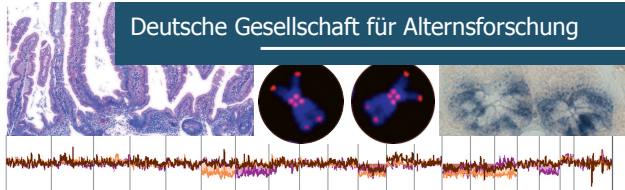


Deutsche Gesellschaft für Alternsforschung



Annual Meeting of the German Association for Aging Research 02. – 03. December 2016 Ulm



CEMMA

cellular and molecular mechanisms in aging



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PREAMBLE

The German Society for Aging Research (DGfA) is delighted to welcome you to this year's meeting here in Ulm. It is a great pleasure to host the German community of aging researchers in our small but beautiful Ulm University. We are honoured to welcome our three outstanding keynote lecturers Judith Campisi, Darren Baker and Valter Longo. Furthermore, we welcome all members of the DGfA without whom organization of the meetings wouldn't be possible. We are looking forward to interesting talks and lively discussions at the posters and during the scientific sessions. One of the goals of the DGfA is connecting researchers in the field of aging, promoting networking and integrating young scientists at the beginning of their career. The annual meeting of the DGfA is the perfect platform to establish collaborations and discover synergies between research groups to gain further insight into the biology of aging and aging-associated diseases. On behalf of the DGfA we wish you a successful meeting and a good time here in Ulm.

**Prof. Björn
Schumacher**
President of
the DGfA
University of
Cologne

**Prof. Karin
Scharffetter-
Kochanek**
Department of
Dermatology and
Allergology
University Clinic Ulm

**Prof. Hartmut
Geiger**
Institute for
Molecular
Medicine
Ulm University

**Prof. Hans
Kestler**
Institute of
Medical Systems
Biology
Ulm University

Friday, 2nd of December 2016

12.00 – 01.00 pm
01.00 pm

REGISTRATION + SNACKS
OPENING REMARKS

01.05 – 01.50 pm

KEYNOTE LECTURE

Darren Baker

Kogod Center on Aging, Mayo Clinic, Rochester, USA

SESSION 1

Chair: Maria Ermolaeva & Wolfgang Wagner

01.50 – 02.05 pm

Thomas von Zglinicki, Newcastle University

Cellular senescence drives age-dependent hepatic steatosis

02.05 – 02.20 pm

Ani Grigoryan, Ulm University

Cdc42-LaminAC axis regulates nuclear architecture changes and drives HSC aging and rejuvenation

02.20 – 02.35 pm

Johanna Heid, Goethe University Frankfurt

Up-regulation of microRNA-29 family protects from cardiac hypertrophy and fibrosis modulating DNA methyltransferases in *Nothobranchius furzeri*

02.35 – 02.50 pm

Ilwook Kim, Leibniz Institute on Aging, Jena

Intestine-specific Sirt1 deletion improves aging phenotypes in telomere dysfunctional mice

02.50 – 03.05 pm

Thomas Wilhelm, IMB Mainz

Post-reproductive inhibition of autophagosome formation extends lifespan in *C. elegans*

03.05 – 03.30 pm

COFFEE BREAK

03.30 – 04.15

KEYNOTE LECTURE

Valter Longo

Longevity Institute, University of Southern California,
Los Angeles, USA

SESSION 2

Chair: Carolina Florian & Thomas von Zglinicki

04.15 – 04.30 pm

Novella Guidi, Ulm University

Stroma-derived osteopontin regulates and attenuates aging-associated phenotypes of HSCs

04.30 – 04.45 pm

Rudolph Wiesner, University of Cologne

Mitochondrial DNA deletions in muscle stem cells lead to impaired regeneration capacity with fibrosis and adipocyte infiltration

04.45 – 05.00 pm

Simon Schwörer, Leibniz Institute on Aging, Jena

Epigenetic stress responses induce muscle stem cell aging by Hoxa9 developmental signals

05.00 – 05.15 pm

Rajiv Lochan Tiwari, Ulm University

Wnt5a-Cdc42 signaling regulates aging of hair follicle stem cells

05.15 – 05.35 pm

COFFEE BREAK

SESSION 3

Chair: Gabriele Saretzki & Sebastian Iben
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05.35 – 05.50 pm

Prerana Chaudhari, Leibniz Institute on Aging, Jena

Using *C. elegans* as a model for studying host-microbiome interactions

05.50 – 06.05 pm

Manfred Gogol, Lindenbrunn Hospital, Coppenbruegge
Cognition, Outcome and Advanced Glycation
Endproducts in Geriatric Inpatients

06.05 – 06.20 pm

**Cornelis Calkhoven, European Institute for the Biology
of Ageing (ERIBA), Groningen**
Reduced expression of C/EBP β -LIP extends health-
and lifespan

06.20 – 07.35 pm

POSTER SESSION with SNACKS and BEER
(Foyer N27)

07.35 pm

**DEPARTURE to the ULMER MUSEUM for FUN, FOOD,
BEER and WINE and GUIDED TOURS through the
MUSEUM: LÖWENMENSCH, HfG and COLLECTION
FRIED**
(Busses wait in front of N27 building)

Saturday 3rd of December

08.30 – 09.30 am ARRIVAL + SNACKS

09.30 – 10.15 am KEYNOTE LECTURE

Judith Campisi

Buck Institute for Research on Aging, Novato, USA

SESSION 4

Chair: Julia von Maltzahn & Rudolph Wiesner

10.15 – 10.30 am

Karin Scharffetter-Kochanek, University Clinic Ulm

In-silico modeling of the Senescence Associated
Secretory Phenotype

10.30 – 10.45 am

Kim Zarse, ETH Zürich

Global Impairment of Insulin Signalling in Adult Mice
Fails to Extend Lifespan

10.45 – 11.00 am

Gabriele Saretzki, Newcastle University

Beneficial effects of telomerase activators on
balance and motor function-a possible treatment for
Parkinson's disease?

11.00 – 11.15 am

Gilbert Weidinger, Ulm University

Zebrafish heart regeneration requires alleviation of genomic stress by BMP signalling

11.15 – 11.30 am

Meenakshi Ravichandran, ETH Zürich

T25B9.1/GCAT: a novel modulator of species-independent aging

11.30 – 12.00 pm

COFFEE BREAK

SESSION 5

Chair: Karin Scharffetter-Kochanek & Holger Richly

12.00 – 12.15 pm

Andreas Brown, Ulm University

P53 deficiency promotes early leukemic transformation of HSCs

12.15 – 12.30 pm

Martin Burkhalter, Ulm University

Xpg limits the expansion of haematopoietic stem and progenitor cells after ionising radiation

12.30 – 12.45 pm

Hui-Ling Ou, CECAD, University of Cologne

eIF4E translation initiation factor IFE-4 in somatic niche cells regulates CEP-1/p53-mediated DNA damage response in primordial germ cells

12.45 – 01.00 pm

Mubashir Ahmad, Ulm University

Inhibition of Axl Receptor Tyrosine Kinase increases osteoblast differentiation and is a promising target to treat age-related osteoporosis

01.15 – 01.30 pm

**YOUNG INVESTIGATOR
POSTER and TALK AWARD**

01.30 – 02.00 pm

SNACKS + DEPARTURE

02.00 – 03.00 pm

DGfA Member Assembly

ABSTRACTS – ORAL PRESENTATIONS

Abstract No. T 1

Cellular senescence drives age-dependent hepatic steatosis

Mikolaj Ogrodnik¹, Diana Jurk¹, Wilbert Vermeij², Satomi Miwa¹, and Thomas von Zglinicki^{1,*}

¹Newcastle University Institute for Ageing ²Erasmus University Rotterdam

Non-alcoholic fatty liver disease (NAFLD) is an age-related condition characterized by excess hepatic fat and is a leading contributor to liver disease worldwide. The mechanisms driving this process are largely unknown. Cellular senescence is a state of irreversible cell-cycle arrest characterized by a pro-inflammatory phenotype and mitochondrial dysfunction. It is a major contributor to age-related tissue degeneration. We found a close correlation between hepatic fat accumulation and markers of hepatocyte senescence in ageing mice exposed to different dietary interventions and in NAFLD patients. Furthermore, global gene expression analysis by RNA-seq revealed that senescent markers and fat accumulation correlated with aberrant expression of lipid metabolism and inflammatory genes. Induction of cell senescence exclusively in hepatocytes in Alb-XPG KO mice caused massive steatosis. Conversely, elimination of senescent cells by suicide gene-mediated ablation of p16Ink4a-expressing senescent cells in INK-ATTAC mice or combined treatment with senolytic-drugs dasatinib plus quercetin (D+Q) reduced overall hepatic steatosis. Mechanistically, mitochondria in senescent cells lose the ability to metabolize fatty acids efficiently. Thus, our study demonstrates that cellular senescence drives hepatic steatosis and targeting senescent cells may offer a novel pharmacological strategy for patients with NAFLD.

Abstract No. T 2

Cdc42-LaminAC axis regulates nuclear architecture changes and drives HSC aging and rejuvenation

Ani Grigoryan^{1,*}, Novella Guidi¹, Katharina Senger¹, Yolanda Markaki², Heinrich Leonhardt², Christian Buske³, Nadezda Kosyakova⁴, Thomas Liehr⁴, Daniel B. Lipka⁵, Christoph Plass⁶, Yi Zheng⁷, Medhanie A. Mulaw³, Hartmut Geiger⁸, and Maria Carolina Florian¹

¹Institute of Molecular Medicine and Stem Cell Aging, University of Ulm, Germany ²Department of Biology II and Center for Integrated Protein Science Munich (CIPSM), Ludwig Maximilians University Munich, Germany ³Institute of Experimental Cancer Research, Comprehensive Cancer Center Ulm, University Hospital Ulm, Germany ⁴Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Germany ⁵Regulation of Cellular Differentiation Group, Division of Epigenomics and Cancer Risk Factors, German Cancer Research Center (DKFZ), Heidelberg, Germany ⁶Division of Epigenomics and Cancer Risk Factors, German Cancer Research Center (DKFZ), Heidelberg, Germany ⁷Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center and University of Cincinnati, Cincinnati, OH, USA ⁸Institute of Molecular Medicine and Stem Cell Aging, University of Ulm, Germany & Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center and University of Cincinnati, Cincinnati, OH, USA

Mechanisms driving aging of hematopoietic stem cells (HSCs) and their possible epigenetic drift are not fully understood while it is known that aged HSCs contribute to the aging-associated immune remodeling as well as to leukemia. Here we report that the decrease of LaminAC regulates HSC aging specific changes in the nuclear architecture involving the nuclear volume and shape, the deposition of the epigenetic mark H4K16ac and the localization of chromosome 11. These changes in the epigenetic and genomic architecture and in the nuclear morphology are linked to Cdc42 activity levels defining a novel Cdc42/LaminAC axis. As inhibition of Cdc42-activity functionally rejuvenates HSCs, these observations imply an important role for reversible changes in the nuclear organization in driving the epigenetic drift during aging and rejuvenation of HSCs. In summary, our data shed lights on a new prominent role for Cdc42-LaminAC-H4K16ac-Chr11 axis in driving critical alterations in the nuclear architecture upon aging and rejuvenation of murine HSCs.

Abstract No. T 3

Up-regulation of microRNA-29 family protects from cardiac hypertrophy and fibrosis modulating DNA methyltransferases in *Nothobranchius furzeri*.

Johanna Heid^{1,*}, Francesco Spalotta², Sandra Atlante², Roberto Ripa³, Chiara Cencioni⁴, Mario Baumgart⁵, Giuseppe Milano⁶, Alessandro Scopece⁶, Carsten Künne⁷, Stefan Günther⁷, Giulio Pompilio⁸, Thomas Braun⁷, Andreas M Zeiher⁴, Alessandro Cellerino⁵, and Carlo Gaetano²

¹Cardiovascular Epigenetics, Goethe Universität, Innere Medizin III, Frankfurt am Main, Germany

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³Scuola Normale Superiore, BIO@SNS, Pisa, Italy ⁴Goethe Universität, Innere Medizin III, Frankfurt am

Main, Germany ⁵Leibniz Institute on Aging, FLI, Jena, Germany ⁶Centro Cardiologico Monzino - IRCCS,

Milan, Italy ⁷Max Plank Institute for Heart and Lung Research, Bad Nauheim, Germany ⁸Centro

Cardiologico Monzino - IRCCS, Milan, Italy

INTRODUCTION & HYPOTHESIS: The short-lived (6 months) turquoise killifish *Nothobranchius furzeri* (NF) is a novel and convenient model organism for aging studies in which the impact of microRNAs (miRs) in cardiovascular aging emerged clear only recently. In this system, however, the engagement of miRs in the aging-associated cardiovascular degeneration is still unknown. The present study investigates the significance of miR-29 in the heart upon NF aging.

METHODS & RESULTS: Changes in miRs expression in the heart of young (5 weeks) and aged (27 weeks) NFs were assessed by RNA sequencing. In the heart, members of the miR-29 family turned out among the most up-regulated. To study the functional role of miR-29 we took advantage of a most suitable model for genetic manipulation as the zebrafish. To this end, we generated a transgenic zebrafish in which miR-29 family expression was down-modulated by a specific sponge targeting miR29: Actb1:eGFPsponge-29 (ZF sponge-29). miR-29 deficiency determined morphological and histological cardiac alterations, including hypertrophy and fibrosis. In addition to that, echocardiography revealed significant reduction in the ejection fraction area of ZF sponge-29 hearts. Consistently, transcriptome analysis revealed an increase of collagen genes in the heart of ZF sponge-29 compared to controls. To get insights at

molecular level on the role of miR29 in NF, cardiac fibroblasts (NF-CFs) were isolated, cultured and exposed to fibrotic stimuli. Specifically, prolonged (>24 hours) hypoxia (1% O₂) significantly decreased miR-29 expression. Consistently, known miR29 targets such as collagens and DNA methyltransferases (DNMTs) increased.

CONCLUSIONS: Our data suggest that up-regulation of miR-29 family might represent an endogenous mechanism aimed at preventing/reducing the age-dependent cardiac damage leading to hypertrophy and fibrosis.

