of her research is the identification of functional deficiencies in DNA damage response and to analyze their impact on cancer risk and radiation therapy outcome, focusing on the occurrence of adverse side effects. Currently, our group is concentrating on studies how epigenetic patterns affect DNA repair activity and whether such patterns can be modulated by radiation or further endogenous or exogenous influences.

Mechanisms of epigenetic reprogramming in the mouse zygote

Mark Wossidlo1, Julia Arand1, Vittorio Sebastiano2,*, Konstantin Lepikhov1, Michele Boiani2, Hans Schöler2 and Jörn Walter1,4

1. Dept. of Genetics/Epigenetics, Campus Saarbrücken, Saarland University, 66123 Saarbrücken, Germany
2. Max-Planck-Institut für molekulare Biomedizin, Röntgenstraße 20, 48149 Münster, Germany

The genomes of sperm and egg are structurally and epigenetically reprogrammed during the earliest phases of development. This (re)programming assures that pluripotency can be established in the developing cleavage stage embryos. A major molecular alteration during this phase of reprogramming is the genome wide loss of 5-methylcytosine (5mC) during the first cell cycle(s) in the mammalian zygote (embryos). The precise molecular mechanisms of such active epigenetic reprogramming processes are not yet defined and the traget regions in the genome are not yet understood. We recently have shown that a great proportion of 5mC of the sperm and egg chromosomes is rapidely and extensively oxidised to 5-hydroxymethylcytosine (5hmC)[1]. The role of Tet mediated oxidation of 5mC in stem cells biology has recently been shown by several groups[2]. A knockdown of the 5hmC generating dioxygenase Tet3 strongly affects this conversion of 5mC into5hmC in the zygote[1]. This establishes a functional link between DNA-demethylation and oxidation reactions. Based on sequencing data we also find that a small proportion of the 5mC/5hmC in the zygote genome is actively converted to cytosine before DNA replication commences during the first cell cycle [3]. Base excision repair has been discussed to be linked to such proposed active demethylation mechanisms. Indeed we find the appearance of DNA strand breaks and DNA repair markers such as gH2A.X and PARP-1, respectively at prereplicative stages[3]. In my presentation I will discus our present knowledge on such possible links between DNA repair and DNA-demethylation.

1. M. Wossidlo, T. Nakamura, K. Lepikhov, C.J. Marques, V. Zakhartchenko, M. Boiani, J. Arand, T. Nakano, W. Reik and J. Walter. 5-hydroxymethylcytosine in the mammalian zygote is linked with epigenetic reprogramming. *Nat Commun 2011, 2: 241.*

2. Walter J. An epigenetic tet a tet with pluripotency. *Cell Stem Cell. 2011 Feb 4;8(2):121-2.*

3. M. Wossidlo, J. Arand, V. Sebastiano, K. Lepikhov, M. Boiani, R. Reinhardt, H. Schöler and J. Walter.Dynamic link of DNA demethylation, DNA strand breaks and repair in mouse zygotes. *Embo J. 2010, 29(11):1877-88.*

Biography data
Scientific Career

1990-92	Postdoc, with Thomas Trautner, MPI für molekulare Genetik, Berlin
1992-1994	Postdoc (EU/EMBO-Fellowship) with W. Reik, BBSRC Cambridge, UK
1994-2000	Group leader at the MPI für molekulare Genetik, Berlin
1994-2000	Group leader (Head of laboratory), MPI für molekulare Genetik, Berlin
1999-2000	Habilitation in Genetics and Privat-Dozent, Humboldt-Universität zu Berlin
since 11/2000	Chair of Genetics at the Saarland University, Saarbrücken

External scientific activities

1999	Co-founder of the Epigenomics AG, Berlin/Seattle and Member of the Science Advisory Board
2008-2010	Member Faculty of 1000 (Genetics)
since 2011	Member of the AG Gentechnologiebericht of the Akademie der Wissenschaften zu Berlin

The function of Rad9 in the antibody generation.

