Epigenetics:

Playing with the Game of Life

Heart Centre
University Hospital Halle
Ernst-Grube-Str. 40
06120 Halle (Saale)

Keynote Lecture
Steve Horvath
Los Angeles, USA

Interdisciplinary Centre on Ageing Halle (IZAH)

German National Academy of Sciences Leopoldina

DGGG - German Society of Gerontology and Geriatrics

RTG 2155: ProMoAge

From Friday,

September 13th, 2019, 6 pm

till Sunday,

September 15th, 2019, 2 pm

Medizinische Fakultät
der Martin-Luther-Universität
Halle-Wittenberg

Opening and Conference Site:
Concert hall Ulrichs Church
Christian-Wolff-Straße 2, 06108 Halle (Saale)

INTERNATIONAL SYMPOSIUM

Epigenetics:
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September 13th – 15th 2019

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in cooperation with

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Epigenetics:
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- Meeting language English -

Friday September 13th 2019

18:00 Opening

Andreas Simm

Address

M. Gekle, Dean of the Medical Faculty
T. Moesta, Medical Director of the University Hospital

Keynote lecture and Schober award

Laudation Steve Horvath

by Claudio Franceschi, Bologna, I

Keynote lecture:

Steve Horvath
University of Los Angeles, Los Angeles, USA

"DNA methylation-based biomarkers and the epigenetic clock theory of ageing"

20:00 Come Together (Concert hall Ulrichs Church)

Saturday September 14th 2019

08:00 – 10:00 Session 1

Ageing and Environment

Chair: Steve Horvath, Andreas Simm

Epigenetics and Aging Research: Perspectives from the Social Sciences	Ruth Müller
Psychological Stress and Telomere Shortening	Irina Spivak
Stress Hormones and DNA Damage Response	Maria Moreno-Villanueva
The role of mitochondrial TERT in the cardiovascular system	Judith Haendeler

10:00 – 10:30 Coffee Break

10:30 – 12:30 Session 2

Personalized Medicine

Chair: Joachim Altschmied, Maria Moreno-Villanueva

Impact of the Epigenome within cardiac fibrosis	Elisabeth Zeisberg
Epigenetics and precision medicine in cardiovascular patients	Sarah Costantino
Transgenerational effects of late reproduction: new insight from multiomics	Oleh Lushchak
Employing Bioinformatics to Personalize Senotherapies	Georg Fuellen

12:30 – 13:30 Lunch Break

Saturday September 14th 2019

13:30 – 15:00 Session 3

Epigenetic Regulation: The Cardiovascular System

Chair: Christiane Ott, Susanne Rohrbach

Histone methylation in cardiac remodelling	Hywel Llewelyn Roderick
Chromatin interactions in cardiac myocytes	Ralf Gilsbach
NON-coding RNAs in cardiovascular disease and ageing	Yvan Devaux

15:00 – 16:30 Poster Presenters – one slide-one minute (even poster numbers)

Chair: Rüdiger Horstkorte

Poster Session / Coffee Break

16:30 – 18:30 Session 4

Posttranslational Modifications: possible Interventions

Chair: Hywel Llewelyn Roderick, Johannes Backs

Quantitation of non-enzymatic protein acylation	Tim Baldensperger
Epigenetic regulation of DNA repair by histone modifications	Eva Bártová
The Effects of Aging on the Heart	Christiane Ott
From longevity signatures to longevity interventions	Vadim N. Gladyshev

20:00 Conference-Dinner (Halloren- und Salinemuseum)

Sunday September 15th 2019

08:30 – 10:00 Session 5

Nutrition, Ageing and Diseases

Chair: Claudio Franceschi, Irina Spivak

Epigenomics, Mediterranean Diet and Ageing	Dolores Corella Piquer
Impact of obesity on atrial fibrillation progression	Susanne Rohrbach
Genetic Modulations of Variegated Gene Expression and its Significance for the Pathobiology of Aging	George M. Martin

10:00 – 11:30 Poster Presenters – one slide-one minute (uneven poster numbers)

Chair: Rüdiger Horstkorte

Poster Session / Coffee Break

11:30 – 11:45 Posterprice

11:45 – 13:45 Session 6

DNA Methylation and Biomarkers

Chair: Eva Bártová, George M. Martin

The role of the external environment in modifying epigenetic biomarkers of aging and mortality	Cavin Ward-Caviness
Role of histone deacetylases in cardiometabolic disease	Johannes Backs
Age-related changes of DNA methylation as a complex system	Claudio Franceschi
An epigenetic mortality risk score and survival prediction	Yan Zhang

13:45 – 14:15 Farewell

DNA methylation-based biomarkers and the epigenetic clock theory of ageing

Steve Horvath

Finding reliable biological measures of aging has been a longstanding research priority, based on the premise that these biomarkers would lead to a better understanding of how aging increases susceptibility to certain diseases, along with identifying strategies for promoting healthy aging.

DNA methylation based biomarkers of aging known as collectively as "epigenetic clock" can be used to measure the age of any human tissue, cell type, or fluid that contains DNA. DNA methylation age captures aspects of biological age, e.g. it predicts lifespan and healthspan in large scale epidemiological studies.

These ‘epigenetic clocks’ link developmental and maintenance processes to biological ageing, giving rise to a unified theory of life course. Epigenetic biomarkers will help to address long-standing questions in many fields, including the central question: why do we age?

I will describe new epigenetic clocks for humans and many other mammals.

Arguably the strongest predictor of human lifespan, DNAm GrimAge, is a composite biomarker based on seven DNAm surrogates of plasma protein levels and a DNAm-based estimator of smoking pack-years. Using large-scale validation data from thousands of individuals, we demonstrated that DNAm GrimAge stands out among existing epigenetic clocks in terms of its predictive ability for time-to-death (P=2.0E-75), time-to-coronary heart disease (P=6.2E-24), time-to-cancer (P=1.3E-12), its strong relationship with computed tomography for fatty liver/excess visceral fat, and age-at-menopause (P=1.6E-12).

Our large scale epigenetic clock analysis of blood confirms the conventional wisdom regarding the benefits of eating a high plant diet, physical activity, and education, as well as the health risks of obesity and metabolic syndrome.

Epigenetics and Aging Research: Perspectives from the Social Sciences

Ruth Müller

In this talk I will explore the growing importance of epigenetics in aging research from a social science perspective. I will ask what kind of new knowledge about aging emerges with epigenetics and what the social and political implications of this knowledge might be. I will show that, currently, there are two different perspectives on how aging and epigenetics might be linked: one that emphasizes the continuing biological malleability of aging processes across the life course, and another that focuses on how early life experiences and exposures delimit aging trajectories. I will show that each of these perspectives focus on different aspects of the „aging problem“ and proposes different solutions for different groups in society. With this talk, I want to encourage interdisciplinary dialogue between the social science and the life sciences about the social, political and ethical dimensions of life science research.

Psychological Stress and Telomere Shortening

Irina Spivak

Irina Spivak[1], Tatiana Smirnova[2], Arina Urazova[3], Tatiana Dolinina[4], Dmitri Spivak[5]
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Telomeric theory of aging holds currently a leading position. Telomeres are ending regions of chromosomes, shortened during each cell division. According to the telomeric theory, telomere length is considered as an indicator of overall health, and possibly longevity. A large number of studies have shown that telomere shortening is accelerated under the influence of psychological and social factors. At the same time, works appeared that demonstrated reversibility of 'telomeric' aging. Meditation, healthy lifestyle, proper nutrition - all these factors could slow down the shortening of telomeres, sometimes even contributing to their elongation. Positive influence of music of different types upon this process was studied by us. Three groups of normal Russian young urban dwellers were studied, each one comprising from 20 to 22 subjects, prior to passing a two-week course of audiotherapy (1.5 hours a day), and right after it. The first group listened to light classical European music, the second one, to modern designer music, the third one (control group) passed a course of nature sounds. Before and after the two-week course, telomere length and telomerase activity in blood samples were measured. Statistically reliable, although moderate, increase in both the telomere length and in the telomerase activity was demonstrated to have occurred as a result of passing a music course. Music course containing sound stimuli which were stereotypic for our Ss (light classical music), tended to induce positive mood and general relaxation, as well as increase in both the telomere length, and the telomerase activity. Music course containing non-stereotypic sound stimuli (designer music), tended to bring about moderate stress and fall in the telomere length. In order to cope with this difficulty, telmerase was most probably activated. Similar reaction to stress was described by us basing on a survey of operators of hazardous chemical industry: their telomere length was significantly longer, and the telomerase activity was higher, than in members of control group. It looks quite plausible that stress tended to activate defense reactions, including the telomerase activity.