Abstract No. T 4

Intestine-specific Sirt1 deletion improves aging phenotypes in telomere dysfunctional mice

Ilwook Kim^{1,*}, Yohei Morita¹, Omid Omrani¹ and Karl Lenhard Rudolph¹

¹Leibniz institute for age research - fritz lipmann institute

Sirt1, a member of sirtuin family, is an NAD-dependent deacetylase and known as a longevity gene. Sirt1 deacetylates many targets that are involved in cell resistance to stress, metabolism, differentiation, aging and tumor suppression. It is also reported that Sirt1 contributes to telomere maintenance. However, the functional consequences of inhibition of Sirt1 in intestinal organ in response to telomere dysfunction during aging are largely unknown. Here we crossed late-generation telomerase knockout mice (G3 mTerc^{-/-}) carrying a heterozygous floxed allele of Sirt1 with Terc^{+/-} Sirt1^{fl/+} mice carrying an inducible Cre-recombinase transgene under control of the villin promoter, which is specifically active in the intestinal epithelium. The crosses generated intercross F1 Terc^{+/-} (iF1), iF1 Sirt1^{fl/fl}, intercross G4 mTerc^{-/-} (iG4) or iG4 Sirt1^{fl/fl} mice. In the study, we analyzed effects of Sirt1 deletion on intestinal organ in telomere-dysfunctional mice. iG4 mice show premature aging phenotypes predominantly affecting proliferative organs, such as intestinal stem cells and Hematopoietic stem cells. In contrast, iG4 Sirt1^{fl/fl} mice show improvement of lifespan, intestinal stem cell maintenance. We have found that the Sirt1 deletion attenuates cell apoptosis and chromosome end fusion, resulted from impaired DNA damage response (DDR), which lead to better maintenance of number of intestinal stem cells in mice carrying critically shorten telomeres. Furthermore, histone proteins involved in DDR are hyper-acetylated by Sirt1 deletion, indicating that impairment of histone deacetylation by Sirt1 deletion attenuates initiation of DDR. Our results demonstrate that sirt1 plays an important role for DDR in intestinal organ during aging. Moreover, impairment of DDR may rescue intestinal organ from apoptosis in response to telomere shortening.

Abstract No. T 5

Post-reproductive inhibition of autophagosome formation extends lifespan in *C. elegans*

Thomas Wilhelm^{1,*}, Jonathan Byrne¹, Rebeca Medina¹, Ena Kolundžić², Johannes Geisinger¹, Martina Hajduskova², Baris Tursun², and Holger Richly¹

¹Institute of Molecular Biology (IMB) ²Berlin Institute for Medical Systems Biology (BIMSB)

In the year 1957 George C. Williams formulated the antagonistic pleiotropy hypothesis of aging. It predicts that some genes mediate beneficial effects early in life when natural selection is strong, but are detrimental late in life when natural selection is weak. Accumulation of these harmful effects late in life could explain the aging process in the light of evolution. In an RNAi screen designed to discover novel genes that exhibit antagonistic pleiotropy, we identified the forkhead box (FOX) A transcription factor pha-4. Inactivation of pha-4 from L1 larval stage and from the first day of adulthood reduced *C.elegans* lifespan. In contrast, post-reproductive inactivation of pha-4 resulted in a significant lifespan extension. As PHA-4 is known to regulate autophagy, we investigated the effects of late-life inactivation of autophagy related genes on lifespan. Post-reproductive inactivation of genes required for the autophagosome nucleation, such as bec-1, led to a strong lifespan increase of more than 60 %. Just like pha-4, these genes reduced lifespan when inactivated early in life. Interestingly, post-reproductive inactivation of genes that are involved in later steps of the autophagosome formation had no positive effects on *C.elegans* lifespan. Besides the extension of lifespan, we did also observe a significant improvement in healthspan upon post-reproductive inactivation of the nucleation complex. Autophagy is mostly described with its advantageous cytoprotective and longevity-promoting effects. Yet, some studies characterize autophagy as a double-edged sword that, under certain conditions, can be detrimental to the organism. Our data suggests that aging dysregulates autophagy and thereby alters this process from advantageous to harmful. Further, we identified a strong neuronal contribution to the observed lifespan as well as healthspan extension.

Abstract No. T 6

Stroma-derived osteopontin regulates and attenuates aging-associated phenotypes of HSCs

Novella Guidi^{1,*}, Mehmet Sacma¹, Ludger Ständker², Karin Soller¹, Gina Marka¹, Johannes Weiss³, Frank Kirchhoff⁴, Tanja Weil⁵, Jose A. Cancelas⁶, Maria Carolina Florian¹ and Hartmut Geiger¹

¹Institute for Molecular Medicine, Ulm University ²Kompetenzzentrum Ulm Peptide Pharmaceuticals, Ulm University ³Department of Dermatology and Allergic Diseases, Universitätsklinikum Ulm

⁴Institute of Molecular Virology, Universitätsklinikum Ulm ⁵Institute of Organic Chemistry III, Ulm University ⁶Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Hematopoietic stem cell (HSC) aging is one underlying cause of aging-associated immunosenescence as well as leukemia. Upon aging HSCs undergo changes in function and structure, including skewing to myeloid lineages, lower reconstitution potential and loss of protein polarity. While stem cell intrinsic mechanisms are known to contribute to HSC aging, little is known on whether aged-related changes in the BM niche regulate HSC aging. Heterochronic transplantation of young BM cells into young or old recipients revealed that an aged environment supports aging-associated lineage skewing of HSCs (myeloid over lymphoid), a decrease in the overall level of engraftment and an increase in the frequency of long term HSCs (LT-HSCs) with associated apolarity. Osteopontin

(OPN) is a secreted glycoprotein expressed by osteoblasts located close to the endosteum. OPN was decreased in aged stroma, both in terms of expression and secretion. We then tested whether the decrease of OPN in stroma upon aging might be causatively linked to aging of HSCs. Transplantation of young BM cells into young OPN KO recipients, similarly to aged recipients, resulted in a significant decrease in stem cell engraftment with an increase of LT-HSC frequency with loss of polarity. Moreover, a brief ex-vivo exposure to Thrombin-cleaved OPN functionally rejuvenated old HSCs, resulting in increased engraftment, decreased HSC frequency, increased stem cell polarity and a restored balance of lymphoid and myeloid cells in peripheral blood. Finally, with the use of peptide inhibitors we have identified integrin $\alpha 9\beta 1$ as a downstream effector of Thrombin-cleaved OPN activity in initiating a signaling cascade regulating Cdc42 activity and HSC polarity and consequently stem cell aging and attenuation of aging. Therefore, our data suggest a critical causative role for reduced stroma-derived OPN in promoting HSC aging and show that addition of OPN digested by thrombin ameliorates aging of HSCs.

Abstract No. T 7

Mitochondrial DNA deletions in muscle stem cells lead to impaired regeneration capacity with fibrosis and adipocyte infiltration

Sammy K. Kimoloi¹, Olivier R. Baris¹, and Rudolf J. Wiesner^{1,*}

¹Center for Physiology and Pathophysiology, Institute of Vegetative Physiology, Medical Faculty, University of Köln

The adult skeletal muscle can regenerate upon injury due to the enormous capacity of resident muscle satellite cells (MuSCs). Their regenerative capacity declines with ageing, leading to replacement of contractile fibers with non-contractile fibrous connective and adipose tissues. However, the exact mechanisms driving this decline currently remain unclear. Previous studies have shown increased mitochondrial biogenesis during MuSC differentiation, suggesting that mitochondrial dysfunction in MuSCs might be detrimental for regeneration. mtDNA deletion mutations and mitochondrial dysfunction are also known to increase with ageing in various cell types. We therefore aimed at testing the hypothesis that mitochondrial dysfunction induced by mtDNA deletions impairs MuSC regenerative capacity. To this end, we generated a mouse strain which expresses, upon Tamoxifen-Pax7-driven Cre-recombination (Rosa26-Stop-construct), a dominant-negative mutant of the mitochondrial helicase Twinkle, thus strongly enhancing the generation of Δ mtDNA species in MuSCs. Histochemical analysis of cardiotoxin-injured muscles from these mice revealed mitochondrial dysfunction and markedly impaired regeneration characterized by increased fibrosis, fiber atrophy and adipocyte infiltration. These findings indicate that Δ mtDNA-induced mitochondrial dysfunction in MuSCs might be a key intrinsic factor in the decline of MuSC function and might contribute to ageing sarcopenia. Similar negative impacts of mtDNA defects have been observed in neural and hematopoietic stem cells by others. This raises the possibility that strategies which can maintain the integrity of mtDNA in stem cells might potentially sustain the regenerative capacity of muscle and other tissues during ageing. The generated mouse model is thus a valuable

model to test such strategies in the future, as well as investigating the molecular mechanisms linking mitochondrial dysfunction in MuSCs with muscle fibrosis and fat infiltration during ageing.

Abstract No. T 8

Epigenetic stress responses induce muscle stem cell aging by Hoxa9 developmental signals

Simon Schwörer^{1,*}, Friedrich Becker¹, Christian Feller², Ali Baig¹, Ute Köber¹, Henriette Henze¹, Johann Kraus³, Beibei Xin⁴, André Lechel⁵, Daniel Lipka⁶, Christy Varghese¹, Manuel Schmidt¹, Remo Rohs⁴, Ruedi Aebersold², Kay Medina⁷, Hans Kestler⁵, Francesco Neri¹, Julia von Maltzahn¹, Stefan Tümpel¹, and Karl Lenhard Rudolph¹

¹Leibniz-Institute on Aging - Fritz-Lipmann-Institute ²ETH Zürich ³Ulm University ⁴University of Southern California ⁵Ulm University 6DKFZ 7Mayo Clinic Rochester

Muscle stem cells (MuSCs) are responsible for growth and regeneration of skeletal muscle in response to exercise and injury. Their number as well as functionality is reduced in age, thus contributing to impaired regeneration and mobility during aging. Alterations in developmental pathways have been associated with declines in stem cell function during aging but the nature of this process remains not well defined. Hox genes are key regulators of stem cells and tissue patterning during embryogenesis with an unknown role in aging. Epigenetics describe mechanisms that orchestrate dynamic changes of gene activity by altering structural properties of the chromatin during development, differentiation and disease. In our study, we found that an altered epigenetic stress response is responsible for the induction of Hoxa9 expression and a consecutive upregulation of numerous developmental pathways in aged MuSCs, constituting a maleficent burden for their functionality. We hypothesize that cell intrinsic aging of MuSC is the outcome of an epigenetically driven recapitulation of developmental stages.

Abstract No. T 9

Wnt5a-Cdc42 signaling regulates aging of hair follicle stem cells

Rajiv Lochan Tiwari^{1,*}, Pratibha Mishra¹, Nicola Martin¹, Nikhil O. George¹, Vadim Sakk¹, Karin Soller¹, and Hartmut Geiger¹

¹University of Ulm

Somatic stem cell aging is supposed to be one of the underlying causes of attrition of skin with age. Upon aging the onset of anagen is delayed and one underlying cause might be aging of A-6highCD34+ (HFSC) stem cells, which reside in the bulge of the hair follicles. The Wnt signaling pathway contributes to anagen onset in mouse skin during normal homeostasis. Our data demonstrate that upon aging there is decrease in canonical Wnt signaling and a shift towards non-canonical Wnt signaling in HFSC. The activity of the small RhoGTPase Cdc42 is regulated, among others, by Wnt-signaling. Cdc42 activity was increased in aged HFSC, which correlated with a high percentage of aged HFSC stem cells being apolar for Cdc42 distribution, while in young most stem cells presented with a polar distribution. Our data show elevated levels of expression

of Wnt5a in aged HFSC and we confirmed that Wnt5a antagonizes canonical Wnt signaling when given to young HFSCs while a lentiviral mediated knockdown of Wnt5a in aged HFSC increased canonical Wnt signaling. In-vitro treatment of aged HFSC with a specific inhibitor for Cdc42 activity (CASIN) changes the frequency of aged cells polar for Cdc42 distribution to a youthful level and restores canonical Wnt signaling. In-vivo treatment of aged mice with CASIN at a dose of 2.4mg/kg twice a day for five days induced anagen onset and increased the percentage of back skin area with anagen hair follicle. In summary, aging of hair follicle stem cells is linked to changes in a Wnt5a-Cdc42 signaling axis with elevated non-canonical signaling upon aging and elevated activity of the small RhoGTPase Cdc42. Aged phenotypes of hair follicle stem cells like elongated telogen time can be reverted to a youthful phenotype by inhibition of the activity of the small RhoGTPase Cdc42.

Abstract No. T 10

Using *C. elegans* as a model for studying host-microbiome interactions

Prerana Chaudhari^{1,*}, Tetiana Poliezhaieva¹, and Maria A. Ermolaeva¹

¹Leibniz Institute on Aging - Fritz Lipmann Institute (FLI)

Commensal microbiota exerts significant effects on multiple aspects of the host physiology. Mechanisms of this interaction on both the host and the microbial side are poorly understood. We aim to identify microbes and microbial metabolites capable of influencing host systemic homeostasis and longevity by using *C.elegans* as a model. In a pilot test, we exposed nematodes to a mixed microbiome of 4 healthy young humans and observed significant lifespan extension in comparison to animals grown on control *E.coli* diet. In attempt to identify the microbe responsible for the pro-longevity effect of the sample, we treated nematodes with the most abundant species found in this sample. Surprisingly, exposure to this single bacterium significantly reduced survival of nematodes, contrary to the effect of the complete microbiome. This result indicates that microbiome complexity may be a critical factor for the pro-homeostatic effect of commensal microbiota. Interestingly, analysis of intestinal microbiota obtained from human centenarians revealed that microbiomes of long-lived humans have higher than average complexity. Nematodes exposed to the distinct microbial samples will be analyzed by high-throughput proteomics. In cooperation with the Leibniz Institute for Natural Product Research (HKI) we conduct unbiased screens for microbes with potential pro-longevity properties by feeding nematodes with single non-pathogenic isolates. In a pilot screen, we exposed nematodes to ten different isolates of *Pseudomonas* and identified one which promoted significant lifespan extension. Nematodes exposed to this isolate are being analyzed by high-throughput proteomics while our collaborators are performing genome sequencing and comparative genomic analysis of the bacterial isolate. Isolation of secondary metabolites produced by the candidate microbe is also in progress. Intriguingly, *Pseudomonas* species are one of most potent producers of secondary metabolites among microbes; at the same time microbiome analysis of Chinese centenarians revealed that presence of *Pseudomonas* in the intestine was highly predictive of extended life.

Abstract No. T 11

Cognition, Outcome and Advanced Glycation Endproducts in Geriatric Inpatients

Manfred Gogol^{1,*}, Hilde Hartmann¹, Andreas Walz¹, and Andreas Simm²

¹Lindenbrunn Hospital ²University Halle

Purpose:

Measuring AGEs in skin and serum and test for correlation of cognition and functional outcome in geriatric inpatients.