Lili An ‡§ , Yulan Wang ‡§ , Yuheng Liu ‡§ , Xiao Yang ‡§ , Chunchun Liu ‡§ , Zhishang Hu ‡§ , Wei He ‡§ , Wenxia Song ¶ , and and Haiying Hang ‡§[1]

From the ‡ National Laboratory of Biomacromolecules, and the § Center for Computational and Systems Biology, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China and the ¶ Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, Maryland 20742. [1]Corresponding author.

B cell maturation and B cell-mediated antibody response require programmed DNA modifications such as the V(D)J recombination, the immunoglobulin (Ig) class switch recombi-nation, and the somatic hypermutation to generate functional Igs. Many protein factors involved in DNA damage repair have been shown to be critical for the maturation and activation of B cells. Rad9 plays an important role in both DNA repair and cell cycle checkpoint control. However, its role in Ig generation has not been reported. In this study, we generated a conditional knock-out mouse line in which Rad9 is deleted specifically in B cells and investigated the function of Rad9 in B cells. The Rad9-/- B cells isolated from the conditional knock-out mice displayed impaired growth response and enhanced DNA lesions. Impaired Ig production in response to immunization in Rad9-/- mice was also detected. In addition, the Ig class switch recombination is deficient in Rad9-/- B cells. Taken together, Rad9 plays dual roles in generating functional antibodies and in maintaining the integrity of the whole genome in B cells. The mechanisms underlying the role of Rad9 in antibody generation will be discussed.

Selected Publications:
He W, Ma X, Yang X, Zhao Y, Qiu J, Hang H*. A role for the arginine methylation of Rad9 in checkpoint control and cellular sensitivity to DNA damage. *Nucleic Acids Res.* 2011 Feb 14. [Epub ahead of print]
An L, Wang Y, Liu Y, Yang X, Liu C, Hu Z, He W, Song W and Hang H*. Rad9 is required for B cell proliferation and immunoglobulin class switch recombination. *J Biol Chem.* 2010 Nov 12;285(46):35267-73. Epub 2010 Aug 20.
He W, Zhao Y, Zhang C, An L, Hu Z, Liu Y, Han L, Bi L, Xie Z, Xue P, Yang F, Hang H*. Rad9 plays an important role in DNA mismatch repair through physical interaction with MLH1. *Nucleic Acids Res*. 36:6406-17 (2008).

Biography
Birthday: 1957-9-20
Education and Career:
2004-present, Professor, DNA repair and cell cycle control, Institute of Biophysics, Chinese Academy of Sciences.
1994-2004, Postdoc, Research Associated Scientist and Assistant Professor DNA repair and cell cycle control Columbia University, New York.
1988-1994 Ph. D. Radiation Oncology, Colorado State University.
1983-1986 MS. Reproduction Biology, Institute of Zoology, Chinese Academy of Science.
1978-1982, BS., Biochemistry, Sichuan University, China.

Research interest:
*Hus1, Rad1*and *Rad9* are a group of cell cycle checkpoint genes conserved from yeast to human. The three proteins form a ring structure complex (9-1-1protein complex). The complex can circle and slide on DNA duplex. It physically interacts with multiple cell cycle control and DNA repair proteins. We are currently dissecting the mechanisms how the genes work in cell cycle control, DNA repair and tumor prevention.

Applications of class III histone deacetylase in cancer research

Ying Zhao, Xiangyu Liu, Donglai Wang and Wei-Guo Zhu
Department of Biochemistry and Molecular Biology,
Peking University Health Science Center, Beijing 100191, China