Stress Hormones and DNA Damage Response

Maria Moreno-Villanueva

Philipp Palombo[1], Anita Grath[1], Michael Laumann[2], Judy Salzwedel[1], Namni Goel[3], Stephanie Krieger[4], Honglu Wu[5], Iris-Tatjana Kolassa[6], Andreas Krammer[7], Markus Gruber[7], Alexander Bürkle[1], Maria Moreno-Villanueva[1,5,7]
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Chronic stress is associated with a higher risk for carcinogenesis as well as age-related diseases and immune dysfunction. Stress hormone-mediated DNA damage has been discussed as a possible mechanism. When DNA damage exceeds DNA repair capacities, cells undergo either apoptosis or senescence. These mechanisms preclude the proliferation of cells with heavily damaged DNA, thus protecting the organism against tumor development. On the other hand, the senescence associated secretory phenotype (SASP) has the ability to promote tumor progression.

We investigated the effect of “stress” on DNA damage response in vivo as well as ex vivo. Both, high epinephrine concentration and DNA damage have been associated with post-traumatic stress disorder (PTSD), cognitive decline and acute intensive exercise. Our in vivo studies showed (i) an increased in endogenous DNA strand breaks in patients suffering from PTSD, (ii) higher radiation-induced DNA strand breaks in sleep-deprived, cognitively vulnerable individuals than sleep-deprived cognitively resistant individuals, (iii) and an increase in acute intensive exercise-induced DNA strand breaks in untrained, but not in trained, individuals. In our ex vivo experiments, peripheral blood mononuclear cells (PBMC) were treated with the epinephrine analogue isoproterenol. We found significant induction of DNA strand breaks that remained unrepaired 24 h after incubation. Isoproterenol-induced DNA strand breaks could be partially prevented by pre-treatment with the β-adrenergic receptor antagonist propranolol. Isoproterenol treatment also inhibited phytohemagglutinin-induced cell proliferation and affected the expression of genes involved in cell cycle regulation and DNA repair. Furthermore, isoproterenol treatment led to strong cellular adhesion and morphology changes and increased senescence induced ß-galactosidase activity. Although further experiments are necessary, our results suggest a stress-mediated induction of cellular senescence-like phenotype. These findings provide new insights into the molecular consequences of stress and stressassociated diseases, such as PTSD, opening new perspectives for future research.

The role of mitochondrial TERT in the cardiovascular system

Judith Haendeler

Background - The catalytic subunit of telomerase, Telomerase Reverse Transcriptase (TERT), has been demonstrated to display protective functions in the cardiovascular system. TERT is not only present in the nucleus, but also in mitochondria. However, up to now it has not been investigated whether nuclear or mitochondrial TERT is responsible for the protective effects. Therefore, we generated two unique new mouse models containing TERT exclusively in either the nucleus or the mitochondria and derived cells from these animals to unequivocally differentiate between the role of mitochondrial and nuclear TERT in the cardiovascular system.

Methods and Results – To understand the role of nuclear and mitochondrial TERT in the cardiovascular system, mice carrying TERT targeted exclusively to the mitochondria (mitoTERT) or to the nucleus (nucTERT) were crossed to heterozygous TERT-deficient animals to obtain first generation offspring expressing mitoTERT or nucTERT on an otherwise TERT-deficient background. Heart mitochondria from mitoTERT mice, but not from nucTERT mice, were able to compensate for the reduced mitochondrial respiration observed in the hearts of TERT-deficient animals. Ischemia/reperfusion injury demonstrated that TERT-deficient mice displayed increased infarct size compared to wildtype animals. Astonishingly, mitochondrial TERT, and not nuclear TERT, improved the outcome after ischemia/reperfusion. Infarct size in mitoTERT mice was even smaller than in wildtype animals. Functional analysis after one week of reperfusion demonstrated that mitoTERT mice display full recovery of the ejection fraction. Mechanistically, mitochondrial TERT protected cardiomyocytes from apoptosis and rescued the defect of TERT-deficient cardiac fibroblasts to differentiate into myofibroblasts. In endothelial cells, introduction of mitochondrial TERT improved migratory capacity and phosphorylation of endothelial NO synthase.

Conclusions - This study identifies mitochondrial TERT, and not nuclear TERT, as required and sufficient to improve cardiovascular functionality. Thus, mitochondrial TERT plays a previously unappreciated protective role in heart and vessels.

Impact of the Epigenome within cardiac fibrosis

Elisabeth Zeisberg

Every form of chronic heart disease is associated with cardiac fibrosis. While cardiovascular mortality is increased as a result of cardiac fibrosis, there is no specific anti-fibrotic therapy in clinic as of yet. Silencing of anti-fibrotic genes by aberrant DNA methylation has been shown to drive progression of organ fibrosis. Hydroxymethylation of these anti-fibrotic genes is able to rescue their expression and thereby inhibit fibrosis. We here propose both molecular gene-specific as well as pharmacologic means to target aberrant gene methylation in organ fibrosis.

Epigenetics and precision medicine in cardiovascular patients

Sarah Costantino

Abstract Cardiovascular diseases (CVDs) remain the leading cause of mortality worldwide and also inflict major burdens on morbidity, quality of life, and societal costs. Considering that CVD preventive medications improve vascular outcomes in less than half of patients (often relative risk reductions range from 12% to 20% compared with placebo), precision medicine offers an attractive approach to refine the targeting of CVD medications to responsive individuals in a population and thus allocate resources more wisely and effectively. New tools furnished by advances in basic science and translational medicine could help achieve this goal. This approach could reach beyond the practitioners 'eyeball' assessment or venerable markers derived from the physical examination and standard laboratory evaluation. Although many studies have focused on the genes that impact CVD, their “nongenetic regulation” is gaining increasing attention. A growing body of evidence suggests that epigenetic modifications - changes to the genome that do not involve changes in DNA sequence - may significantly derail transcriptional programs implicated in angiogenesis, oxidative stress and inflammation, thus fostering maladaptive pathways and cardiovascular dysfunction. Most importantly, the changes imparted by non-genetic factors appear to be relatively long lasting, suggesting that certain epigenetic mechanisms, which can be relatively stable in nature, are a pivotal component of this regulation. Notably, adverse epigenetic signals can be transmitted to the offspring, and may contribute to early CVD phenotypes in the young generations. Environmental factors are potent inducers of epigenetic variations and altered gene expression. Cigarette smoking, pollution, and poor dietary habits all contribute to alter chromatin architecture, DNA methylation as well as circulating and tissue levels of non-coding RNAs. The “epigenetic landscape” may provide a real post-genomic snapshot offering the tools to build individual maps of CV risk. Hence, epigenetic changes accumulated during the lifetime may be employed to customize diagnostic and therapeutic approaches in primary and secondary prevention of CVD. Individual epigenetic maps will be invaluable to define new risk scores predicting beyond traditional calculators, as well as to use chromatin modifying drugs able to erase plastic chromatin changes, thereby restoring gene expression in endothelial cells, cardiomyocytes and bone marrow-derived angiogenic cells. The present lecture aims to acquaint the cardiovascular community with the rapidly advancing and evolving field of epigenetics and its implications in cardiovascular precision medicine.