Methods:

We measured skin AGEs with AGE Reader by DiagnOptics through autofluorescence (AF) and in serum (carboxymethyllysine (CML), arginine/pyridine(A/P)) and total AGEs by 330/405, 440/520, 280/350, and 360/440 nm. For functional measurement, we used the Barthel Index (BI) at admission and discharge. For cognitive assessment, we used the Mini Mental Status Examination (MMSE).

Results:

We analyzed 166 patients out of 196 included (mean age 80.5±7.8 y, women (W) 81.8±7.1, men (M) 77.8±8.6 y, p=0.0018). 30 patients were excluded for medical reasons, e.g. delirium. AF in W/M were 2.8±0.72/3.16±0.65, p=0.0019, while serum levels show no sex difference (CML W 365±134, M 372±133, A/P W 464±123, M 489±126 ug/ml; 330/405 nm W 2.21±0.98, M 2.35±1.13, 440/520 nm W 9.44±5.75, M 9.68±5.9, 280/350 nm W 34.5±5.6, M 34.6±5.8, 360/440 nm W 3.14±0.95, M 3.26±0.98 mg/ml, all n.s.). Mean BI at admission was 50.4±18.9 in W and 47.3±20.9 in M (n.s.), at discharge W 73.8±21, M 77.8±20.8 (n.s.). No significant correlation was found except for A/P in M for BI at discharge (r=-0.2909, p=0.05).

Mean MMSE was 23.1±4.8 (W 22.9±4.8, M 23.3±4.9, n.s.) and show no correlation to AGEs, but MMSE subscore immediate recall do to CML (r -0.1666) and 280/350 nm (r 0.2035, p=0.05 each). MMSE subscores in M were n.s., while in W CML (r -0.209) and 280/350 nm (r 0.2464) for immediate and A/P (r 0.1977) for delayed recall showed significant correlations (p=0.05 each).

Conclusion:

Sex differences in AGE skin measurements were not confirmed by serum analysis. We found no correlation between AGEs and functional outcome and no sustained correlation between AGEs and cognition in our cohort. In women, different MMSE subscores show some significant correlation for different subclasses of AGEs.

Abstract No. T 12

Reduced expression of C/EBPβ-LIP extends health- and lifespan

Christine Müller¹, Laura Zidek², Tobias Ackermann¹, Sabrina Eichwald², Gertrud Kortman¹, Julia von Maltzahn², Alain de Bruin³, Zhao-Qi Wang², and Cornelis Calkhoven^{1,*}

¹ERIBA, Groningen ²FLI, Jena ³DMPC, Utrecht

Aging is associated with physical decline and the development of age-related diseases including metabolic disorders and cancer; conditions that can be attenuated by calorie restriction (CR) (also known as dietary restriction). Understanding the molecular mechanisms that act downstream of CR may reveal novel therapeutic strategies to

defeat aging-associated decline and diseases. We have shown earlier that translation of the C/EBP β -mRNA into the metabolic transcription factor isoform C/EBP β -LIP is stimulated by the nutrient and energy sensitive mTORC1 pathway, which critically depends on a short upstream open reading frame (uORF) within the C/EBP β -mRNA sequence. We generated mice that are deficient in LIP expression through ablation of the uORF (C/EBP β Δ uORF mice). These mice display CR-like metabolic improvements, including enhanced fatty acid oxidation and lack of steatosis, improved insulin sensitivity and glucose tolerance and higher adiponectin levels (Zidek et al., 2015 EMBO Rep. 16, 1022). Now we present that these C/EBP β Δ uORF mice show improvements a broad spectrum of ageing parameters, including cancer incidence, motor coordination, glucose tolerance and memory/naïve T-cell ratio. Moreover, female C/EBP β Δ uORF mice display an extended median lifespan of 20.6% and an increase in maximum lifespan of 9%. We also show that during normal aging LIP levels are increased independently of changes in mTORC1. Our data demonstrate an important role of C/EBP β in the aging process and suggest that restriction of LIP expression sustains health and fitness during ageing. Thus, therapeutic strategies targeting the LIP isoform may offer new possibilities to treat age-related diseases and to prolong health span.

Abstract No. T 13

In-silico modeling of the Senescence Associated Secretory Phenotype

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Here we present an in-silico gene regulatory network of senescence and the senescence associated secretory phenotype (SASP) incorporating published interaction data of signaling pathways like IL-1, IL-6, p53 and NFkappaB under the assumption of DNA damage.

Cells are subjected to continual stresses from exogenous and endogenous sources. These events can cause a number of responses, ranging from complete recovery to malfunction and ultimately cell death. Permanent cell-cycle arrest or senescence is a protection mechanism that helps cells recover from this damage and seems to be a fundamental mechanism of aging and development. However, cellular senescence can be accompanied by a SASP that causes chronic inflammation and paracrine senescence. Our Boolean model can predict different in-silico knock-outs that prevent key SASP-players, like IL-6 and IL-8, from getting activated upon cell cycle arrest. We found different gene knock-outs and knock-out combinations that prevent the activation of IL-6 and IL-8 signaling, factors that among others seem to be responsible for spreading and retaining the SASP. We could single out the NFkappaB Essential Modifier (NEMO) as a potential target. To validate these results in-vitro we used NEMO-floxed mice to isolate murine dermal fibroblasts that were afterwards transfected with a Cre recombinase-expressing plasmid. We subsequently introduced DNA damage and analyzed mRNA expression and protein secretion of IL-6 and murine IL-8 homologues using qPCR and ELISA. Verifying our in-silico results, we could show that a NEMO

knockout inhibits IL-6 and IL-8 homologue mRNA expression and protein secretion in murine dermal fibroblasts after DNA damage in-vitro, enabling us to lower the contagiousness of the SASP for neighboring cells. Consequently the combination of in-silico models and in-vitro benchwork gives us the power to create in-vitro and in-vivo models that might help to understand the dynamics of the SASP.

Abstract No. T 14

Global Impairment of Insulin Signalling In Adult Mice Fails to Extend Lifespan

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Impaired insulin/IGF1 signalling has been shown to extend lifespan in model organisms ranging from yeast to mammals. Here we sought to determine the effect of targeted disruption of the insulin receptor (IR) in non-neuronal tissues of adult mice on the lifespan. We induced hemizygous (PerIRKO+/-) or homozygous (PerIRKO-/-) disruption of the IR in peripheral tissue of 15 wk old mice using a tamoxifen-inducible Cre transgenic mouse with only peripheral tissue expression, and subsequently monitored glucose metabolism, insulin signalling, and spontaneous death rates over four years. Complete peripheral IR disruption resulted in a diabetic phenotype with increased blood glucose and plasma insulin levels in young mice. Although blood glucose levels returned to normal, and fat mass was reduced in aged PerIRKO-/- mice, their lifespan was reduced. By contrast, heterozygous disruption had no effect on lifespan. This was despite young male PerIRKO+/- mice showing reduced fat mass and enhanced insulin sensitivity. In conflict with findings in metazoans like *C. elegans* and *D. melanogaster*, our results suggest that heterozygous impairment of the insulin signalling limited to peripheral tissues of adult mice fails to extend lifespan despite increased systemic insulin sensitivity, while homozygous impairment shortens lifespan.

Abstract No. T 15

Beneficial effects of telomerase activators on balance and motor function-a possible treatment for Parkinson's disease?

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Telomerase is a reverse transcriptase best known for its canonical function of telomere maintenance. However, recently various non-canonical functions of the telomerase protein TERT have been described. Our group described beneficial effects of mitochondrial TERT where it decreases oxidative stress, apoptosis and DNA damage. While telomerase activity is downregulated during brain development TERT is still expressed in adult brain. We published recently increased mitochondrial TERT localisation in hippocampal neurons of late stage Alzheimer's disease and protection from pathological tau in vitro. We hypothesise that boosting TERT levels could be beneficial in delaying or ameliorating brain ageing as well as neurodegenerative diseases. Here we use a transgenic Parkinson's disease (PD) mouse model for oral

treatment with 2 telomerase activators. We employ qPCR for analysing TERT expression in mouse brains and cultivated neurons. We use various behavioural tests such as rotarod, stride length analysis, open field test and novel object recognition in order to analyse motor behaviour, balance, anxiety and curiosity. We also analyse the generation of reactive oxygen species from isolated brain mitochondria and will analyse brain pathology. We show that TERT expression decreases with age in wildtype mouse brains and this expression can be enhanced by telomerase activator treatments in vivo and in cultured primary neurons in vitro. We found significant improvement of balance and motor function, anxiety and movement in mice that received the telomerase activators. We will present data on mitochondrial function in brain tissue and analyse alpha-synuclein pathology and dopamine related proteins in these mice in the future. We conclude that increased TERT expression in the brain of wild type and PD mice has a protective effect on brain function during ageing and neurodegeneration. This might lead to novel therapeutic approaches to prevent or delay changes connected to brain ageing and neurodegenerative diseases such as Parkinson's disease.

Abstract No. T 16

Zebrafish heart regeneration requires alleviation of genomic stress by BMP signaling

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Accumulation of DNA damage and replication stress are key factors in declining tissue homeostasis and regeneration during ageing. Some vertebrate species, including zebrafish, display highly elevated regenerative capabilities relative to mammals even at old age. In contrast to mammals, zebrafish can regenerate their heart via proliferation of cardiomyocytes. Surprisingly, we have found that a large fraction of proliferating cardiomyocytes in regenerating zebrafish hearts accumulate gammaH2a.x. The temporal profile of DNA damage in cardiomyocytes closely follows that of cell cycle re-entry, and EdU incorporation experiments show that virtually all cardiomyocytes that become gammaH2a.x positive have entered the cell cycle. Thus, we hypothesize that cardiomyocytes experience replication stress during heart regeneration. Nevertheless, regeneration proceeds and cardiomyocytes can proliferate, implying that zebrafish hearts possess efficient mechanisms of alleviating genomic stress. Indeed, we find that ATM and ATR activity is required for regenerative cardiomyocyte proliferation. Furthermore, we have recently found that Bone Morphogenetic Protein (BMP) signaling is essential for regenerative cardiomyocyte proliferation and heart regeneration. Interestingly, cardiomyocyte proliferation during heart development is not dependent on BMPs, suggesting that BMP signaling regulates regeneration-specific cellular process that are a pre-requisite for cardiomyocyte proliferation. Indeed, we find that inhibition of BMP signaling increases the number of cardiomyocytes displaying gammaH2a.x, while BMP2 overexpression reduces it. In summary, our work surprisingly shows that genomic stress, which is a hindrance for tissue regeneration in aged mammals, is prevalent in efficiently regenerating zebrafish hearts at young age. It further suggests that BMP signaling is one molecular mechanism

by which genomic stress can be alleviated, allowing for regeneration to proceed. We propose that zebrafish regeneration is a useful model to identify such potential anti-ageing pathways.

Abstract No. T 17

T25B9.1/GCAT: a novel modulator of species-independent aging

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Within the JenAge/GerontoSys consortium, we previously used an unbiased screening approach to identify aging related genes that were regulated similarly during physiological aging in three different organisms, namely nematode (*C. elegans*), zebrafish (*D. rerio*) and mouse (*M. musculus*) (doi: 10.1038/ncomms10043). The candidate genes obtained from this screen were tested for the effect on lifespan by performing RNA interference (RNAi) against them in young adult nematodes. T25B9.1, the highly conserved *C. elegans* ortholog of Glycine C-Acetyltransferase (*gcat*) was downregulated in all three species and had the second most pronounced lifespan extension, increasing the mean lifespan by 22% upon knockdown. *gcat*/T25B9.1 is a pyridoxal-phosphate-dependent enzyme involved in the degradation of L-threonine to glycine. Further characterization indicates that the observed lifespan increase is mediated by non-linear action of the byproducts methylglyoxal and reactive oxygen species. In addition, this lifespan extension requires the NRF-2 homolog SKN-1 by protecting the cells from protein damaging stress through the activation of ubiquitin proteasome pathway. Altogether, these results indicate a hormetic effect of methylglyoxal and ROS in mediating lifespan promoting effects through increased stress response.

Abstract No. T 18

p53 deficiency promotes early leukemic transformation of HSCs

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The tumor suppressor p53 has major functions in DNA damage response pathways. Hence, p53 mutations are associated with large numbers of hematopoietic neoplasms. In hematopoietic stem cells (HSCs) p53 has been claimed to be crucial for quiescence, self-renewal and senescence. Although many studies have shown the involvement of p53 mutations or altered expression in cancer progression, their role in early initiation steps towards cancer especially in stem cells has not extensively been studied yet. Here, we report an unexpected new role of p53 in preventing initial steps of transformation of HSCs. As previously reported, HSCs from wild type mice in response to irradiation enter the active cell cycle while simultaneously initiating apoptosis. HSCs from mice lacking p53, however, preserve their quiescent state, enabling their protection from cell death. Whereas wild type mice lose almost all the HSCs residing in

the bone marrow shortly after irradiation, the corresponding number of HSCs in mice devoid of p53 remains unaffected. These HSCs are still able to self-renew and, moreover, they show a constitutive preference for the error-prone non-homologous end joining pathway. Notably, this behavior is of intrinsic nature, primarily stem cell specific and not present in less primitive progenitors. We have data suggesting that p53-deficient HSCs display persistently high p57(Kip2) levels after DNA damage, offering one possible functional explanation for the observed behavior. Intriguingly, transplanted p53 KO HSCs expand massively in vivo several months after sublethal irradiation and severely lose their differentiation potential, with the corresponding recipient mice developing symptoms of preleukemia. In compliance with our previously published data we believe that we have found evidence for a novel p53-dependent and stem cell specific mechanism responsible for removing DNA-damaged HSCs from the stem cell pool. This proposed mechanism may turn out to play a crucial role in maintaining its genomic and functional integrity.

Abstract No. T 19

Xpg limits the expansion of haematopoietic stem and progenitor cells after ionising radiation

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Reduced capacity of genome maintenance represents a problem for any organism, potentially causing premature death, carcinogenesis, or accelerated ageing. Strikingly though, loss of certain genome stability factors can be beneficial, especially for the maintenance of tissue stem cells of the intestine and the haematopoietic system. We therefore screened for genome stability factors negatively impacting maintenance of haematopoietic stem cells (HSC) in the context of ionising radiation (IR). We found that in vivo knock down of Xeroderma pigmentosum, complementation group G (Xpg) causes elevation of HSC numbers after IR treatment, while numbers of haematopoietic progenitors are elevated to a lesser extent. IR rapidly induces Xpg both on mRNA and on protein level. Prevention of this induction does not influence activation of the checkpoint cascade, yet attenuates late checkpoint steps such as induction of p21 and Noxa. This causes a leaky cell cycle arrest and lower levels of apoptosis, both contributing to increased colony formation and transformation rates. Xpg thus helps to adequately induce DNA damage responses after IR, thereby keeping the expansion of damaged cells under control. This represents a new function of Xpg in the response to IR, in addition to its well-characterised role in nucleotide excision repair.