Histone deacetylases (HDAC) play a critical role in regulating gene expression and thus are considered to be an important target of anti-neoplastic therapy. Among the three classes of HDACs, class III HDACs, also called Sirtuins, are quite different from other classes of HDACs in biological functions. Especially, class III HDACs need NAD+ as a co-enzyme to deacetylase histone and non-histone proteins. Here we report that SIRT2 is a key enzyme to block autophagic process in human cancer cells. SIRT2 binds to FoxO1 in untreated cells and keeps FoxO1 as a deacetylated status, and disassociation of SIRT2 from FoxO1 in response to serum starvation or oxidative stress makes FoxO1 as an acetylated form, by which in turn elicits autophagy. We also found that SIRT1 specially interacts with histone methyltransferase Set7/9 and becomes methylated. However, the Set7/9-mediated methylation of SIRT1 is dispensable for SIRT1's inhibitory function on p53. Further study demonstrate that the interaction between SIRT1 and Set7/9 induces a possible changes in ability of SIRT1 binding with p53, and relatively increases p53 transactivity.

Histone deacetylases play a critical role in regulating gene expression and thus are considered to be a target of anti-neoplastic therapy. Among the three classes of histone deacetylases, class III histone deacetylases, Sirtuins, are quite different from other classes of histone deacetylases in biological functions. Especially, class III histone deacetylases need NAD+ as a co-enzyme to deacetylase histone and non-histone proteins. Here we report that Sirtuin 2 is a key enzyme to block autophagic process in human cancer cells. Sirtuin 2 binds to FoxO1 in untreated cells and keeps FoxO1 as deacetylated status, and disassociation of Sirtuin 2 from FoxO1 in response to serum starvation or oxidative stress makes FoxO1 as an acetylated form, by which in turn elicits autophagy. We also found that Sirtuin 1 specially interacts with histone methyltransferase Set7/9 and becomes methylated. However, the Set7/9-mediated methylation of Sirtuin 1 is dispensable for Sirtuin 1's inhibitory function on p53. Further study demonstrates that the interaction between Sirtuin 1 and Set7/9 induces a possible changes in ability of Sirtuin 1 binding to p53, and relatively increases p53 transactivity.

Bilateral Research Projects

http://www.sinogermanscience.org.cn

The funding program "Sino German Research Projects" offers the opportunity for mid-term bilateral co-operation (up to three years) between at least one researcher from Germany and one from China.

Within this funding scheme the Sino German Center provides financial support for consumables, smaller scientific equipment as well as travel and accommodation costs. Basically the Sino German Center can not provide money for personnel (with the only exception within this program for the German applicants because of a particular agreement with DFG).

Precondition for applications is a previously received funding by the Sino German Center in another funding program, e.g. the participation in a Center-funded workshop. Chinese applicants must be holder of a current or previous grant from the National Natural Science Foundation of China*. German applicants must be eligible to apply at the Deutsche Forschungsgemeinschaft.

* This precondition needs not to be fulfilled for young researchers below the age of 35.

Sino-German Co-operation Groups

(http://www.sinogermanscience.org.cn)

Sino-German Co-operation Groups bring together several research groups with complementary expertise from a small number of universities or research institutions in China and in Germany. They aim to support a concentrated joint research effort in a specific field. They can, e.g. be a vehicle for preparing a large-scale collaborative research endeavor.

Funding is provided for workshops and the exchange of scientists, to some extent also for research materials and joint publications.

Agreement between NSFC and DFG on support of Sino-German joint interdisciplinary research programs (SG-JIRP) (“ “in Chinese)

On the basis of the “Agreement on Cooperation between the National Natural Science Foundation of China and Deutsche Forschungsgemeinschaft” as executed on July 19, 2004, the National Natural Science Foundation of China NSFC (hereinafter referred to as “NSFC”) with its address at 83, Shuangqing Road, Haidian District, Beijing, 10085, P.R. of China and the Deutsche Forschungsgemeinschaft (hereinafter referred to as “DFG”), with its address at Kennedyallee 40, 53170 Bonn, Germany, hereinafter collectively referred to as “the Parties”, agree to support Sino-German joint interdisciplinary research programs as follows:

Definition

This program, established by NSFC and DFG, will be a platform for scientists from both countries to conduct interdisciplinary joint research. Groups of researchers at a limited number of research institutions, based at one principal location in China and one principal location in Germany, can combine their expertise, agree on a common research topic, identify common research goals and submit joint proposals to the Parties for funding , within the spheres of competence of the Parties, to undertake ambitious long-term research at an internationally competitive level, promote interdisciplinary cooperation, and advance young researchers. For the researchers in China, the regulations and procedures of the NSFC are applicable. For the researchers in Germany, the regulations and procedures of the DFG program “Collaborative Research Centre / Transregio” are applicable.