Transgenerational effects of late reproduction: new insight from multiomics

Oleh Lushchak

Department of Biochemistry and Biotechnology, Vasyl Stefanyk Precarpathian National University, Ivano-Frankivsk, Ukraine

An average parental age of child birth is increasing in modern societies. Average age of mothers, delivered the first child, was observed to be increased for about five years in the last decade. Many traits in offspring are affected by parents. Thus, we tested the effects of late reproductive age using the fruit fly Drosophila as a model. Briefly, initial flies were kept till late reproductive age where only less than 10% were able to lay eggs (24 days). Collected eggs were used to get flies of next generation. This procedure was repeated for 5 generations and then flies of control and experimental flies were kept for about 100 generations (~7 years) on regular food. Physiological traits such as lifespan, reproduction and fecundity, as well as transcriptome, proteome, and metabolome were analyzed. The lifespan and fecundity of experimental flies were significantly reduced independently of diet that varied by yeast content at fixed sucrose concentration. Transcriptome analysis showed that steady state mRNA levels of more than 100 genes were changed by more than two-fold. Strong upregulation was observed in genes that belong to GO terms related to development. KEGG analysis depicts an activation of Foxo, Wnt and Hippo pathways. Down regulation was observed for gene related to metabolism and energetics. Pathways involved in fat and amino acid metabolism were significantly suppressed at the transcriptional level. Remarkable amounts of proteins and peptides were affected in experimental flies. These flies have increased protein amounts of GO categories related to carbohydrate and amino acid metabolism with respect pathways activated. All the transcriptome and proteome changes resulted in remodeling of fly metabolism with significant increase of free carbohydrates and decreased amounts of metabolites of TCA circle. Thus, reproduction at older age has huge transgenerational impact by affecting physiology, transcriptome, proteome and metabolome in offspring.

Employing Bioinformatics to Personalize Senotherapies

Georg Fuellen

Senotherapies targeting the detrimental effects of senescent cells (e.g., senolytics) are offering exciting prospects, now based on a large array of mouse studies and two human pilot trials.

A precision medicine approach based on lab and omics data, to determine (epi-)genetic predispositions, may be key to improve upon the "therapeutic" window between desired effects and potential side effects. We developed "pathway maps" for human health, cellular senescence, and fibrosis, to define and interpret desired changes in omics-based characterizations, and to then match these with the known effects of interventions. These maps and other bioinformatics approaches shall be employed in a major senescence biomarker study

(SASKit) and a major preclinical development effort (AntifibrotiX) commencing in Rostock this year, and in a large-scale effort to identify biomarkers for personalizing senotherapies currently under development and/or based on natural compounds. To reposition interventions based on their ability to reverse age-related changes in the transcriptome, epigenome, etc, is an important focus, but we ask the question: how can we distinguish the detrimental from the protective?

Histone methylation in cardiac remodelling

Hywel Llewelyn Roderick

Malina Doynova, Konstantinos Chatzieleftheriadis, Patrick Brien and **Hywel Llewelyn Roderick**

Changes in epigenetic landscape have been invoked as key to the alterations in transcription required to bring about the phenotypic changes of cardiac myocytes associated with development, disease and ageing. By analysing the epigenomes and transcriptomes of cardiac myocytes purified from rat hearts, we have recently uncovered a role for loss of histone 3 (H3) lysine 9 (K9) in the substantial transcriptional changes observed during pathological remodelling. This contrasted with the that of cardiac myocytes that were hypertrophic following exercise, where no such changes were observed. A disease-associated reduction in the euchromatic histone methyl transferase Ehmt2 during disease was found to be responsible in the reduction in H3K9me2. Suggestive of a role for changes in H3K9me2 also in development, many of the genes reactivated by loss of H3K9me2 during disease overlapped with those silenced during post-natal development. By profiling changes in H3K9me2 and transcription at key stages during late pre-natal and early post-natal cardiac development, we are now gaining insights into the mechanisms by which alterations in H3K9me2 governs the transitions of the cardiac myocyte from its mitotic pre-natal state to its post-natal terminally differentiated phenotype.

Chromatin interactions in cardiac myocytes

Ralf Gilsbach

Martin Schwaderer[1,2], Stephan Nothjunge[1,2], Joachim Wolff[3], Theresa Kehl[1], Björn A. Grüning[3], Lutz Hein[1,4], **Ralf Gilsbach[1]**
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Background: Epigenome studies in cardiac myocytes revealed dynamic establishment of active regulatory sites during development and disease (Gilsbach et al., Nat. commun. 2018). The aim of this project was to identify target promoters of distal regulatory sites and to prove the functional relevance of chromatin interactions in cardiac myocytes.

Methods: We performed Hi-C and Promoter Capture Hi-C experiments to analyze higher order chromatin organization and promoter interactions in cardiac myocytes. We perturbed active regulatory sites using the CRISPRi system. CRISPRi utilizes a programmable catalytic-deficient Cas9 fused to the KRAB repressor domain. ChIP-seq, RNA-seq and Western Blot analysis were carried out to access the functional consequences of promoter and enhancer perturbation.

Results: Our Hi-C experiments revealed that the higher order chromatin organization, including TADs and A/B compartments, remains stable in cardiac myocyte postnatal development and disease. We furthermore generated a promoter interaction map for newborn, adult and diseased cardiac myocytes. In total we detected more than 30.000 genomic elements contacting 2432 genes. These interactions were strongly enriched for enhancer-promoter and promoter-promoter interactions. Among those were for example a spatial interaction between the NPPA and NPPB promoter and between a putative enhancer and Gata6. CRISPRi-mediated silencing of the NPPA promoter induced heterochromatin formation at the NPPA promoter and silencing of NPPA and NPPB gene expression, indicating that the NPPA promoter has enhancer function and controls NPPB gene expression. Since Gata6 has been shown to be implicated in pathological cardiac hypertrophy, we further used CRISPRi to silence a putative enhancer 320kb downstream of Gata6. This resulted in reduced gene expression, protein levels and genome-wide binding of Gata6. RNA-seq experiments revealed that enhancer-mediated silencing of Gata6 enhancer affected more than 600 genes. These genes were significantly associated with cardiovascular developmental and stress processes.

Conclusion: This study unraveled promoter interactions in cardiac myocytes and showed that functional (epi)genetic-modulation of distal regulatory elements allows steering of gene expression programs. Furthermore, will this project show the dynamics of promoter interactions in cardiac myocyte development and disease.

Non-coding RNAs in cardiovascular disease and ageing

Yvan Devaux

With an ageing population, cardiovascular disease burden reaches epidemic proportions. Diseases affecting the cardiovascular system are as diverse as numerous and contain an important epigenetics component. Indeed, all levels of epigenetics regulation have been shown to be associated with cardiovascular disease development and progression. Here, the focus will be on non-coding RNAs and their regulation and role in cardiovascular disease and ageing. Non-coding RNAs are encoded by a large part of the genome and constitute a large family with multiple features and functions. Non-coding RNAs have been arbitrarily classified according to their size into small and long non-coding RNAs. The category of small non-coding RNAs includes the well-known microRNAs which regulate gene expression through messenger RNA degradation. The less well-known long non-coding RNAs contain linear and circular forms and regulate gene expression through more diverse and complex mechanisms, mainly occurring at the epigenetics level. While the regulation of microRNAs associated with cardiovascular disease development, as well as their potential therapeutic and predictive value, has been widely addressed, whether long non-coding RNAs constitute a reservoir of novel therapeutic targets and biomarkers of cardiovascular disease remains to be demonstrated. Also, while several studies revealed the regulation of microRNAs with ageing, less is known of the impact of ageing process on long non-coding RNAs. Overall, there is evidence that non-coding RNAs are clinically translatable and have the potential to move personalized healthcare a step forward.