Abstract No. T 20

eIF4E translation initiation factor IFE-4 in somatic niche cells regulates CEP-1/p53-mediated DNA damage response in primordial germ cells

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Maintenance of genome integrity in primordial germ cells (PGCs) is pivotal for the perpetuation of species. In order to faithfully propagate the genomic information, DNA damage checkpoints guard the genome of germ cells in *C. elegans* while they are dispensable in somatic tissues. Persistence of DNA damage due to the deficiency of global genome nucleotide excision repair (GG-NER) leads to the activation of the *C. elegans* p53 homolog CEP-1, resulting in arrest of PGCs thus preventing germ cell proliferation. We uncovered a previously unknown mechanism through which the eukaryotic translation initiation factor 4E (eIF4E) homolog IFE-4 regulates the CEP-1/p53-mediated PGC arrest. We determined that IFE-4 functions in somatic niche cells to activate CEP-1/p53 in PGCs harbouring DNA damage. We implicate fibroblast growth factor (FGF) signalling in the communication between the genomically compromised PGCs and the somatic gonad. We establish that the DNA damage response in PGCs is controlled non-cell-autonomously by IFE-4-mediated translation initiation. Our data suggest that the somatic niche influences the stability of heritable genomes.

Abstract No. T 21

Inhibition of Axl Receptor Tyrosine Kinase increases Osteoblast differentiation and is a promising target to treat age-related osteoporosis

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Osteoporosis is characterized by low bone mass and altered bone microarchitecture, leading to increased risk of fractures in particular in the ageing population. Similar to age-related bone loss glucocorticoids (GCs), widely used to treat acute and chronic inflammatory diseases, lead to glucocorticoid-induced osteoporosis (GIO). GIO is very reminiscent to age-related osteoporosis characterized by low bone turnover. Most of the currently available pharmacological agents to treat osteoporosis are antiresorptive and decrease the risk of fractures by stabilizing the bone mass. However, antiresorptive drugs do not improve bone quality. To identify novel targets for small molecules we performed an large-scale siRNA screen with high content analysis to test osteoblast differentiation decisive for bone formation.

Among 48 candidates, we validated the tyrosine kinase Axl as a novel regulator of osteoblast differentiation. siRNA knockdown or a drug inhibition approach enhanced osteoblast differentiation and abrogated the deleterious effects of glucocorticoid treatment. Currently, we test Axl by chemical inhibitors and in genetic loss of function

studies in mice how they interfere with bone integrity in vivo and results will be presented.

In summary, we identified the tyrosine kinase Axl as a potential target that could be used as anabolic therapeutic target to treat age-related osteoporosis and other bone related disorders.

ABSTRACTS – POSTER PRESENTATIONS

Abstract No. 1

A DNA repair-independent pathomechanism in Cockayne syndrome and trichothiodystrophy

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Progerias like Cockayne syndrome (CS) and trichothiodystrophy (TTD) are caused by recessive mutations in several genes involved in Nucleotide-Excision Repair (NER). They are both characterized by childhood onset of degenerative symptoms reminiscent of ageing, and also show severe neurodegeneration. However, the loss of NER does not provide sufficient evidence in covering the entire spectrum of symptoms of both diseases. Using patient-derived fibroblasts we described an alternative function of the proteins that cause CS (CSA and CSB) and TTD (XPD), transcription of the ribosomal DNA by RNA polymerase I. Here we investigated the cellular consequences of a disturbed RNA polymerase I transcription. In both cases we found a decreased ribosomal biogenesis and low quality of protein translation. Translational inaccuracy creates an instable proteome characterized by a reduced resistance to unfolding. In CS cells, we showed that the presence of unstable proteins and the high levels of ROS generates ER stress and activates the unfolded protein response (UPR). However, both CS and TTD cells showed an increased activation of the protein kinase RNA-like ER kinase (PERK) pathway from the UPR. The highly activated UPR further suppresses the RNA polymerase I activity and the protein translation in an attempt to prevent further damage. This *circulus vitiosus* resulting in the repression of RNA polymerase I can be disrupted using chemical chaperones, and the function of the enzyme improved. Despite certain differences between CS and TTD cells, our data show a common pathomechanism in both cell types, involving ER stress and UPR. We propose here an alternative explanation, but common for the severe phenotype of both CS and TTD patients

Abstract No. 2

Cortisol, oxytocin, and telomere length in immune cell subsets of women with child maltreatment experiences

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Experiencing maltreatment during childhood (CM) has life-long consequences for both physical and mental health. The affected individuals suffer not only from a higher risk for depression, but carry also an increased risk for the development of age-related disorders such as cardiovascular diseases and cancer. Shortened immune cell telomere

length (TL) has been proposed as one mechanistic link between CM and adverse health outcomes later in life. On the level of immune cell subsets, monocytes, naïve and memory cytotoxic T cells seem to be particularly sensitive to stress. Despite this knowledge, no study has assessed TL in specific immune cell subsets so far. Additionally, dysregulations of the hypothalamic-pituitary-adrenal (HPA)-axis were associated with both CM and TL shortening, whereby higher cortisol levels were shown to contribute to TL attrition. It has, however, not been assessed, whether oxytocin, which was shown to dampen HPA-axis reactivity, has the potential to buffer TL shortening. Therefore, we assessed TL in PBMC, monocytes, naïve and memory cytotoxic T cells, as well as peripheral cortisol and oxytocin levels in a study cohort of 15 women with (CM+) and 15 women without CM experiences (CM-). The CM+ group showed significantly shortened TL in memory cytotoxic T cells compared to the CM-group, but not in monocytes and naïve cytotoxic T cells. Across both groups, higher cortisol levels were associated with shorter TL, whereas higher oxytocin levels were associated with longer TL in memory cytotoxic T cells. These findings suggest that long-lived memory cytotoxic T cells, which play not only a central role in immunity to (re)infection but also to cancer, are most sensitive to TL shortening associated with increased biological stress states. TL of memory cytotoxic T cells might serve as a sensitive biomarker that integrates the cumulative effects of both biological adversity and resiliency factors on disease risk.

Abstract No. 3

Sirtuin 3 levels change in mouse models of amyotrophic lateral sclerosis and Huntington's disease during the course of disease

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The loss of upper and lower motor neurons characterizes the progressive and fatal neurodegenerative disease amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease. Weight loss in ALS patients and early changes in mitochondria indicate dysregulation of energy metabolism. These features are also present in Huntington's disease (HD) patients. Three members of the seven mammalian sirtuins are located to mitochondria, regulating energy metabolism. Of these mainly the NAD⁺ dependent sirtuin 3 (SIRT3) deacetylates proteins and is majorly involved in regulating ROS defense. Furthermore, SIRT3 is a sirtuin connected to human aging, which is a risk factor for neurodegenerative diseases. So far hints for the involvement of SIRT3 in neurodegenerative diseases are published, but a systemic analysis examining Sirt3 mRNA and protein levels during the course of disease in a mouse model of ALS and HD is missing. mRNA levels of the mitochondrial sirtuins Sirt3, Sirt4 and Sirt5 were analyzed during the course of disease in male ALS (SOD1(G93A)) and HD (R6/2) mice. We found disease-specific changes of Sirt3 mRNA levels in the disease-affected tissues of end-stage ALS and HD mice. Furthermore, the activity of SIRT3 was determined in the spinal cord of the SOD1(G93A) mice analyzing the acetylation status of the SIRT3 target superoxide dismutase 2. On the cellular level Sirt3, Sirt4, and Sirt5 mRNA levels were examined to identify the brain-derived cell type with the highest levels. In

summary our findings support a disease-stage specific expression of Sirt3 in the affected brain regions of ALS and HD.

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Abstract No. 4

A Study to mimic the dyskeratosis congenita premature aging phenotype by PRDM8 knockout in induced pluripotent stem cells

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Dyskeratosis congenita (DKC) is a rare disease associated with impaired telomere maintenance and a premature aging phenotype. In this study, we analyzed peripheral blood DNA methylation (DNAm) profiles of DKC patients, using the 450K BeadChip platform from Illumina. We observed statistical significant differences in DNAm patterns compared to healthy controls, particularly in CpG sites related to the internal promoter of the histone methyltransferase PR domain containing 8 (PRDM8) which was significantly hypermethylated. Gene expression analyses revealed a decrease of PRDM8 expression in DKC patients - thus uncovering the epigenetic regulatory effect of the promoter methylation. To further investigate the physiological role of PRDM8 in DKC and to define whether the lack of gene expression mimics the premature aging phenotype, we used CRISPR/Cas9 editing technology to delete PRDM8 in induced pluripotent stem cells (iPSCs). Since PRDM8 has an important role in neuronal development, we analyzed expression of neuronal markers of cells derived from embryoid body assays of iPSC knockout clones. The results indicate a reduced differentiation potential of PRDM8 knockout cells into the neuronal lineage. Therefore, we aim to further characterize the PRDM8 knockout clones using directed neuronal differentiation and functional assays.

Abstract No. 5

The role of senescent fibroblast-derived Chemerin in cutaneous squamous cell carcinoma progression

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Ample evidence in recent years has indicated a profound role for aged stroma in tumor progression. Senescent dermal fibroblasts that accumulate in the skin stroma during aging, have been suggested to display a senescence-associated secretory phenotype (SASP) which supports the malignant progression of cutaneous squamous cell carcinoma (cSCC). However, exact mediators and mechanisms have not been fully elucidated. In the present study, we revealed that SASP of replicative senescent fibroblasts enhanced cSCC cell migration, a major hallmark of cancer progression. We

identified that the transcript and protein levels of a chemoattractant protein, termed Chemerin, were upregulated in senescent compared to young fibroblasts in vitro and in the skin sections derived from old healthy individuals and cSCC patients as compared to young humans. While the abundance of Chemerin was downregulated in cSCC cells, the level of its putative receptor, CCRL2, was significantly elevated in cSCC cells compared with normal keratinocytes in vitro and in situ. These findings were suggestive of a causal role for fibroblast-derived Chemerin to trigger cSCC chemotaxis, as confirmed with Transwell chamber migration assays. Knock-down of Chemerin gene in senescent fibroblasts via silencing RNAs resulted in a significant reduction of SASP-stimulated cSCC migration. Moreover, shRNA-mediated depletion of either CCRL2 or GPR1 receptors abrogated cSCC cell migration in response to Chemerin. Finally we demonstrated that Chemerin activated JNK and ERK1/2 MAPK signaling pathways. Inhibition of MAPK pathway impaired SASP- and Chemerin-induced migration in cSCC cells. Taken together, we uncovered a novel tumor-promoting role for Chemerin, as a major SASP factor released from senescent dermal fibroblasts, in mediating cSCC cell migration and possibly progression, relaying its signals through CCRL2 and GPR1 receptors with subsequent MAPK activation. These findings may have important clinical implications to develop strategies to overcome the age-associated cSCC progression in elderly patients.

Abstract No. 6

Analysis of effect of pesticides on survival of rodents in long-term toxicity and carcinogenicity studies

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Long term chronic toxicity and carcinogenicity feeding studies in two species of rodents (mice and rats, males and females) for 70 substances were analysed for a potential impact of treatment on survival. The analysed substances include active substances in plant protection products and biocidal products, e.g. insecticides and fungicides. The majority of tested substances (93%, n=65) showed no improvement on survival of male and female mice. However, statistically significant improved survival (trend-test or pair-wise comparison, $p < 0.05$) was observed in studies for 7% of tested substances (n=5) in at least one dose level and only in male mice. For all 5 substances, this effect was not associated with alterations in reported food consumption. Similarly, 93% of tested substances (n=65) showed no improvement on survival of male and female rats. Statistically improved survival (trend-test or pair-wise comparison, $p < 0.05$) was observed in studies for 7% (n=5) of tested substances in at least one sex and at least one dose level. Effect of 1 substance was not associated with decrease in food consumption and therefore considered independent from calorie restriction. The 6 identified pro-survival substances are of particular interest for ageing research and require further dissection of the mechanisms involved in their effect.

Abstract No. 7

Preparedness of the Mae Hong Son Province for the Aging Society

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This survey study aim 1) to investigate the preparation of Mae Hong Son people for entering into the aging society 2) to study awareness of public health preparedness for the aging society of Mae Hong Son Province Administrative Organization. The samples used in this study were people aged 55 - 60 years in Mae Hong Province. Located at Khun Yuam Sub district, Khun Yuam District, Pang Ma Pha Sub district, Pang Ma Pha District, Thung Yao Sub district, Pai District, Mae ka Tuan Sub district, Sob Moei District, Mae Sariang Sub district, Mae Sariang District, Mae Tho Sub district, Mae La Noi District. And Huai Pha Sub district, Muang Mae Hong District. The data were collected from 1,088 people by Stratified sampling Method. The instrument used in this study were 36 items of questionnaire that contains three parts: 1) Sample's general information 2) The Interview of Mae Hong Son people's preparation before entering aging society. 3) The Interview about preparedness of health for the aging society of Mae Hong Son Province Administrative Organization. Then analyzed the data by using percentage and standard deviation. The research found that Mae Hong Son people are preparing for an aging society as followed; psychological, residence, physical health, careers and leisure time on a large scale with an average of 3.81 (SD=0.88), 3.66(SD=0.99), 3.53(SD=1.04) and 3.51(SD=0.89), respectively. However finances and saving were prepared on moderate scale with an average of 2.84(SD=0.89) and in the awareness of public health preparedness for the aging society of Mae Hong Son Province Administrative Organization were moderate with an average of 2.99 (SD=1.07)

Abstract No. 8

Multimodal imaging of cellular redox balance and mitochondrial function in Alzheimer's disease

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Aging is associated with a shift in cellular redox balance, with mitochondrial dysfunction as one underlying reason. Likewise, Alzheimer's disease (AD), the most prominent disease in the aged population, as well as other neurodegenerative disease are characterized by alterations in brain energy metabolism. Indeed, metabolic deficits are not uniform in the brain but there is a selective vulnerability of different brain regions, cell types and even mitochondrial populations. Current methods in assessing mitochondrial function are limited with respect to their capability of spatially separating these metabolic differences. Tough, NADH exhibits autofluorescence, allowing a

marker-free detection with high spatial resolution. In surplus, NADH redox state encodes the information of cellular redox balance and mitochondrial function, possessing the potential of an ideal marker in aging research. However, as an indirect parameter of mitochondrial function, NADH redox state is further influenced by a range of cellular and environmental factors. Our aim was to decode NADH redox state in terms of mitochondrial respiration to establish a metabolic imaging technique with subcellular resolution. For this purpose we determined NADH redox state by measuring its autofluorescence lifetime and correlated it with high-resolution respirometry in an Oroboros Oxygraph. Here, we carved out mitochondrial matrix pH as the most important confounding factor in metabolic imaging using NADH. Combining both parameters improves quantitative correlation of NADH lifetime and cellular respiration, as demonstrated in metabolically-modified cells as well as in an APP-overexpressing model of Alzheimer's disease. Further we demonstrate the suitability of this highly innovative approach in primary neurons, astrocyte-neuron co-cultures and brain slices providing novel insight into the complex energy metabolic interactions between cell types of the brain. By combining redox state and mitochondrial function, our multimodal imaging approach will shed light onto bioenergetic alterations during aging and age-associated pathologies.