The Parties agree to take joint responsibility for the evaluation of proposals by peer review, and separate responsibility for the funding of the participating researchers in their country. The funding period runs for four years, with the possibility of maximum two extensions. In addition, the following guidelines for the joint support of SG-JIRP apply.

Preparation of Pre-proposals

The Parties shall encourage researchers in China and Germany interested in the implementation of a SG-JIRP to inform them in the early stages of planning and to define coordinators for the Chinese and German side, and to make use of the following forms of cooperation to prepare their joint proposal:

(1) exchange of researchers,

(2) joint seminars,

(3) joint individual projects,

(4) joint workshops for the preparation of proposals for SG-JIRP, based on the “Agreement on Cooperation between the NSFC and DFG” (July 19, 2004). Also “Sino-

German Cooperation Groups" supported by the "Sino-German Centre for Research Promotion" can be suitable elements of the preparatory phase. The Parties shall give advice based on their regulations and procedures.

When a group of researchers in China and Germany has agreed to a common research program, they may submit their initiative as a joint pre-proposal to the Parties, including an outline of the joint research plan and the type and extend of funding required, information about the institutional background, outlines for the individual contributions of the participating scientists, and their curriculum vitae and lists of publications. The parties shall consult each other and the coordinators on issues such as eligibility for funding, the suitable form of cooperation, and the further processing of the initiative.

If the Parties agree to proceed, they will organize a joint workshop in Germany or China, including representatives of the applicants, the Parties, and additional scientific experts as jointly nominated by the Parties, for the presentation and discussion of the scientific merits of the pre-proposal and its suitability for a SG-JIRP. The results of this workshop, based on the advice given by the scientific experts, will be summarized in minutes jointly agreed upon by the Parties.

Based on these results of the workshop, the Parties shall consult each other and jointly decide if the formulation of a full proposal will be admitted, and will inform the coordinators about this decision.

Prepapration of Full-proposals

If the Parties agree to proceed, the researchers or their research institutions may submit a joint full proposal, based on their respective regulations and procedures. In particular, the full proposal shall contain detailed information on the joint research plan, the institutional background, the individual contributions of the participating scientists, the level and procedures suggested for the Sino-German cooperation, and the level of support requested.

Evaluation and Decision Procedure

If the Parties agree to proceed, the full proposal shall be evaluated in the framework of a site visit at either the principal Chinese or the principal German location. The Parties will jointly nominate the international independent review panel and organize the site visit. The advice given by the review panel shall be summarized in minutes jointly agreed upon by the Parties.

The Parties take separate funding decisions through their responsible decision-making bodies, following their respective regulations and procedures, based on the scientific quality of the proposal as described in the minutes of the review panel meeting. The SG-JIRP can

be established only if the Parties both take a positive decision and agree on the first time period of funding.

During the last year of a current funding period, the applicants may be invited to submit a renewal proposal for the next funding period. For renewal, a pre-proposal will not be required.

For the nomination of the scientific experts participating in the joint workshop and the review panel for the evaluation of the full proposal, the guidelines of the Parties for conflict of interest are applicable.

Intellectual Property Rights

The Parties encourage researchers and institutions to enter into agreements to ensure the effective protection and correct distribution of intellectual properties resulting from research funded under this agreement.

Miscellaneous

In addition to the joint support of SG-JIRP as described above, other procedures for parallel support of cooperation between researchers funded by the DFG in Germany and the NSFC in China will be possible. In particular, the Parties may support the coordination of research programs, exchange of researchers and joint workshops between researchers based on individual agreements.

Executed in Beijing on April 11, 2005

NSFC DFG