Quantitation of non-enzymatic protein acylation

Tim Baldensperger

Enzymatic acetylation of lysine residues is considered a major regulatory process in epigenetics and metabolism. Consequently, recently discovered structurally related acylation by activated thioesters is expected to be another key mechanism in cellular regulation. Established methods like Western blotting and proteomics lack the ability to detect the plethora of acylation structures in a single analysis and do not allow absolute quantitation. We developed a HPLC-MS/MS method for the simultaneous detection and quantitation of 14 acylated lysine species. The syntheses and structure elucidation of authentic reference standards were a prerequisite for the successful method development and quantitation by standard addition calibration. Furthermore, a work-up procedure based on total enzymatic hydrolysis of proteins with efficiencies of hydrolysis between 85-88 % was established for mouse liver, kidney, heart and brain. Extensive effort was invested into the method validation resulting in recovery rates between 75-93 % and levels of detection in the nanomolar range. Thus, we were able to quantitate 8 acylation structures in biological samples without enrichment. Further enrichment by HPLC fractionation resulted in the quantitation of 6 additional acylation structures including 4 novel structures N^6-acetoacetyl lysine, N^6-isovaleryl lysine, N^6-(2-methylbutyryl) lysine and N^6-tiglyl lysine. After screening in different organs a procedure for the extraction of mitochondrial, cytosolic and histone proteins from mouse liver was developed and validated. Finally, levels of lysine acylation were quantitated in subcellular compartments, correlated with aging and compared to posttranslational protein modifications like glycation and oxidation.

Epigenetic regulation of DNA repair by histone modifications

Eva Bártová

***Eva Bártová**, Soňa Legartová and Alena Kovaříková Svobodová*
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DNA repair processes are mediated via phosphorylation of histone H2AX as the most prominent epigenetic marker of double-strand breaks (DSBs). Similarly, di-methylation of histone H4 at lysine 20 position (H4K20me2) contributes to the recruitment of the central DNA repair protein 53BP1 to chromatin in the vicinity of DSBs. These data show crosstalk between specific histone signature and a function of the 53BP1 protein at DNA lesions. We analyzed in which extent H4K20me1/me2/me3 play a role in 53BP1-dependent repair pathway and how mutations in the TP53 gene affect recruitment kinetics of 53BP1 protein at micro-irradiated chromatin. We observed that 53BP1 protein binds to not only H4K20me2 but also H4K20me3 and that kinetics of the 53BP1 protein at DNA lesions is different in various TP53 mutant cells. We found that in the cells with distinct mutations in the TP53 gene, γ-irradiation did not change the level of 53BP1, but we observed radiation-induced down-regulation of the MDC1 protein. Our data contribute to the knowledge of recruitment kinetics, nuclear organization of the 53BP1 protein, and we show epigenetic features regulated the function of 53BP1 at DNA lesions.

This work was supported by the Czech Science Foundation; grant number 18-07384S.

The Effects of Aging on the Heart

Christiane Ott

Christiane Ott [1,2]
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The prevalence of cardiac diseases, which are among the main causes of death worldwide, is likely to increase because of an aging population. One important hallmark of the aging process is the accumulation of modified proteins, caused by impaired proteolysis. Failure of protein quality control, among others autophagy, can lead to the accumulation of highly cross-linked protein aggregates, such as lipofuscin (LF). Particularly post mitotic cells, such as cardiomyocytes, can rapidly accumulate protein aggregation products. Comparing heart function and murine heart tissue from 5-month (young) and 25-month (old) old C57Bl/6J (B6) mice we obtained significant differences in ejection fraction, cardiac output, left ventricular mass but also an increase in collagen content and modified proteins. Besides impaired cardiac function, also autophagy is decreased in the old murine heart tissue. In a model of heart failure, induced by transverse aortic constriction, we were able to demonstrate that induced autophagy by rapamycin, a mTOR inhibitor, can improve heart function. To further investigate whether improvement of autophagy could prevent impaired cardiac function in aging we developed a model system to mimic the effect of age-related protein aggregation on cardiomyocyte function using artificial LF. It is characterized to contain covalent cross-linked protein aggregates, consisting of oxidized proteins and lipids. To investigate the impact of LF on cardiomyocyte function, we isolated cardiomyocytes from young B6 mice and treated them with artificial LF. To determine the physiological consequences of LF on cellular function, cardiomyocyte contractility was measured using the Myocyte Calcium and Contractility System of IonOptix, demonstrating a decreased amplitude of contraction comparable to the measured contraction of cardiomyocytes isolated from old murine hearts. Since autophagy and cardiac function decline with age, we aim to investigate how declined autophagy and in consequence increased protein aggregates can impact cardiomyocyte contractility, to clarify molecular processes involved in impaired cardiomyocyte functionality and to develop novel therapeutic strategies, using nutritional interventions.

From longevity signatures to longevity interventions

Vadim N. Gladyshev

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The rate of aging varies significantly across species and cell types and is further modified by genetic and environmental factors. We employ this diversity to shed light on general principles and mechanisms of lifespan control. We apply comparative genomics approaches to short- and long-lived species and carry out analyses across panels of mammals. We sequenced the genomes of several mammals with exceptional lifespan and identified genes that may contribute to their longevity. We also carried out analyses of gene expression, metabolites and elements across large panels of mammals as well as known longevity interventions in mice. These studies point to both private and common adaptations to longevity involving various pathways. In addition, these analyses identify patterns of gene expression and metabolite levels, which we term longevity signatures, that characterize species lifespan and the effects of interventions that adjust lifespan within species. Using these signatures, one may identify new pharmacological, dietary and genetic interventions with the potential to increase lifespan. To characterize longevity interventions in mice, we also developed blood and multi-tissue DNA methylation clocks. Together, these genomics approaches and tools offer a platform for unbiased discovery and validation of longevity interventions in mammals.

Epigenomics, Mediterranean Diet and Ageing

Dolores Corella Piquer

The Mediterranean diet (MedDiet) has shown its favorable effects as a protector against different diseases including cardiovascular diseases, diabetes, several types of cancer, and neurodegenerative diseases, among others. The scientific evidence level for each outcome is variable. Recent meta-analyses, including both observational studies and clinical trials, have gathered the evidence on the favorable effects of the MedDiet on various intermediate (plasma lipid concentrations, fasting glucose, inflammation markers, etc.) and final (total cardiovascular events, stroke, myocardial infarction, etc.) phenotypes of cardiovascular disease. However, despite the good level of epidemiological evidence showing the cardioprotective effects of the MedDiet, new studies are needed to better understand the molecular mechanisms whereby the Mediterranean diet may exercise its beneficial effects. Each of the cardiovascular phenotypes is in turn multifactorial and multigenic, there being many genes and epigenetic factors contributing to the same. The study of gene-MedDiet interactions is, therefore, extraordinarily complex and, at present, we only have very preliminary results on those interactions at the genome and at the epigenome level. We will present meta-analysis results as well as the recent advances in understanding the molecular basis of MedDiet effects, mainly focusing on cardiovascular diseases, but also discussing ageing. There is heterogeneity in defining the MedDiet, and it can, due to its complexity, be considered as a complex exposome with thousands of nutrients and phytochemicals. We will review MedDiet composition and assessment, as well as the latest advances in the epigenomic (DNA-methylation, histone modifications, micro-RNAs and other emerging regulators), aspects of the MedDiet effects (as a whole, and for its most typical food components), and present some results obtained by our research group. We will also present a critical review of the limitations of studies carried out on the topic, also integrating genomic and transcriptomics data, and propose new analyses to better understand the most important mechanisms whereby the MedDiet as a whole, or its main food components, may exercise their protective effects on cardiovascular diseases and aging.

Transcriptomic (selected genes and whole transcriptome) metabolomic and metagenomic aspects of the MedDiet effects (as a whole, and for its most typical food components). We will also present a critical review of the limitations of studies undertaken and propose new analyses and greater bioinformatic integration to better understand the most important molecular mechanisms whereby the MedDiet as a whole, or its main food components, may exercise their protective effects.

Impact of obesity on atrial fibrillation progression

Susanne Rohrbach

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Atrial fibrillation (AF) from paroxysmal to persistent (par/per) AF is accompanied by structural remodeling with increasing atrial fibrosis. Interstitial fibrosis is considered a structural basis for the development and maintenance of AF. Obesity increases AF incidence and progression compared with lean patients, while weight loss decreases AF recurrence following treatment. However, the underlying mechanisms leading to increased AF and atrial fibrosis in obese patients have not been analyzed in detail.