Abstract No. 9

Monitoring nucleolar activity as a markers of disease progression in age-dependent neurodegenerative diseases

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Decreased rRNA synthesis and disrupted nucleolar integrity are associated with age-dependent neurodegenerative disorders such as Parkinson's disease (PD), Huntington's disease (HD) and Alzheimer's disease (AD). Nonetheless their role in disease progression is still unclear. We optimized molecular and histological methods to monitor nucleolar activity in mouse and human tissues. Our aim is it to perform a systematic analysis of nucleolar function in different models of HD to explore the hypothesis that nucleolar deficits are specific marker of disease progression in HD. In addition, we investigate the impact of altered rRNA synthesis on protein synthesis. In a cellular model expressing mutant huntingtin, the expression of factors regulating rRNA synthesis is affected as well as markers of nucleolar integrity and protein synthesis. In a mouse model of HD rRNA synthesis is altered in striatal neurons but not in muscle tissue at early stages, suggesting context-specific networks accounting for a transient neuroprotective response prior to neuronal death in HD. The analysis at later stages is in progress. Our work indicates nucleolar activity might regulate neuronal homeostasis and progressive neurodegeneration, supporting the identification of novel disease modifiers regulating nucleolar function.

Abstract No. 10

Adaptive Immunodeficiency Accelerates Intestinal Aging of Telomere-dysfunctional Mice

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Mice with dysfunctional telomeres suffer from accelerated aging phenotypes. The accumulation of DNA damage triggers checkpoint responses, which prevent genomic instability but compromise stem cell function and contributes to aging pathologies. Recently, several studies provided evidence that oncogene induced stress and DNA damage both engage the immune system preventing tumour formation and the survival of damaged cells 1. Little is known about a crosstalk between aging associated DNA damage responses and the immune system. To this end we set up studies comparing aging wild type mice with Rag2 knockout mice (adaptive immune-deficient mice), and the respective cohorts in a telomerase (Terc) knockout background. Late generation Terc^{-/-} mice develop age related pathologies in highly proliferative tissues like the hematopoietic system and the intestine characterized by stem cell dysfunction, accumulating DNA damage, gastrointestinal crypt atrophy and reduced B and T cell proliferation 2. Our preliminary data show improved survival of aging late generation Terc^{-/-} mice as compared with double knockout mice (Rag2^{-/-} Terc^{-/-}). The histologic analysis reveals aggravated crypt atrophy, fibrosis, senescence and more DNA damage in the gastrointestinal epithelium of Rag2^{-/-} Terc^{-/-} mice compared to Terc^{-/-} G4 mice. There is increase in Cd4+Cd25+ (Treg) population in the gut of Terc^{-/-}G4 after intestine specific sub-lethal irradiation. To our knowledge increase macrophages in lamina propria of intestine and absence of Treg cells in Rag2 Terc (DKO) are responsible for wasting colonic phenotype. Further experiments are ongoing to confirm the functional role of Treg cells in damage clearance in the aging gut.

Abstract No. 11

Characterization of vaccine-induced, asialo GM1-expressing CD8 T-cell responses in aging mice

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Aged humans and mice show reduced levels of immune responses, especially towards new antigens. This condition, known as immunosenescence, is caused by many age-related changes within the immune system. Especially CD8 T-cells show strong alterations upon aging: In contrast to young, most CD8 T-cells of old mice express a memory (CD44^{hi}) surface phenotype and only a low output of recent thymic emigrants replenishes their naïve (CD44^{lo}, CD62L^{hi}) T-cell pool. A phenomenon associated with immunosenescence of memory CD8 T-cells is the surface expression of NK-cell markers like KLRG1 and NKG2D. The ganglioside asialo GM1 (GA1), a known NK-cell marker, was found on the surface of CD8 T-cells but its expression in old mice and its role in the immunosenescence of CD8 T-cells was not investigated yet. We showed that GA1 is

not (or barely) expressed on naïve CD8 T-cells, but rapidly induced on both, young (2-3 months) and old (>18months) murine CD8 T-cells upon activation. Therefore, all activated effector CD8 T-cells express GA1 and were depleted by anti GA1 antibody injections into mice. In particular, this finding is relevant for the depletion of autoreactive effector CD8 T-cells and the inhibition of autoimmune diseases. Interestingly, the memory CD8 T-cell populations in non-immunized young and old mice (corresponding to 20% or >80% of all CD8 T-cells in the spleen, respectively) express GA1. The depletion of these memory CD8 T-cells in young mice by anti GA1 antibody injections had no significant effect on the de novo priming of HBV core-specific CD8 T-cells by DNA-based vaccination. In contrast, our preliminary data indicated that depletion of the major memory CD8 T-cell pool in old mice, enhanced the priming of effector CD8 T-cells from the naïve T-cell pool. This suggested that non-specific memory CD8 T-cells in old mice may suppress de novo priming of CD8 T-cells.

Abstract No. 12

CD4+ T cells from elderly individuals are more susceptible to HIV-1 infection and apoptosis

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Elderly HIV-1-infected individuals progress to AIDS more frequently and rapidly than people becoming infected at young age. Additionally, HIV-1 infection is associated with an accelerated aging of the immune system and many non-AIDS but age-related illnesses (cardiovascular and renal disease, diabetes mellitus, dementia, arthritis) are more frequent in HIV-1-infected individuals than in age-matched uninfected people. HIV-infection and aging share many characteristics: both are associated with a low production of naïve T cells, diminished T cell functionality, an accumulation of aging T cells, a loss of regenerative capacity and an increase of memory CD4+ T cells, which are predominantly infected by HIV-1 and serve as a major cellular reservoir. Furthermore, age is an important factor in AIDS progression because the regenerative capacity of people becoming infected at older age is already reduced. To identify possible reasons for accelerated aging processes in HIV-infected individuals and the differences in clinical progression rates, we infected CD4+ T cells from healthy young and elderly individuals with HIV-1 and performed comprehensive phenotypic and functional analyses. Unstimulated T cells from elderly individuals expressed higher levels of activation markers and death receptors as well as the viral CXCR4 coreceptor but responded poorly to stimulation. Upon stimulation they were highly susceptible to HIV-1 infection but produced less infectious virus, which might be due to a decreased life span. The increased susceptibility of T cells from elderly correlated directly with CXCR4 and inversely with CD4 expression and the level of apoptosis correlated with cell surface expression of FAS. Our results suggest that the immune system of people becoming HIV-infected at older age provides a particular susceptible environment for HIV-1 infection and immune damage.

Abstract No. 13

Aging epigenome of muscle stem cells in response to injury

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Muscle stem cells (MuSCs) are embedded into skeletal muscle tissue and become activated upon injury to repair damaged myofibers or give rise to myofibers de novo. With age, the regenerative capacity and functionality of MuSC declines. The transition from the quiescent to the activated state is parallel to profound changes of the epigenetic landscape in regard of histone modifications and DNA methylation. These epigenetic changes are crucial for the myogenic commitment and self-renewal of MuSC. Our data indicates a global alteration of the epigenome in aged MuSC thereby promoting aberrant gene expression and the induction of developmental pathways upon activation. Importantly, functionality of aged MuSC could be enhanced by siRNA or chemical compound treatment targeting epigenetic modifiers being involved in the age related alteration of the epigenome. Our aim is to identify upstream mechanisms that lead to the altered epigenome of aged MuSC and identify approaches for epigenetic therapies.

Abstract No. 14

Cell-nonautonomous Regulation of Proteostasis and Longevity by the microRNA mir-71

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The maintenance of protein homeostasis (proteostasis) during aging is crucial for a healthy and long life. Despite the identification of multiple factors involved in the response to proteotoxic stress, the role microRNAs play in the regulation of proteostasis and longevity has been understudied. Therefore, we analyzed the role of microRNAs on proteasomal degradation as well as additional proteostasis pathways and sensitivity on proteotoxic stress in *Caenorhabditis elegans*. In doing so, we identified the microRNA mir-71 as a highly influential regulator of these processes. mir-71 is indispensable in a specific subset of chemosensory neurons, so called amphid wing C (AWC) cells, where it controls the toll and interleukin 1 receptor domain protein TIR-1; an adapter protein that is well described in the innate immune response and neuronal development. However, TIR-1 has not yet been associated with proteostasis. Moreover, we demonstrate that mir-71 is dependent on the release of neuropeptides and thereby provides an uncharacterized cell-nonautonomous signaling between AWC neurons and the intestine. Rescue of mir-71 in AWC neurons is sufficient to overcome proteostasis defects and decreased lifespan. Our findings reveal a mir-71 dependent cell-nonautonomous signaling through TIR-1 that originates in AWC neurons with a strong impact on organism wide proteostasis and lifespan. This broadens our

understanding of the importance of the connectivity between different tissues and its profound influence on processes relevant in aging.

Abstract No. 15

Induction of the progeroid/cancer prone XP-like phenotype by a medical drug is mediated via reversible downregulation of DNA repair.

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Prophylactic protection of patients with severe immunosuppression is of vital importance to shield the patient from opportunistic fungal infections. It has been reported, that patients treated with a broad spectrum antimycotic drug (AD) develop adverse effects such as phototoxicity followed by pigmentary changes and the development of ultraviolet radiation (UV) associated non melanoma skin tumors. Thus, patients closely resemble the phenotype of the progeroid disorder xeroderma pigmentosum (XP), known to be caused by a defect in the DNA repair mechanism nucleotide excision repair (NER). So far the underlying molecular mechanisms by which this drug leads to the XP-like clinical phenotype have not been clarified. Therefore, we investigated if the antimycotic drug leads to a reduction of DNA repair and increases DNA damage. We found that long term treatment lead to suppression of unscheduled DNA synthesis as well as increased comet formation while double strand breaks were not induced. Importantly repair suppressive effects were transient since removal lead to normalization of all repair associated parameters. Furthermore, compound treatment did not cause significant transcriptional regulation of mRNA levels of NER proteins such as XPA – G, ERCC1 and RAD23 A/B and of DNA damage signaling factors (ATM and ATR). Interestingly electronmicroscopy revealed AD induced changes in Chromatin density and location of AD at the chromatin. Furthermore DNA damage dependent histone acetylation was reduced upon AD treatment and could be rescued with the addition of inhibitors of the histone deacetylases (HDAC). Finally the simultaneous treatment with AD and a HDAC inhibitor rescued the repair defect and the accumulation of damage. Taken together these results indicate that the broad spectrum antimycotic suppress NER, via inhibition of histone acetyltransferases, increase DNA damage and thus, within months lead to photosensitivity, pigmentary changes and ultimately skin tumors.

Abstract No. 16

RBP-J associated cofactor SHARP/Mint as an epigenetic regulator of tissue repair and aging

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In the absence of a Notch signal, transcription factor RBP-J recruits corepressors, that deacetylate and demethylate histones at Notch target genes. We identified the cofactor SHARP as a direct interaction partner of RBP-J that recruits the corepressor NCoR. Recently, we identified that SHARP not only interacts with repressing NCoR-complex but also with activating methyltransferase KMT2D/ASCOM, the mammalian Trithorax group ASC-2 complex (Oswald et al., Nucleic Acids Res., 2016). In worm and fly, mutations in components of Trithorax and the antagonistic Polycomb complexes are associated with altered histone modifications and lifespan. Knockdown of SHARP/Mint perturbs methylation of H3K4 and H3K27 indicating an essential role of SHARP/Mint in that context. Mice deficient for the ASCOM subunit ASC-2 are embryonic lethal whereas adult ASC-2^{+/-} mice exhibit spontaneous wound healing defects. A pilot study using young and aged wildtype mice already showed that expression of Polycomb repressive complex 2 components are downregulated during wound healing whereas H3K27 demethylase Kdm6b (Jmjd3) is upregulated. This correlates with previously discovered epigenetic reprogramming and derepression of wound repair genes during tissue regeneration. Additionally, we observed changes in Notch target genes underlining a role of Notch signaling in wound healing. By using a conditional SHARP/Mint knockout model, we analyzed the role of SHARP/Mint in tissue regeneration. SHARP/Mint^{flox/flox} mice were crossed with Col1 α 2-Cre mice expressing Cre in the connective tissue and skin fibroblasts, as well as with K14-Cre mice showing epidermal Cre expression. The wound healing capacity after skin injury was monitored over 10 days in SHARP/Mint^{del/del}, SHARP/Mint^{flox/del} and wildtype mice using a full-thickness excisional wound healing approach. Indeed, K14-Cre x SHARP/Mint^{del/del} mice show significantly impaired wound healing capacity compared with wildtype mice. Taken together, we propose that SHARP/Mint plays a pivotal role as an epigenetic regulator of wound/tissue repair and, potentially, also in aging.