In a prospective all comers study patients with paroxysmal or persistent AF underwent standardized ablation (bipolar radiofrequency, loop recorder (LR)) additive to baseline cardiac surgery procedure. Patients were followed-up for 2 years (electrocardiogram, LR readout, echocardiography, blood sampling, and physical examination). MiRNA expression in right and left atrial tissue (RA, LA) was analyzed by microarray and qPCR. Pro- and anti-fibrotic miRNAs were overexpressed in primary human cardiac fibroblasts by lentiviral transduction and cellular consequences of these differentially expressed miRNAs were analyzed. In addition, cells were incubated with serum from normal weight or obese patients or with selected adipokines and analyzed with regard to transdifferentiation into hypersecretory myofibroblasts.

Our analyses show that obesity influences AF progression and maintenance as well as therapeutic success after surgical MAZE. Both, the RA and the LA of patients with AF are undergoing similar expressional miRNA changes, which are more pronounced in patients with persistent AF. The increase in different pro-fibrotic miRNAs induces a phenotype switch in cardiac fibroblasts towards hypersecretory myofibroblasts. Obesity facilitates this switch, an effect mediated by differentially expressed adipokines. Structural atrial remodeling in response to weight loss, which is known to increase endogenous release of the well-known cardioprotective adipokine adiponectin, contributes to reverse remodeling. The effects might offer additional and supporting metabolic therapeutic options in AF ablation therapy.

Genetic Modulations of Variegated Gene Expression and its Significance for the Pathobiology of Aging

George M. Martin

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Variegated Gene Expression (VGE) is used to refer to observations of cell to cell differences in the expressions of genetic loci among families of apparently identical cell types. This phenomenon has also been referred to as Epigenetic Drift. The term VGE is preferred, however, because the underlying mechanisms may also involve post-transcriptional mechanisms. In 2009, the author suggested that this phenomenon may be considered to be an example of an antagonistic pleotropic mechanism of aging as such variation might protect members of the population with the right combinations of gene expressions to survive an unpredictable environmental challenge. Given the observations on increasing degrees of VGE during aging, however, that phenomenon is likely to lead to a loss of physiological homeostasis and could therefore be one of the canonical mechanisms of aging and age-related diseases. We shall review some examples of that hypothesis, including potential roles in cardiovascular and neurodegenerative disorders. Finally, we shall summarize recent research using hTERT immortalized human diploid fibroblasts for the discovery of groups of genetic loci whose knockdowns lead to either substantial increases or substantial decreases in VGE of a reporter gene of significance to the biology and pathobiology of aging (SIRT1).

The role of the external environment in modifying epigenetic biomarkers of aging and mortality

Cavin Ward-Caviness

Authors: Cavin Ward-Caviness, Chantel Martin, Shirley Pu, Sandra Alvarez, Cierra Dungee, Tarek Zikry, Sandro Galea, Monica Uddin, Derek Wildman, Karestan Koenen, Allison Aiello

Background: With the recent creation of epigenetic biomarkers of the aging process, researchers now have validated, quantitative measures of biological age and a means to assess age acceleration, i.e. deviations of biological (epigenetic) age from chronological age. A recent review of epigenetic aging biomarkers revealed that external environmental exposures play a large role in determining epigenetic age acceleration. However, the impact of subjective perceptions and protective factors remain poorly understood.

Methods: Using 157 participants of the Detroit Neighborhood Health Study, we assessed the relationship between neighborhood characteristics and a validated DNA methylation-based epigenetic mortality risk score (eMRS) as well as three epigenetic aging biomarkers: Horvath age acceleration (AA), extrinsic epigenetic age acceleration (EEAA), and phenotypic age acceleration (PhenoAA). Associations were adjusted for age, race, sex, ever smoking, ever alcohol usage, education, employment, and years residing in neighborhood. A secondary model additionally adjusted for personal neighborhood perception and associations were stratified on the presence of greenspace. We summarized 19 objective neighborhood quality indicators into 9 principal components which served as metrics of objective neighborhood quality exposures.

Results: Of the nine principal components utilized for this study, one (PC7) was associated with the eMRS (β = 0.15; 95% confidence interval = 0.05-0.3; P = 0.003), AA (β = 2.05 y; 95% confidence interval = 1.01-3.08 y; P = 1.6×10^{-4}), EEAA (β = 2.55 y; 95% confidence interval = 1.32-3.37 y; P = 8.2×10^{-5}), and PhenoAA (β = 2.59 y; 95% confidence interval = 1.15-4.02 y; P = 6.9×10^{-4}). PC7 was an indicator of the presence of abandoned cars, poor streets, and non-art graffiti in the neighborhood. Subjective neighborhood perception was not associated with the epigenetic aging biomarkers. We observed a protective effect of large mature trees in the neighborhood for the eMRS and a protective effect of community gardens in the neighborhood for PhenoAA.

Conclusion: Objective measures of neighborhood disadvantage are significantly associated with epigenetic aging and mortality risk. Associations were independent of an individual's perception of their neighborhood and in some cases were attenuated by neighborhood greenspace features.

Role of histone deacetylases in cardiometabolic disease

Johannes Backs

Over the last years class II histone deacetylases, in particular HDAC4, emerged as critical regulators of cardiometabolic disease. We discovered that HDAC4 is regulated by a lipid droplet associated protein and in turn regulates glucose handling including the hexosamine biosynthesis pathway. Genetic mouse models and gene addition approaches confirmed that HDAC4 plays an important role in the maintenance of cardiac function during exercise and in diabetes. New mechanistic insights led to the development of small molecule screening approaches to disrupt signaling events that regulate HDAC4. The talk will focus on the role of lipid droplet associated proteins and how they regulate gene expression through an HDAC4-dependent mechanism.

Age-related changes of DNA methylation as a complex system

Claudio Franceschi

Claudio Franceschi and Mikhail Ivanchenko
University of Bologna, Italy and Lobachevsky State University of Nizhny Novgorod, Russia

In recent years an in depth analysis of age-related DNA methylation changes has been possible owing to the availability of arrays that interrogate over 850,000 methylation sites quantitatively across the genome at single-nucleotide resolution in different cells and organs. Thus, and a number of dataset are available on a large number of healthy and non-healthy subjects. Taking advantage of this opportunity we will present showing that in humans age-related DNA methylation changes are quite complex, and can be linear and nonlinear, paying particular attention to blood cells and liver. Taking into account also data from different domains (proteomics), we will argue that when the aging process is analyzed at a fine level of granularity by high-dimensional technologies (DNA methylation, proteomics) its nonlinearity, difficult to grasp at a coarse level, emerges. Thus, molecular aging may follow different nonlinear or discontinuous trajectories in the different organs and cells of the body, resulting in a very complex mosaic. These considerations can be relevant: i) to put into the right perspective the different clocks that have been proposed to evaluate biological *versus* chronological age; ii) to understand how different stressors can affect the transition from a health state to a disease state, a condition that poses high-dimensional nonlinear reconstruction problem; iii) to appreciate that the aging nonlinear trajectories can be different in different subjects, according to their individual, lifelong (genetics) x (environment) interaction, thus explaining, at least in part, the large heterogeneity of the phenotype of old people; iv) to evaluate the non-linearity of the age-related changes of the gut microbiota and of inflammaging.

Acknowledgements: This work was supported by the grant of the Ministry of Education and Science of the Russian Federation Agreement No. 075-15-2019-871

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An epigenetic mortality risk score and survival prediction

Yan Zhang

Survival predictors are of potential use for informing on biological age and targeting prevention of aging-related morbidity. Using an epigenome-wide approach, we developed a mortality risk score (MRscore) based on 10 blood DNA methylation (DNAm) markers. It strongly predicted all-cause, cardiovascular disease, and cancer mortality, and strongly correlated with other well-established aging indicators, such as telomere length, oxidative stress, and frailty index, while outperforming these indicators in survival prediction. The MRscore was first derived and validated in two large German cohorts, and has been subsequently confirmed in multiple large cohort studies from the US, such as the Framingham Heart study, the Women's Health Initiative, and the Normative Aging Study. The MRscore also correlated with different generations of the epigenetic age measures, including the Horvath and Hannum epigenetic clock, and the DNAmphenoAge, but showed superior performance to those epigenetic age measures for survival prediction. These findings suggest that the MRscore is a reliable and robust epigenetic marker of health outcomes and bears potential of a surrogate endpoint for clinical research and intervention.

Posters

(in alphabetical order)

Sunday 15th of September from 11:30 to 11:45

The Poster Award Ceremony

(1) Glycation of macrophages induces expression of pro-inflammatory cytokines and reduces phagocytic efficiency

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Glycation and the accumulation of advanced glycation end products (AGEs) are known to occur during normal aging but also in the progression of several diseases, such as diabetes. Diabetes type II and aging both lead to impaired wound healing. It has been demonstrated that macrophages play an important role in impaired wound healing, however, the underlying causes remain unknown. Elevated blood glucose levels as well as elevated methylglyoxal (MGO) levels in diabetic patients result in glycation and increase of AGEs. We used MGO to investigate the influence of glycation and AGEs on macrophages. We could show that glycation, but not treatment with AGE-modified serum proteins, increased expression of pro-inflammatory cytokines interleukin 1β (IL-1β) and IL-8 but also affected IL-10 expression, resulting in increased inflammation. At the same time, glycation reduced phagocytic efficiency and led to impaired clearance rates of invading microbes and cellular debris. Our data suggest that glycation contributes to disturbed wound healing during aging and diabetes due to changes of macrophage activity and cytokine expression.

(2) The role of β-catenin K49 trimethylation/acetylation in aging

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The Armadillo repeat protein β-catenin originally was identified as a component associated with the cytosolic domain of E-cadherin where it together with β-catenin is involved in the association of the E-cadherin molecules with the actin cytoskeleton. Later on a second role in the canonical Wnt signaling pathway was identified. Accumulation of β-catenin in response to activation of the Wnt pathway results in its nuclear translocation where it acts as a transcriptional cofactor together with LEF/TCF HMG-box proteins or other transcription factors. Wnt signaling plays an essential role during multiple steps of embryonic development but also in adulthood by regulating wound healing, regeneration, stem cell fate, metabolism and carcinogenesis.

Recently, it was reported that β-catenin-K49 is a target of Ezh1 and Ezh2 methyltransferases in the polycomb repressive complex 2 (PRC2) resulting in trimethylation of β-catenin thereby repressing neuronal differentiation of ES cells. Alternatively, β-catenin-K49 can be acetylated by CREB-binding protein (Cbp) inducing mesodermal differentiation. Interestingly, β-catenin appears to be trimethylated during early steps in the formation of E-cadherin-mediated cell-cell contacts (Hoffmeyer et al., 2017). From these observations it appears that trimethylation versus acetylation of β-catenin-K49 acts as a switch in β-catenin function.

In this MD project we want to analyze the role of these β-catenin-K49 modifications in aging using antibodies specifically detecting trimethylated or acetylated β-catenin.

Hoffmeyer K, Junghans D, Kanzler B, Kemler R (2017) Cell Reports 18, 2815-2824.

(3) A Human Tissue-Specific Transcriptomic Analysis of the Relationship between Ageing, Cancer and Cellular Senescence

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Ageing is the biggest risk factor for cancer, but the mechanisms linking these two processes remain unclear. Using publicly available RNA-Seq data, we compared genes differentially expressed with age and genes differentially expressed in cancer among nine human tissues. In most tissues, ageing and cancer gene expression surprisingly changed in the opposite direction, except in thyroid and uterus. These overlapping gene sets were related to several processes, mainly cell cycle and the immune system, suggesting the tissue-specific relationship between gene expression changes in ageing and cancer. Moreover, cellular senescence signatures derived from a meta-analysis of 20 publicly available microarray datasets changed in the same direction as ageing and in the opposite direction of cancer signatures. Therefore, transcriptomic changes in ageing and cellular senescence might relate to a decrease in cell proliferation, while cancer transcriptomic changes shift towards an increase in cell division. Our results highlight the complex relationship between ageing, cancer and cellular senescence and suggest that in most human tissues ageing processes and senescence act in tandem while being opposite to cancer.

(4) Influence of the adherence to the Mediterranean diet on the effect of smoking on genomewide methylation among subjects with metabolic syndrome

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Tobacco smoking is an important risk factor for lung cancer and other diseases. Moreover, smoking can speed up the normal aging process increasing the biological age. Changes in methylation due to smoking have been demonstrated at several loci. The most consistent association reported has been decreased methylation in smokers at the CpG cg05575921 in the aryl hydrocarbon receptor repressor (**AHRR**). Another consistent association has been found with coagulation factor II (thrombin) receptor-like 3 (F2RL3, cg03636183). Despite the numerous studies examining changes in methylation at the genome-wide level depending on the smoking behavior, very few studies have examined the additional influence of the diet in modulating the smoking effects. Thus, our aim was to examine whether a higher adherence to a Mediterranean diet (MedDiet) modulated the effect of smoking on genome-wide methylation among older subjects with metabolic syndrome. We analyzed 88 participants in the PREDIMED PLUS-Valencia study (mean age 64+/-5 years, all with metabolic syndrome). DNA was isolated from blood. We performed genome-wide DNA methylation (EWAS) using the Illumina Infinium MethylationEPIC array. Smoking status was assessed according to the WHO classification. Adherence to the MedDiet was determined by a 17-item questionnaire. Tobacco smoking was associated methylation of several genes: cg05575921-AHRR (P=1.57E-09); cg21566642 (P=4.12E-09); cg17739917-RARA (P=7.73E-08); cg01940273 (P=2.68E-07), and cg03636183-F2RL3 (P=1.34E-06). Adherence to MedDiet was associated with differential methylation of several genes and some heterogeneity by smoking was detected. In subjects with low adherence to MedDiet, the top-ranked CpG associated with tobacco smoking was cg05575921-AHRR (P=8.91E-07), however, in subjects with high MedDiet adherence, some of the top-ranked SNPs differed, specifically, the cg05575921-AHRR was not among the 500 top-ranked SNPs, decreasing differences in methylation (P=0.0058). In conclusion, our results suggest that MedDiet could be an additional modulator of the smoking effects on DNA-methylation.

(5) Characterization of the AMPK pathway in endothelial cells under senescence, ageing and after exposure to high glucose

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AMP-activated protein kinase (AMPK) represents an important regulator of cellular energy status and homeostasis and integrates multiple longevity pathways such as FOXO, SIRT1 and inhibition of mTORC1. Our aim is to understand how alterations of the AMPK pathway in endothelial cells contribute to vascular ageing and whether protein modifications are involved in these processes.

We characterized the AMPK pathway in primary human endothelial cells from umbilical cord veins (HUVEC), which were propagated to reach replicative senescence, and in endothelial cells prepared from 24 months old mice. Alternatively, young HUVEC were exposed to $_{H2O2}$ (50 or 100 µM in serum-containing growth medium, 8 days) in order to generate premature stress-induced senescence. In addition, HUVEC were treated with high glucose (25 mM) or $_{H2O2}$ (100 µM) to test whether AMPK or its upstream kinase calcium/calmodulin-activated kinase kinase 2 (CaMKK2) are targets for stress-mediated protein modifications, especially modifications by O-linked β-N-acetylglucosamine (*O*-GlcNAc). *O*-GlcNAcylation was detected in immunoprecipitates of AMPK or CaMKK2 using a specific antibody against *O*-linked β-N-acetylglucosamine.

Our data show an upregulation of AMPK expression and activity in replicative senescence and chronological ageing of endothelial cells. Premature stress-induced senescence had no effect on AMPK expression but led to an increase of AMPK activation induced by vascular endothelial growth factor (VEGF). Treatment of endothelial cells with high glucose did not induce *O*-GlcNAcylation of AMPK. This was also not seen when an inhibitor of *O*-GlcNAcase was applied in addition. However, *O*-GlcNAcylation of immunoprecipitated CaMKK2 was demonstrated after treatment with H_2O_2 or high glucose. The latter was paralleled by an increased CaMKK2 activity.

Together, our data show that the AMPK pathway is altered under conditions of senescence and ageing and that CaMKK2, an important upstream kinase of AMPK is prone to *O*-GlcNAcylation. Future experiments will reveal whether these two observations are linked and whether CaMKK2 modification may contribute to altered AMPK activity in senescence.