Abstract No. 17

Accelerating telomere shortening and premature aging in telomerase deficient mice upon deletion of H2AX or MDC1 component of DNA damage machinery

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Telomere shortening limits the proliferative capacity of human cells and tissues by induction of DNA damage checkpoints. Studies on telomerase deficient mice revealed that telomere shortening impairs the maintenance of adult stem cells and organ

homeostasis leading to premature aging and a shortened lifespan. Telomeres protect ends of chromosomes from being detected as DNA lesions and at the same time any type of repair at telomeres causes deleterious chromosomal fusions and genomic instability. However, many DNA damage proteins, like ATM, ATR and MRN complex, play an essential role in telomere replication and function, are localized to functional telomeres and their deletions leads to accelerated telomere shortening and de-protection. In this study we were interested to investigate the role of H2AX and MDC1, early DNA damage sensing and signaling proteins, at dysfunctional short telomeres. Here, we show that the deletion of H2AX or MDC1 in aging telomere dysfunctional mouse compromise stem cell function, organ homeostasis and shorten the lifespan of telomere dysfunctional mice without inducing tumor formation. H2AX or MDC1 deletions lead to increased checkpoint activation and premature intestinal failure. Furthermore, these mice show accelerated telomere shortening and increased anaphase bridges in intestinal crypts, reflects a morphological sign of dysfunctional telomeres. MDC1 deletion induces chromosomal instability in stem and progenitor cells of the small intestine and accelerates the loss of stem cells in response to telomere dysfunction. Mechanistically, deletion of H2AX or MDC1 increase chromosomal fusion through increasing of Exo1 mediated end-resection at dysfunctional short telomeres. Moreover, MDC1 deletion showed reduced repopulation activity of hematopoietic stem cells in competitive transplantations. Together, these results provide the first evidence for the role of H2AX and MDC1 in stem cell telomere maintenance and their deletion can cause accelerated aging, organ system failure and premature death in the setting of stem cell aging mouse model.

Abstract No. 18

DNA Replication Fidelity, Error Catastrophe and Aging

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DNA replication represents an optimized, highly accurate process, but is at the same time the main contributor of genome instability. The number of replication determines the mutational load of a cell and is determined mainly by the rounds of cell divisions in an organism. Thus, increasing the number of population doublings ad libitum does not increase life span, but rather may lead to cancer and age-related pathologies. Whereas microorganisms have an extremely low error rate of about 0.004 mutations per genome and generation (i.e. only one out of 250 offspring cells bear a mutation), this number is estimated at a surprisingly high rate of 70-140 mutations per genome and generation in humans. Spontaneous mutation rates in the human soma appear to be even higher. Thus, mutation accumulation poses a serious threat to large and long-living animals such as humans. This is reflected by the accumulation of stem cells with clonal mutations and the high incidence of cancer in the old. The present poster contemplates the determinants of replication error rates from a theoretical point of view. We have also established syngenic human HAP1 cell models created by CRISPR-Cas9 genome editing defective in different mechanisms contributing to DNA replication fidelity. Combined with fluctuation analysis, these cell lines are utilised to determine

upper limits of replication error rate tolerated in cancer cells. With these studies we aim to develop new strategies to study and to interfere with mutation accumulation in stem cells and in cancer.

Abstract No. 19

Knockout of p53 and tert in the turquoise killifish *Nothobranchius furzeri*

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The turquoise killifish *Nothobranchius furzeri* has proven to be an increasingly useful model organism in the field of aging research. Its extremely short vertebrate lifespan of only up to one year goes along with rapid growth and early sexual maturation. Still the teleost fish shows typical signs of aging, like shortening of telomeres or the impairment of mitochondrial function. Telomeres and p53 play critical roles in ensuring genomic stability and in the aging process. We have used TALEN and CRISPR/Cas9 technology in *N. furzeri* to analyze the effects of deleting p53 and tert (the enzymatic component of telomerase) in an age-dependent context. Even though no change on the expression levels of tert was detected in the tert knockouts, the telomerase activity is completely abolished and the telomeres are subsequently shortened. Conceivably due to this shortening, the lifespan of the homozygous tert knockouts is significantly reduced. Furthermore, we analyzed the effects of the tert knockout on the naturally high regenerative capacity of the fish. Interestingly, even after multiple amputations of the tail fin, the knockout does not affect this ability. However an age-dependent decline was observed. CRISPR/Cas9 technology was used to generate p53 knockout mutants. In wildtype *N. furzeri*, a subset of p53 target genes involved in cell cycle control and apoptosis was up-regulated upon irradiation, which was lost in p53 knockout mutants. Immunohistological analyses revealed a stop of cell proliferation in wildtypes after irradiation, whereas proliferation still continued in irradiated p53^{-/-} animals. The median lifespan of p53-deficient fish was significantly reduced. Visible tumors were found in the p53 knockouts, which probably contribute to this lifespan reduction. Additionally, respective tert/p53 double mutants promise to reveal further insights into the regulation of telomeres and DNA damage, as well as subsequent cellular and organismal effects such as apoptosis, cell cycle control and tumorigenesis.

Abstract No. 20

Attenuation of IGFBP7 Disrupts Hematopoiesis and Causes Leukemia

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Insulin-Like Growth Factor Binding Protein 7 (IGFBP7) is a secreted protein shown to induce apoptosis and senescence as well as reduce proliferation in many different cancer cell lines including acute myeloid leukemia, breast, prostate, hepatocellular, lung, and colorectal cancers. In an aneuploidy screen IGFBP7 was one of the genes identified in controlling aneuploidy. What is IGFBP7's role in controlling cancer and aneuploidy in vivo? To answer this we are employing an inducible and reversible shRNA

mouse model to knockdown expression of IGFBP7. We observed that after knockdown of IGFBP7 hematopoietic stem and progenitor cell populations were changed. Specifically, HSC and MPP populations were decreased while all progenitor populations were increased. We found an increase in pro growth signaling including increased phosphorylated IGF1 receptor and increased expression of Cyclin C and IGF1r. We also observed less quiescence of HSCs and increased cycling of KSL cells. HSPCs with a knock down of IGFBP7 develop aneuploidy. These mice eventually develop myeloid leukemia which includes higher white blood cell counts, splenomegaly, weight loss, and an expansion of myeloid cells in the peripheral blood and spleen. Is this leukemia caused by elevated self-renewal of progenitor cells or by an inability to remove pre-leukemic cells that become clonal? These questions are being addressed now

Abstract No. 21

Expression and activity of the small RhoGTPase Cdc42 in blood cells of older adults are associated with age and cardiovascular disease

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The expression level of the small RhoGTPCdc42 in human lymphoblastoid cells from the Utah CEU cohort was previously reported to correlate with age and to be a significant predictor of survival in humans. In mice, the activity of Cdc42 is increased in multiple types of tissues and organs upon aging and mice genetically engineered to present with elevated levels of Cdc42 activity already in young animals display premature aging. Inhibition of the aging-associated elevated Cdc42 activity in for example murine blood forming stem cells (hematopoietic stem cells, HSCs) rejuvenates HSCs and thus reverts unwanted aging-associated changes in hematopoiesis. These data imply that Cdc42 is mechanistically linked to aging and rejuvenation and might serve as a novel biomarker of aging. Here we determined Cdc42 activity and expression levels in peripheral blood (PB) cells from a cohort of 196 older adults. We investigated the association of these parameters with both chronological and biological aging. We also tested in this cohort a recently published algorithm determining chronological age based on DNA methylation profiles. A positive correlation with chronological age was found for both the level of Cdc42 mRNA and the level of active Cdc42 protein (the GTP bound form). The level of Cdc42 mRNA as well as total protein showed also a strong association to cardiovascular disease (CVD) and Cdc42 mRNA levels also to a history of myocardial infarction (MI). In summary, these data validated Cdc42 as a blood biomarker of both chronological aging as well as aging-associated diseases like CVD and MI.

Abstract No. 22

AGEs in the hematopoietic system

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Advanced glycation end-products (AGEs) are protein modifications that emerge due to purely chemical reactions with sugars, a process called glycation. We focuss CML, the most studied AGE, and use antibody detection to determine its levels in different cell types of the hematopoietic system.

Abstract No. 23

Identification of novel Hox target genes with essential roles in hematopoietic stem cell self renewal and differentiation

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Hematopoietic stem cells (HSCs) are able to restore the entire hematopoietic system in vertebrates. During aging, the number of hematopoietic stem cell is increased while their regenerative potential is decreased. In addition, skewing in the differentiation process of old HSCs is observed, resulting in enhanced myelopoiesis and a decreased lymphopoiesis. It is of key importance to understand how the equilibrium of self-renewal and differentiation is maintained in HSCs. Members of the Hox gene family have been shown to be involved in this process. In order to understand the function of Hox genes in HSCs we dissected the downstream effect of Hox genes. For this purpose we applied both in vitro and in vivo approaches to investigate the role of Hox genes and their downstream pathways in HSCs. We conducted an unbiased in vivo screen using a focused shRNA pool targeting all Hox gene members in HSCs in serial transplanted mice. This screen revealed a comprehensive map of Hox genes that are required for the maintenance of HSCs including Hox genes that have not been implicated in HSCs control. The functional role of Hox genes in HSC self renewal was validated by transplantation experiments. Since Hox genes have very similar DNA binding motifs and show similar phenotype in our study, we investigated whether they share common molecular targets. Using microarray-based transcription profiling on single Hox gene knock-downs that affect HSCs maintenance, novel target genes were identified in hematopoietic cells that are regulated by 2 or multiple Hox genes. Of note, the knockdown or overexpression of individual target genes phenocopies the known effects of Hox gene overexpression or knockdown on HSCs self renewal and differentiation. Together, this study identifies novel essential regulators of HSCs that require a combinational transcriptional input of Hox genes for their proper expression and function in HSCs.

Abstract No. 24

Investigating the effect of hematopoietic stem cell aging on immune function

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Aging of hematopoietic stem cells is thought to contribute to aging-associated immune remodelling which among others manifests itself in a decreased responsiveness to vaccines. In this project we further characterize the influence of HSC-aging on the reconstitution of the immune system and its function by analyzing vaccine-induced immune responses. To this, we established a new transplantation model: young and aged CD45.1+ long term hematopoietic stem cells (LT-HSCs) purified from B6.SJL-Ptprca Pepcb/BoyJ mice were adoptively transferred into irradiated T- and B-cell-deficient CD45.2+ RAG1-/- mice. Using this model, we can ensure that all cells of the adaptive immune system arise from the transplanted LT-HSCs. In mice which had received old LT-HSCs the percentage of naïve CD4+ and CD8+ T-cells in the spleen was decreased compared to those transplanted with young LT-HSCs. Mice which had received aged LT-HSCs had an increased proportion of FoxP3+ CD3+CD4+ T-cells compared to those which had been transplanted with young LT-HSCs. Besides, after immunization with plasmid DNA, antigen-specific CD8 T-cells were efficiently induced in mice which had received young LT-HSCs, whereas significantly less antigen-specific cells could be detected in mice which had been transplanted with aged LT-HSCs. Thus, the reconstituted immune system of mice which had received aged LT-HSCs resembled an old immune system. In previous experiments it could be shown that aged LT-HSCs can be rejuvenated by treatment with CASIN, an inhibitor of the GTPase Cdc42, which reprograms the epigenetic status of aged HSCs. In first preliminary experiments we could show that the proportion of antigen-specific CD8+ T-cells after DNA immunization was significantly increased in mice transplanted with rejuvenated aged LT-HSCs, compared to mice which had received non-treated old LT-HSCs. In ongoing experiments we are further investigating the effect of rejuvenation of aged LT-HSCs on the repopulating immune system and its function.

Abstract No. 25

DNA double-strand break repair in human hematopoietic cells during aging

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Hematopoietic stem and progenitor cells (HSPC), maintaining the hematopoietic system for a lifetime, and even shorter lived peripheral blood lymphocytes (PBL) are known to accumulate DNA damage with age, suggesting a decline of DNA repair activities. Age-related changes of the DNA damage response have been proposed to critically limit stem cell survival and thus lead to aging of tissues and ultimately the organism. From these links between DNA repair, stem cell maintenance and aging, the

question arises how HSPC deal with DNA damage compared to mature blood cells and how repair processes change during aging. As this issue has been addressed mostly in murine models so far, we analyze different aspects of DNA double-strand break (DSB) repair in primary cycling human HSPC and PBL derived from donors of varying age. Applying an EGFP-based reporter system we detected a change in DSB repair pathway usage in both cell types upon aging. Monitoring the DNA damage markers gammaH2AX and 53BP1 after damage induction supported the age-dependent differences in DSB repair activities, as seen with the reporter assay. Elevated numbers of 53BP1 foci in cells from old individuals post Mitomycin C treatment indicate an accumulation of repair intermediates, pointing to a deficiency in downstream homologous repair (HR). Since HR represents a major bypass mechanism of replication stress, a decrease in HR activity, in combination with an accumulation of basal gammaH2AX signals without DSB formation, likely indicates replication damage. Therefore, cells from old individuals might be compromised in their ability to remove replication lesions, ultimately leading to accumulation of stalled replication forks. Altogether, we show that DSB repair processes are indeed changing upon aging in both, primitive and mature cells of the human hematopoietic system, which might contribute to the aging process and the development of age-associated diseases, such as anemia and leukemia.

Abstract No. 26

Revealing the positive effects of p21 deletion

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Since its discovery more than 20 years ago, our view on p21 has changed: rather than being a simple "cell cycle inhibitor, senescence inducer, and tumor suppressor" it is now appreciated as a much more complex and broader regulator of different cellular programs. For example, p21 can act both as a tumor suppressor and as an oncoprotein, and accordingly, studies on various model systems demonstrated that p21-deficient mice are characterized by decreased susceptibility to tumor formation than wt mice. Also, it was shown that loss of p21 has an anti-aging effect and can be beneficial for tissue regeneration. That is why the deletion of p21 can be beneficial in anti-cancer, anti-aging, and pro-regeneration medicine and experiments on inducible conditional p21 knockout mice seem to represent a particular interest. In our lab such mouse was created, and due to chosen Cre recombinase under Mx promoter, we are able to induce p21 deletion in liver, spleen and bone marrow. As first experiments on this system, we checked p21 involvement in aged liver regeneration after 2/3 partial hepatectomy, and in maintaining quiescent status, proliferation and differentiation of HSCs (hematopoietic stem cells). Experiments on p21 involvement in leukemia development will be presented as well. Summarizing, we developed an interesting mouse model, which can be used for revealing p21 role and its downregulation in cancer, aging and regeneration studies.