(6) Meaningfulness of a biological marker (advanced glycation endproducts) measured by noninvasive skin-scan (autofluorescence measurement) in geriatric-oncological patients

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Background: Older patients with cancer differ regarding their biological age, functionality, organ reserve and comorbidities. Therefore, a comprehensive geriatric assessment (CGA) e.g. to assess physical and cognitive functioning, nutritionals status, mood, self-care and social support is recommended before planning oncological therapies. In addition, a valid assessment of the biological age could add valuable information.

Objective: To investigate the possible benefit of the autofluorescence measurement of advanced glycation endproducts in the skin (AGE value) in the clinical context of geriatric oncology. So far, AGE measurement has been used as a screening tool for diabetes mellitus, renal failure and cardiovascular diseases.

Methods: Exploratory analysis of AGE data of a heterogeneous sample of older cancer patients to generate hypotheses regarding the clinical relevance of AGE. The AGE value of the skin was determined by autofluorescence measurement in n=100 cancer patients. Based on of age-specific reference values, AGE values were categorized as age-appropriate or pre-aged. Descriptive analyses regarding sex, comorbidities, cancer diagnoses and results of geriatric assessments were performed. To explore the predictive properties of AGE values survival data were analyzed with simple Kaplan-Meier estimates and Cox regressions with log-rank tests.

Results: The analyses of the AGE values were performed on 97 patients (47 women and 50 men) with a mean age of 75 ±5 years. Main cancer sites comprised lung n = 27 (28%), hematological n = 16 (17%), breast n = 14 (14%), head/neck n= 14 (14%), skin n = 12 (13%) and other =14 (14%). AGE values were Ø 3.1 Arbitrary Units (AU) ±0.8 (min. 1.7, max. 6.1). According to the age-specific reference values n =38 patients were categorized as age-appropriate and n =59 as the pre-aged. In the study population men were more often categorized as pre-aged than women. The analysis showed that a high calendar age is not automatically associated with pre-ageing. It is a new aspect about this older population. The survival time analyses indicated a survival advantage for tumor patients with high AGE values (p = 0.036, KI =1.038- 2.937, n = 97).

Discussion: Due to the exploratory design, the results are not generalizable. To the best of our knowledge the result that high AGE value were associated with a better survival of older cancer patients have so far not been puplished elsewhere.

Conclusion: These results should be examined in further studies comprising larger and more homogeneous samples of older cancer patients.

(7) EZH2 expression and mutation status as well as effects of EZH2 inhibition in T-cell lymphomas and leukemia

Elisabeth Groß, Thomas Weber

Enhancer of Zeste Homologue 2 (EZH2) is a methyltransferase which is part of the Polycomb Repressive Complex 2 (PRC2). It influences hematopoietic stem cell differentiation and due to the regulation of HomebOX (HOX) genes also cell type intrinsic gene expression (1; 2). EZH2 is frequently overexpressed in solid tumors or mutated with gain-of-function in B-cell neoplasia (3; 4). Therefore, it is considered as a therapeutic target. However, inactivation of the protein is linked to increased chemotherapy resistance in e. g. Acute Myeloid Leukemia (AML) (5). Due to this dual role of EZH2 in malignancy, we aimed to investigate its mutation and expression status in Monomorphic Epitheliotrophic Intestinal T-cell Lymphoma (MEITL) patient samples. We also aimed to analyze the consequences of EZH2 inhibition on tumor control and chemotherapy resistance in T-cell lymphoma and leukemia cell line models.

Two of the analyzed 32 MEITL patients (6 %) carried genetic aberrations of EZH2 with predicted altered protein function. Semi-quantitative immunohistochemistry revealed high EZH2 expression in T-cell Acute Lymphoblastic Leukemia (T-ALL), moderate expression in Diffuse Large B-Cell Lymphoma (DLBCL), Peripheral T-Cell Lymphoma Not Otherwise Specified (PTCL NOS) and MEITL, as well as relatively low expression in Enteropathy Associated T-cell Lymphoma (EATL), Angioimmunoblastic T-Cell Lymphoma (AITL) and Anaplastic Large-Cell Lymphoma (ALCL). We also tested the influence of EZH2 inhibition on chemotherapy resistance and gene expression in tumor cell lines with T-ALL origin as well as other hematopoietic backgrounds. Oxaliplatin resistance was increased by EZH2 inhibition especially in immature T-cell tumor cell lines as well as B-cell tumor cell lines with activating EZH2 mutation or EZH2 overexpression.

In conclusion, this study helps to elucidate the role of EZH2 function and its pharmacological inhibition in T-cell neoplasia.

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(8) Potential of AGE-modified peptides as an early diagnostic marker for Alzheimer's disease – A pilot study

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The research community strives for early diagnostic markers in Alzheimer's disease (AD) to prevent disease-driven neuronal damage. A major known risk factor for AD, diabetes, is characterized by the carbohydrate-induced glycation of proteins, forming long-lasting, non-reversible and often cross-linking advanced glycation end products (AGEs) which are also implicated in the development of AD.

Thus, the question arose whether such protein-alterations could serve as biomarkers for AD-development in a sub-set of patients.

CSF of 5 control and 5 patients with assured AD diagnosis was tryptically digested and analyzed by an LC-MS/MS-workflow. The results were examined for AGE-containing peptides, which were unambiguously identified and could be quantified by their MS^1-signals. Of those, potential biomarker-candidates were determined by statistical analyses.

We quantified 164 glycated peptides in this study of which 139 are possible biomarker-candidates. AUC-analysis of those indicated that the data-set might include two interesting peptides, containing sugar- and glyoxal-induced modifications, that discriminate between AD and controls. These two candidates will be investigated in more detail. Additionally, sample size estimation based on this pilot study indicated, that with a reasonable number of samples, statistical significance could be reached for the top-candidates.

In conclusion, AGE-modified peptides show the potential to be used as biomarkers in AD.

(9) Clonality and heterogeneity of intestinal stem cells upon aging

Ali Hageb, Bettina Möhrle and Hartmut Geiger
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Intestinal stem cells (ISCs) are among the most proliferative stem cells in the body. ISCs are located at the base of the intestinal crypt base are one of the best studied stem cell models for normal homeostasis, aging, regeneration and cancer. The intestinal tract shows a high prevalence for cancer and the determination of the tumour-initiating cell is important to understand intestinal cancer biology. It is thought that aging of ISCs might be linked to many intestinal diseases ranging from malabsorption to cancer. Experimental approaches to investigate clonal evolution and heterogeneity of ISCs upon aging are necessary to test this concept. Our hypothesis that we are testing is that aging results in reduced clonality, but at the same time increased heterogeneity among intestinal stem cells. We established for the determination the extent of changes in clonality in ISCs upon aging tracing of multiple fluorescent proteins reporters (confetti mice express 4 colours or more). For the tracing experiment based on confetti mice, the four colours in ISCs were clearly identified and traced by our novel established protocol of analysis. Secondly, heterogeneity of crypts cells and more specifically Lgr5+ stem cells of young and old mice will be investigated by culturing single Lgr5+ stem cells to form organoids as a functional assay, histochemistry and immunohistochemistry, lineage tracing and analysis of RNA seq data from Lgr5+ stem cells. Single Lgr5+ stem cell culture assays revealed the Lgr5+ cells from young animals show a higher frequency of organoid potential. With respect to lineage tracing experiment, we established novel protocols in which ISCs in crypts were successfully transduced by a lentivirus with very high efficiency and stability in secondary organoid assays.

Key words: Intestinal stem cell, aging, clonality and heterogeneity

(10) Generation of disease-specific induced pluripotent stem cells and differentiation into a blood-brain barrier system for the analysis of APOE4 and its role in Alzheimer's disease

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One pathological characteristic of late-onset Alzheimer's disease (AD) ft he accumulation of amyloid-β (Aβ) peptides. Dysregulation ft he blood-brain barrier (BBB) contributes to AD disease progression. The most associated genetic risk factor is the ε4 allele of apolipoprotein E (APOE). APOE is involved in several metabolic pathways including lipid transport, Aβ aggregation, and Aβ clearance. However, the molecular and cellular signaling pathways regulated by APOE4 are currently poorly understood. The differentiation of BBB cells using patient-derived induced pluripotent stem cells (iPSCs) is a promising approach to analyze AD disease mechanisms in the context of APOE4 and ITS role in BBB breakdown.