Abstract No. 27

Septin 7 is a novel downstream effector of Cdc42 for regulating aging of HSCs

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Aging functionally impairs hematopoietic stem cells (HSCs) yet the underlying molecular mechanisms are poorly understood. Changes in the activity of the small Rho GTPase Cdc42 regulate actin and tubulin organization as well as cell polarity in distinct cell types. Cdc42 activity is increased upon aging of HSCs. This increased activity results in an apolar distribution of cytoplasmic and nuclear polarity proteins and causes decreased stem cell function. Polarity in the cytoplasm is organized, among others, by septins, a family of 13 GTP-binding proteins. Septins form filaments and act as scaffolds or diffusion barriers and it was shown that they can act downstream of Cdc42 via effector proteins called borgs. Hyperactive Cdc42 causes a loss of septin filament assembly, probably by inhibiting the interaction between borgs and septins. We thus hypothesized that septins play a role downstream of Cdc42 and borgs in establishing and maintaining polarity in young LT-HSCs while borgs might link Cdc42 and septins with respect to signal transmission. Our data showed that expression of septin 7 mRNA was decreased in aged long-term repopulating HSCs (LT-HSCs). Among all the septins tested, only septin 7 displayed a polar distribution in young LT-HSCs, which was LT-HSC specific and regulated by Cdc42 activity. Using proximity ligation assays, we verified and quantified a direct interaction between septin 7 and borg 4 as well as between Cdc42 and borg 4 in LT-HSCs. The extent of these interactions was dependent on Cdc42 activity, thus further supporting our hypothesis. The functional role of septin 7 in LT-HSCs is currently determined in bone marrow transplantation experiments. The identification of mechanisms that control polarity and thus cytoskeletal remodeling upon aging will contribute to our understanding of aging-associated hematopoietic dysfunction and disease.

Abstract No. 28

The Role of the Miz-1 Transcription Factor in the Aging of Haematopoietic Stem Cells.

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The haematopoietic system in vertebrates consists of cells of the adaptive and innate immune system. Haematopoietic stem cells (HSCs) produce all blood cells of the haematopoietic system, but also have the ability to self-renewal throughout life. Nevertheless, during aging HSCs lose their self-renewal potential and are more biased to the myeloid lineage, the cells of the innate immune system. The helix-loop-helix transcription factor c-Myc is one of the key factors in maintaining HSC homeostasis in

controlling the proliferation, differentiation, and migration of HSCs from the stem cell niche. Upon expression of c-Myc, HSCs globally increase mRNA expression and decrease expression of α - and β -integrins on the cell surface, allowing them to migrate out of the stem cell niche and to differentiate. The Myc-interacting zinc-finger protein 1 (Miz-1) transcription factor and c-Myc bind to form a complex, which represses expression of the negative cell cycle inhibitor p21, allowing the cell to progress through the cell cycle. Furthermore, Miz-1 is essential allow proper lymphocyte development by regulating cytokine signalling pathways and apoptosis in common lymphoid progenitor cells (CLPs). Interestingly, we found in young mice that absence of Miz-1 results in an increase of long term (LT)-HSCs in mice with loss of self-renewal capacity as well as altered developmental potential. This effect resembles the phenotype in aged mice, pointing to a crucial role of Miz-1 and the Miz-1/Myc-complex in the aging process of the hematopoietic system.

Abstract No. 29

Glycolysis in young and aged hematopoietic stem cells: Flux, Key players and Localization

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Adult stem cells can self-renew and differentiate into all lineages, maintaining tissue homeostasis and regenerative capacity of the organ. Declining stem cell function upon aging leads to reduced regeneration and increased susceptibility to cancer and degenerative diseases. For a long time, stem cell metabolism has only been viewed as a status of the cell, not as a regulatory entity. In the last several years, glycolytic flux, oxidative phosphorylation (OXPHOS), fatty acid oxidation and ROS levels have become strong indicators for stem cell function and fate. Hematopoietic stem cells (HSCs) are mostly quiescent and perform anaerobic metabolism. Upon entry into active cell cycle a switch to OXPHOS is essential. Aged HSCs are less functional, mitochondrial dysfunction and higher ROS levels are observable, but so far there are barely any reports on glycolytic changes. In this study we would like to do an in-depth comparison of young and aged HSCs for differences in glycolytic flux and subcellular localization of glycolytic enzymes. The aim thereby is to test for glycolytic centers similar to the metabolon in plants, to correlate location patterns of glycolytic enzymes to anaerobic or aerobic metabolism, to analyze the influence of stereoscopic changes on glycolysis upon aging and to detect recurrent patterns in young and old HSCs. Using fluorescence microscopy, we will analyze localization and co-localization patterns of key enzymes of glycolysis, cytoskeleton and downstream pathways like OXPHOS and pentose phosphate pathway. We will also work on establishing a method to trace glucose in HSCs. In a last step, we would like to examine how the usage of cytoskeleton modulating reagent, Casin, is influencing glycolysis flux and localization. The aim is, to identify another mode of action for Casin and to find a new point of application in rejuvenation therapies of aged HSCs – changing stereoscopic arrangement of enzymes.

Abstract No. 30

Integrative approach for discovery of novel pathways in stem cell aging

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An increasing number of available aging related studies allows creation of mathematical models and hypothesis-generating methods in biological systems. Among others, high-throughput datasets in individual studies often focus on the identification of candidate probes and associated gene sets that may be involved in aging. The integration of these candidate probes poses a major challenge and requires specialized methods that can handle the heterogeneity, inherent high-dimensionality, and small sample size in high-throughput experiments. We present an integrative approach for merging ranked lists of candidate probes across different platforms and species via rank aggregation. These ranked lists reflect the influence of single candidate probes to a specific study question. Combining such ranked lists of candidate probes among the different studies may reveal influences common in aging. Rank aggregation methods are the natural choice to combine the different ranked candidate probes. These methods compute a consensus ranking that reflects a ranking with least disagreement to the input lists. Hence, candidate probes that are persistently ranked high in individual studies will also be present in top positions a consensus ranking. This ranking information is then used in a gene set analysis with a novel AUC-like measure. This measure characterizes the influence of gene sets to the aging process and may reveal a gene set's significance in the common pathways of aging. Furthermore, this approach reveals new gene sets that would not be identified by the individual study's analysis. In a show-case we analyze twelve different stem cell aging related high-throughput studies. The analysis reveals novel hypotheses about gene sets that were not identified in the analysis of single studies. Although, one of these additional gene sets and genes within are known to be associated with aging, other gene sets pose new hypotheses about the common processes in stem cell aging.

Abstract No. 31

Genetic and compound screens for mediators of ER stress tolerance

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Accumulation of molecular damages along with decline in repair capacities are prominent hallmarks of aging. Particularly, protein damage and disruption of proteostasis are amongst most pronounced age-related features. Improved protein quality control has been linked to stress tolerance and lifespan extension suggesting that proteostasis enhancers could serve as mediators of healthy aging. Protein folding stress in the endoplasmic reticulum (ER) is particularly relevant in aging as it is fueled by chronic baseline inflammation which develops at old age. We use screens in human cells and *C.elegans* to identify novel genetic factors and chemical compounds which promote cell survival under protein folding stress in the ER. To date genetic screens for factors implicated in protein quality control were almost exclusively performed in

invertebrate models such as nematodes and flies. We used CRISPR/Cas9 genome editing for genome-wide gene inactivation in mammalian cells. This approach allows us to perform genetic screens for mediators of ER stress tolerance in the mammalian system. We transduced human fibroblasts with a lentiviral CRISPR/Cas9 library targeting 19,050 human protein coding genes and 1,864 miRNA precursors. Candidate clones were selected based on enhanced survival of DTT treatment, validated by a second round of DTT exposure and sequenced to search for enrichment of specific gRNAs in these clones. In *C.elegans* we address inflammation-induced systemic ER-stress which has high relevance in aging and in age-linked disorders such as inflammatory bowel disease. We use inflammation-induced developmental arrest of ER-stress sensitive xbp-1 mutants as a model system to screen for chemicals which protect from ER-stress. We use advanced larval stage-specific GFP expression to detect animals which resume development upon incubation with protective compounds. The high-throughput compound screen will be performed in collaboration with the screening unit of the Leibniz Institute for Molecular Pharmacology which developed a platform for microscopy-based compound screens in *C.elegans*.

Abstract No. 32

Lifespan Data Analysis – 3D Gene Network Visualization with JSnet3D-AgeFactDB

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The annotated ageing factor database AgeFactDB of the JenAge project contains over 7,000 lifespan observations. They provide detailed information on the effects of over 2,700 aging factors like genes and chemical compounds on the lifespan of different model organisms under different experimental conditions, such as gene inactivation, overexpression, and dietary restriction. For a single aging factor there can be hundreds of observations made under different conditions, involving many interactions with other ageing factors. Network models or graph representations can assist users in analyzing such complex data in many different ways. They provide compact representations that can easily be interpreted by the visual system of a human being. They allow for analyzing the data in multiple scales via zooming, rotating and filtering. In this work we present JSnet3D-AgeFactDB, an interactive 3D – network viewer specialized in the visualization of ageing-related data from AgeFactDB, combined with data from other databases and data provided by the user. It can be used to analyze lists of differentially expressed genes obtained from ageing-related studies. As an example we present the analysis of a list of differentially expressed genes from a study on the effect of D-Glucosamine (GlcN) on the lifespan of nematodes and ageing mice by Weimer et al., based on a series of RNA-seq experiments within the JenAge project. As a proof of concept we show how the viewer was used to identify new ageing-related genes for the integration into AgeFactDB. For this task the network model was expanded using KEGG pathway / NCBI Gene crosslinking information. This resulted in 48 candidate genes for a literature search, which revealed 13 ageing-related genes not already included in AgeFactDB. Our viewer is based on the open source chemical and

biomolecular 3D structure viewer Jmol. The AgeFactDB-specific HTML/Javascript graphical user interface runs within a web browser.

Abstract No. 33

Multi-class classification for the identification of specific biomarkers for age-related diseases

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Suitable for recording the activity of tens of thousands of molecular markers, high-throughput Omics technologies allow for a more and more detailed characterization of individuals or subjects and for a growing insight into the molecular background of diseases or phenotypes. They especially allow for a more fine-grained categorization of individuals into specific patient classes. As a consequence, diagnostic procedures are developing from binary classification schemes to more complex multi-class architectures consisting of multiple comparisons of different entities. The structure of such an architecture depends on the current task and the type of information that should be extracted. In this work we compare several feature selecting multi-class architectures for the purpose of analyzing gene expression profiles. These classifiers follow a common design principle: only a sparse selection of candidate markers from the global expression profile is taken into account. We focus on a comparison of two selection strategies. The first one is designed to utilize the same selection of features for each decision of the base classifiers. The second one utilizes different markers for each decision. We characterize the influence of the individual selection markers in terms of classification accuracy and feature selection stability.

Abstract No. 34

A damage driven model of cellular aging

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On organism level, aging is typically characterized with loss of regeneration, diminished function and eventually death. To this day, the exact cellular mechanisms leading to this progression are poorly understood. However several cellular deleterious traits correlate to aging cells (telomere shortening, increased DNA damage, change in chromatin structure, decreased function of a number of cellular processes). Senescent and cell cycle arrested cells can be discriminated from proliferating cells by specific molecular markers in human cell cultures, thus enabling the measurement of the progression of the number of senescent cells. Cell culture experiments with arrested cells show a hysteresis phenomenon, “premature senescence”, when these cells are transformed back into the proliferating state. The extent of this hysteresis varies among human fibroblast cell strains. As this effect is not reproducible with existing models, we extend our model by internal degrees of freedom, representing a cellular “age load” that mimics the effects of the deleterious traits known to correlate with cellular aging (such as DNA single and double strand breaks). The model is implemented

as an ensemble of cells, each with specific age loads. The cumulative age load monotonically influences the probability of transition from one state to another including proliferation, in repair, quiescent, cell cycle arrested, senescent or apoptotic.

Abstract No. 35

Transfer Learning for Invariant Biomarker Selection in Age-related Diseases

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Age-related diseases are typically caused by a multi-factorial combination of biomarkers. Their identification is one of the most important tasks in bioinformatics. The major ingredient in this context are biomarker selection techniques that allow for the construction of interpretable decision rules from high-dimensional marker profiles. Selecting valuable biomarkers, these techniques suggest potential hypotheses on the molecular background of a phenotype or disease. Often, these hypotheses will not be unique -- several candidate biomarker combinations and explanations exist. In this work, we provide an ensemble biomarker selection technique aiming at the construction of sparsely overlapping marker combinations. The technique is based on a genetic algorithm with diversity preserving methods. The marker combinations are rated by a correlation-based measure. The best marker combinations are finally aggregated to a multi-classifier system. Our algorithms is trained and tuned on marker profiles from varying age-related diseases. The tuning is carried out on multiple datasets in order to identify a configuration for the genetic algorithm that achieves a good performance for the general marker selection problem. After its initialisation the algorithm can be applied to identify marker combinations for specific research questions. Population-based optimization techniques like the genetic algorithm allow a large degree of customisation. Suitable operators or parameter values are typically not evident. Automatic tuning methods are preferable to manual selection in this context. We utilize the irace tuning package to find good parameter and operator choices for the genetic algorithm. The tuning is parallelised on remote computation servers via the Sputnik library.

Abstract No. 36

A screening strategy for the discovery of drugs with potential calorie restriction mimetic activities

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Aging is the major risk factor for cancer formation and metabolic diseases and deregulation of mRNA translation plays a major role in their etiology. However, drugs that target aberrant translation are scarce. mTORC1 stimulates the expression of the metabolic transcription factor CCAAT/Enhancer Binding Protein β (C/EBP β) isoform Liver-specific Inhibitory Protein (LIP). Regulation of LIP expression strictly depends on

a translation re-initiation event that requires a conserved cis-regulatory upstream open reading frame (uORF) in the C/EBP β -mRNA. We have shown recently that experimental suppression of LIP in mice, reflecting reduced mTORC1-signaling at the C/EBP β level, results in CR-type of metabolic improvements. Hence, we aim to find possibilities to pharmacologically down-regulate LIP in order to induce CR-mimetic effects that include anti-cancer effects. For this purpose we engineered a luciferase-based cellular reporter system for the identification of compounds that translationally suppress LIP. We demonstrate that the reporter system acts as a surrogate for C/EBP β -mRNA translation, emulating uORF-dependent C/EBP β -LIP expression under different translational conditions. By using the reporter system in a high-throughput screening (HTS) strategy we identified drugs from an FDA approved drug library that reduce LIP. Thus, in this study we present a reporter system that can be used for HTS screening campaigns for the identification of compounds that reduce C/EBP β -uORF dependent translation re-initiation and consequently LIP levels and that potentially exhibit CR-mimetic and anti-cancer properties.

Abstract No. 37

Mitochondrial respiration shows tissue- and strain-specific aging in short- and long-lived *N. furzeri* strains.

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The turquoise killifish *Nothobranchius furzeri* is a short-lived vertebrate who inhabits transient freshwater ponds in the southeast of Africa. Due to its exceptionally short lifespan and several unique features this species has advanced to a vertebrate model for the biology and genetics of aging. In our present study we quantified mitochondrial respiration in brain, liver, heart, and skeletal muscle of a short- and a long-lived *N. furzeri* strain at sequential points in time covering young to old age. Tissue samples were obtained at day 35, 68, 100 and 147 for the short-lived strain GRZ-D as well as day 35, 68, 100, 147, 231 and 287 in the longer-lived strain MZCS-0403. We measured mitochondrial respiration by means of high-resolution respirometry using an Oxygraph-2k (OROBOROS INSTRUMENTS, Austria) injecting complex I and II substrates (malate, glutamate, pyruvate, and succinate) followed by ADP and FCCP into the Oxygraph chambers. LEAK-respiration, as an indicator of the coupling efficiency, was measured under selective complex I-stimulation. We found a particularly pronounced decrease in mitochondrial respiration with advancing age in the skeletal muscle of both strains; a more moderate decrease was also observed in the brain. In contrast, mitochondrial respiration in liver and heart was almost constant over the analyzed life time. LEAK-respiration did not show regular patterns in the different organs of both strains. Our results suggest that aging-related changes in mitochondrial respiration of *N. furzeri* are organ specific. These results will be discussed in relation to the biology of aging in general, and, in particular, to the most recently identified relevance of Complex I for the lifespan of this species.

Abstract No. 38

Molecular Aspects of in vitro Adipose Stromal Cell Aging

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Background: Adipose tissue homeostasis is tightly linked to adipose stromal cell (ASC) function. With age, but also in the context of obesity, the proliferative and adipogenic capacity of ASCs decreases. Consequently, systemic metabolic control deteriorates and comorbidities such as insulin resistance, type 2 diabetes mellitus, and cardiovascular diseases develop. This study aimed at elucidating the molecular changes occurring during the in vitro aging of ASCs.

Methods: The non-immortal, non-transformed Simpson-Golabi-Behmel syndrome (SGBS) cell strain and human primary ASCs were used as model systems. Cell proliferation, adipogenic differentiation and markers of cellular senescence were monitored up to an in vitro age of 28 weeks.

Results: With serial passaging, SGBS cell proliferation declined steadily. At 28 weeks, the cells displayed clear signs of cellular senescence such as morphological changes and senescence-associated β -galactosidase activity. The expression of cell cycle regulators p16 and p21 increased continuously and a senescence-associated secretory phenotype with an increased expression and secretion of inflammatory cytokines MCP-1, IL-1 β , IL-6, and IL-8 developed. In parallel, the adipogenic differentiation capacity decreased and was ultimately lost completely, which was reflected by a decreased expression of functional adipocyte markers PPAR γ , adiponectin, and GLUT4. Overall results obtained with human primary ASCs were comparable to SGBS cells, yet with a high inter-patient variability.

Conclusion: The cell systems used in this study mimic age-associated molecular changes observed in adipose tissue in vivo. Maintaining ASC function might be a useful strategy to prevent age- and obesity-associated metabolic disturbances by preserving adipose tissue homeostasis. SGBS cells represent a suitable model system to study adipose tissue aging.

Abstract No. 39

Changes in the Dermal Niche as a Determinant for Stem Cell Ageing?

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We have characterized a novel population of multipotent mesenchymal stromal cells (MSCs) from young and old human and murine dermis, defined in situ by a single marker, ABCB5. Functional investigations have shown that ABCB5 is involved in the

maintenance of quiescence. ABCB5+ MSCs co-expressed SSEA-4 and showed functional MSC properties on a clonal level in vitro. A comparison of dermal ABCB5+ stem cells isolated from young and old human donors revealed an age dependent decrease in the percentages of cells expressing Sox2 and SSEA-4 on the protein level and a deviant differentiation potential. Although ABCB5+ MSCs isolated from old individuals presented with significantly increased DNA double-strand breaks, both MSCs derived from young and old individuals repaired exogenous DNA damage with the same efficiency. Besides the functional changes of this dermal ABCB5+ MSC population in both human and murine dermis, the numbers of ABCB5+ MSCs were significantly decreased with organismal ageing. Notably, an age-dependent change in niche preference of ABCB5+ MSCs was observed: while in young individuals, ABCB5+ MSCs are predominantly localized in direct connection to NG2+ pericytes on the abluminal side of dermal micro vessels, with ageing, a change towards a predominantly interfollicular localisation of MSCs was observed. Therefore, we characterized dermal MSCs and their niches in situ of human and murine skin in youth and age. Interestingly, we found an age-dependent decrease of perivascular osteopontin, an extracellular matrix component which is provided by perivascular NG2+ niche pericytes. Using an osteopontin deleted mouse model we found even lower numbers of ABCB5+ MSCs compared to aged WT mice which strengthened our hypothesis that stem cell niche components, especially osteopontin play a pivotal role in dermal MSC ageing. Further experiments will provide mechanistic insight into niche dependent regulation of stem cell biology by different osteopontin isoforms and their receptors on MSCs.

Abstract No. 40

Microglia from the healthy aging mouse brain show a senescent and dysfunctional phenotype but no telomere shortening in vivo

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Microglia are the main innate immune cells of the central nervous system. In the healthy brain, microglia are mainly responsible for the continuous and active surveillance of brain parenchyma. It has been suggested that microglia from the aging brain might become senescent and dysfunctional, and thus not able to further support neuronal functions. The aim of this study was to evaluate the age-associated microglial phenotype in the murine brain by means of well accepted markers including telomere length, p16, p21, p53, the senescence-associated secretory phenotype and telomerase activity. In addition, the functional status of aging microglia was also assessed. Relative telomere length and telomerase activity were evaluated by means of quantitative real – time PCR and telomeric repeat amplification protocol. Senescence and pro- and anti-inflammatory were evaluated using Western blotting and ELISA. Telomere length from aging microglia was not significantly changed compared to adult ones. Telomerase activity was significantly decreased in microglia from aged brains. Aged microglia showed an increase in mRNA levels of p16 (8-fold) but not p21 and p53. Activation markers Cd68 and Tlr2 were increased in aging microglia. Expression of Il1- β , Tnf- α , Tgf- β , and Bdnf was also increased. Cx3cr1, an important receptor for

microglia-neuron crosstalk, was decreased. LPS and ATP stimulation showed reduced release of IL-1 β and IL-10 in aging microglia. Levels of IL-6 were increased and TNF- α was not changed after LPS stimulation. In spite of increased markers of activation, phagocytotic capacity was reduced in aged microglia compared with adult cells. This study demonstrates that microglia from the aging brain have a senescence-associated phenotype, characterized by expression of p16ink4, but not p53. Furthermore, microglial senescence in vivo seems to be independent of telomere shortening. Microglia also showed changed levels of pro- and anti-inflammatory markers as well as an altered response in the healthy aging mouse brain.

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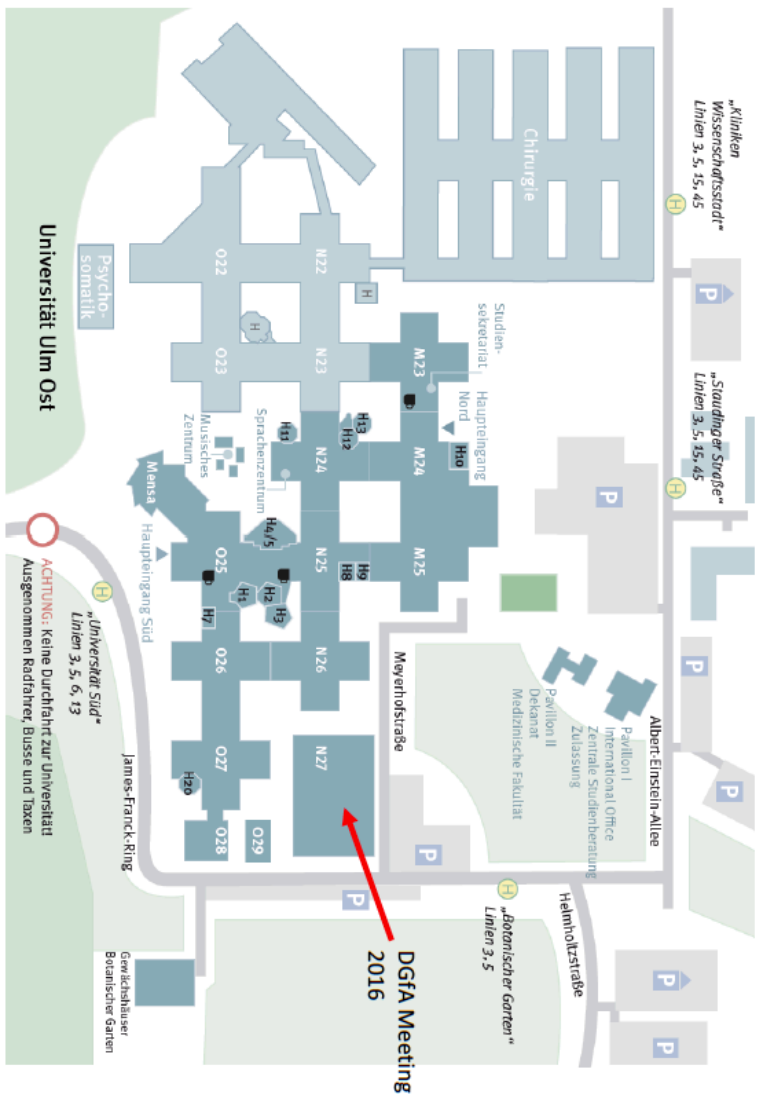
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DIRECTIONS



DIRECTIONS

Finanzamt

Universum Center

Elinger Tor

Neue Straße

Bismarckring

Elinger Straße

Schillerstraße

Ulm Hbf

Roxy

NORD

Ticketautomat

B&R

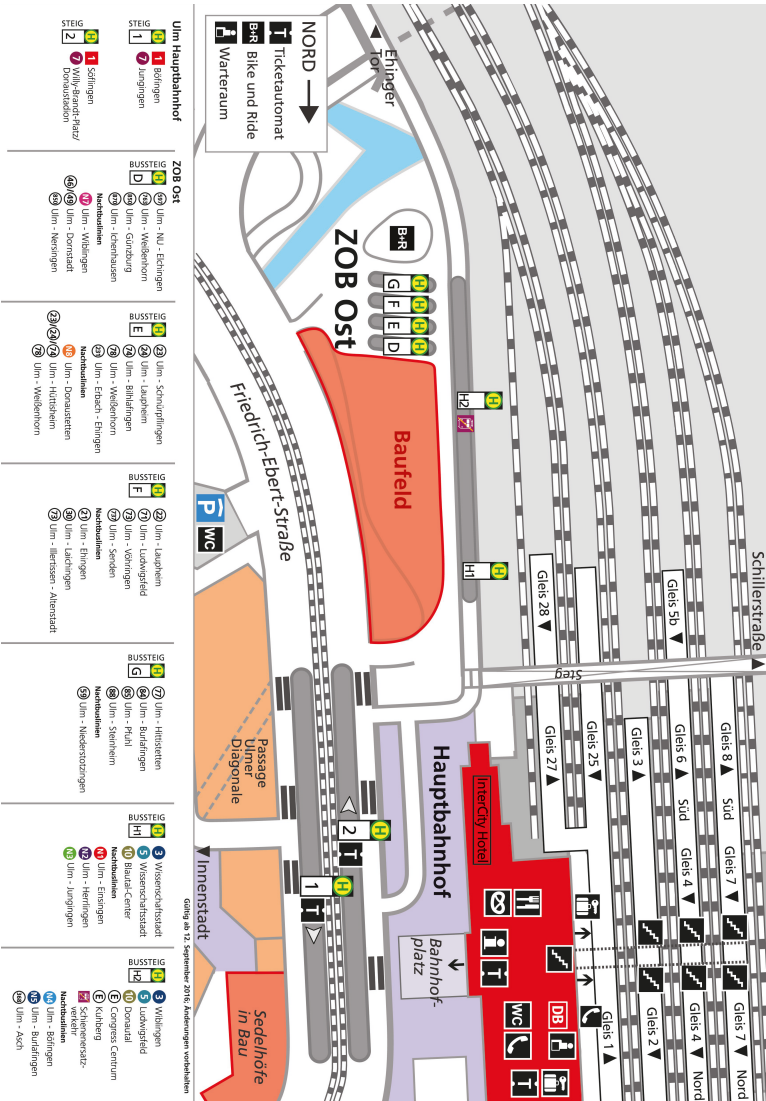
Bike und Ride

Warteraum

Bus Stops and Destinations:

- Elinger Tor**
 - 3 ZOB-Ost
 - 7 ZOB-Ost
 - 29 ZOB-Ost
 - 40 ZOB-Ost
- Finanzamt**
 - 7 ZOB-Ost
 - 29 ZOB-Ost
 - 40 ZOB-Ost
- Universum Center**
 - 1 Bfingen
 - 3 ZOB-Ost
 - 7 ZOB-Ost
 - 10 ZOB-Ost
 - 12 ZOB-Ost
- Elinger Tor**
 - 1 Bfingen
 - 3 ZOB-Ost
 - 7 ZOB-Ost
 - 10 ZOB-Ost
 - 12 ZOB-Ost
- Schillerstraße**
 - 1 Bfingen
 - 3 ZOB-Ost
 - 7 ZOB-Ost
 - 10 ZOB-Ost
 - 12 ZOB-Ost
- Ulm Hbf**
 - 1 Bfingen
 - 3 ZOB-Ost
 - 7 ZOB-Ost
 - 10 ZOB-Ost
 - 12 ZOB-Ost
- Roxy**
 - 1 Bfingen
 - 3 ZOB-Ost
 - 7 ZOB-Ost
 - 10 ZOB-Ost
 - 12 ZOB-Ost
- Other Destinations:**
 - 1 Bfingen
 - 3 ZOB-Ost
 - 7 ZOB-Ost
 - 10 ZOB-Ost
 - 12 ZOB-Ost
 - 13 ZOB-Ost
 - 14 ZOB-Ost
 - 15 ZOB-Ost
 - 16 ZOB-Ost
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 - 36 ZOB-Ost
 - 37 ZOB-Ost
 - 38 ZOB-Ost
 - 39 ZOB-Ost
 - 40 ZOB-Ost

DIRECTIONS



INTERNET

Access to the WLAN “welcome” works via authentication with user name and password in the web browser.

You don’t need a special software.

For registration open the web browser and open a random web page.

You are guided to a so called “Captive Portal” where you have to sign in to the WLAN.

WLAN ACCOUNT:

user name: dgfa.2016@gast.uni-ulm.de

password: RyAWbEPS

NOTES

NOTES