First, we identified the APOE status of our AD patients and healthy matched controls. Then, episomal vectors were used for the generation of iPSCs from B-lymphoblastoid cell lines of patients carrying the APOE4 allele and healthy donors with homozygous APOE3 alleles. After successful generation of AD-specific iPSCs and proven pluripotency, we differentiated them into cells of the BBB system, in particular endothelial cells and astrocytes. Efficient differentiation was shown by the expression of cell- and BBB-specific markers including OCLN and TJP1. Barrier functionality was demonstrated by hindered paracellular transport of sodium fluorescein and transendothelial electrical resistance values > 1000 Ω*cm2 for both AD patients and matching controls.

Overall, we established a patient- and disease-specific BBB model suitable to investigate AD-associated genetic risk variants, resulting pathogenic phenotypes, and underlying disease mechanisms providing potential targets for AD treatment.

(11) Non-enzymatic modifications of the mineralocorticoid receptor during vascular aging

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The mineralocorticoid receptor (MR) and its ligand aldosterone regulate water and electrolyte homeostasis and thereby blood pressure. In the presence of additional permissive micro-milieu factors, it can also induce pathophysiological effects in the cardiovascular system, independently of these physiological effects. The aim of this work was to investigate age-related posttranslational modifications and alterations of MR signaling and function that might mediate changes in the cardiovascular system.

Expression analyses in senescent and young HUVEC cells and in old and young mouse hearts suggest that during aging MR expression is increased, whereas overall nitric oxide synthases (NOS) expression is reduced. Because previous investigations show that pathological MR effects are not exclusively mediated by changes in MR expression, we decided to study the effect of altered NO availability on MR signaling in two different cell lines. Genomic MR activity was markedly diminished when i) applying the NO donor SNAP, ii) overexpressing endothelial and inducible NO synthase (eNOS, iNOS) and iii) stimulating endogenous eNOS in endothelial cells in reporter gene assays. Mechanistically, NO reduces the binding of MR to DNA elements in transcription factor binding analyses, while nuclear translocation monitored by time-lapse recordings of EGFP-tagged MR remained unaffected. The location of this NO induced reduced DNA binding ability is the CDEF-domain of the MR. The large regulatory AB-domain of the MR showed no additional effect. As molecular mechanism leading to altered genomic MR activity we could detect direct S-nitrosylation of the MR-CDEF domain by NO in a biotin switch assay. Site-directed mutagenesis experiments showed that single mutations of cysteines in the first zinc finger of the DNA binding domain of the MR lead to diminished aldosterone responsiveness. Mass spectrometry analyses confirmed direct S-nitrosylation of the MR. Overall, we propose that during aging, MR activity is increased by two mechanisms. Firstly, the MR expression is enhanced during aging. Secondly, direct S-nitrosylation of the MR is diminished due to reduced NO availability, leading to inadequate MR activation with pathophysiological changes in the cardiovascular system during aging.

(12) Aged rabbit adipose-derived mesenchymal stem / stromal cells (ASCs) recapitulate aging biomarkers and show increased histone H3 lysine 27 (H3K27) modifications

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The rabbit is a valuable animal model for a variety of biomedical research areas including embryology, organogenesis, and modelling of diabetes, obesity, or cardiovascular diseases. Embryo and fetal development of the rabbit are often analysed in toxicology studies and drug development. However, little is known about the application of rabbits for modelling aging and age-related disease mechanisms. Adult stem cells such as mesenchymal stem cells are affected by molecular and cellular aging mechanisms. They have the capability to self-renew and can differentiate into multiple cell types of the mesoderm germ layer. One proposed mechanism is the age-dependent alteration in the modification of histones throughout lifespan also called epigenetic drift. Protein modifications of histones as reversible and provide a promising molecular mechanism for deepen our understanding of aging.

The aim of this study was to quantify the histone modification of H3 lysine 27 in primary cultured ASCs from young and old individuals. ASCs were derived from visceral and subcutaneous adipose tissue of young (24 weeks) and old (>108 weeks) female rabbits. Primary ASCs were cultured and analysed for age-dependent differences in H3K27 modifications. Histone modifications of H3 lysine 27 in ASCs were analysed by western blot. The di- and trimethylation of H3K27 was tendetiell increased in aged visceral ASCs. The analysis of aged subcutaneous ASCs revealed a significant elevation of H3K27 acetylation, suggesting that H3K27 modifications are regulated in aged ASCs. Together, analysis of H3K27 was successfully established in young and old ASCs representing an *in vitro* model for the analysis of stem cell aging mechanisms.

(13) Analysing Alzheimer's disease (AD) risk variants in CD33 and TREM2 using microglia-like cells derived from AD-specific iPS cells

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Alzheimer´s disease (AD) is the most common cause of dementia in the elderly. Accumulation of intercellular β-amyloid plaques and intracellular neurofibrillary tangles are two hallmarks of AD that may drive neuronal death and the corresponding dramatic loss of cognitive abilities. A complex interaction between genetic and environmental factors contributes to molecular processes that drive AD. Microglial-mediated processes are key determinants to the accumulation of amyloid deposits in AD, playing roles in amyloid degradation, and initiation and growth of plaques. Environmental and genetic factors contribute to the risk for AD, but the underlying disease mechanisms are poorly understood. In recent years, genome-wide association studies (GWAS) allowed the identification of DNA variations associated with an elevated risk for AD. A number of AD susceptibility genes including CD33, SORL1, ABCA7 and TREM2 point towards the immune system as a player in onset, progression and treatment of AD. The generation of patient specific induced pluripotent stem cell (iPS) lines enables differentiation into microglia. Patient-specific cells can be used as a model to functionally characterize disease associated variants. Potentially functional SNP variants in CD33, SORL1, ABCA7 and TREM2 were tested for association with AD. iPS cells were generated from patients carrying risk variants in these genes. Pluripotency was characterized by alkaline phosphatase staining, the expression of pluripotency markers, and the differentiation into the three germ layers. AD iPS cells were differentiated into microglia characterized by the expression of crucial glia cell markers. Motility, phagocytosis, and behaviour of processes were examined to determine functionality. The protein expression of pluripotency marker genes was successfully induced as shown by IF and WB analyses. Cells were also screened for the most efficient induction of neural cell fates including glia cell fates and the capability to generate derivatives of the three germ layers. We established 4-step protocol for the generation of AD-specific microglia enabling the focused analysis of AD-associated risk variants. The protocol was verified by morphology, FACS analysis, IF analysis, and RNA expression of hematopoietic lineage markers and crucial microglia markers. The established AD-specific iPS cell lines from late-onset AD patients represent a powerful tool for the analysis of molecular and cellular disease mechanisms. Together, combining molecular genetics of AD for the investigation of risk variants and iPS cell technology for the generation of patient- and disease-specific stem cells provides a promising approach to characterize known disease mechanisms, to deepen the understanding of known disease mechanisms, and to discover unknown disease aspects.

(14) Comparative analysis of left and right ventricular adaptations to pressure overload in rats

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Background: Cardiac hypertrophy and subsequent heart failure develop slowly in response to pressure overload in humans. Therefore animal models resulting in a gradual increase in afterload resemble closely the human situation. Significant differences between right ventricular (RV) and left ventricular (LV) adaptations to pressure overload were suggested to exist, but were never compared systematically.

Methods: Pulmonary artery banding (PAB) or aortic banding (AOB) was performed in weanling rats with a non-constricting clip, to study the gradual transition from compensated cardiac hypertrophy (7 weeks post surgery) to heart failure (22 weeks after PAB, 26 weeks after AOB). Cardiac function was characterized by echocardiography (Vevo2100). Heart tissue, blood samples and isolated cardiomyocytes were characterized by histological and mitochondrial analyzes, ELISA or qPCR/Western Blot respectively. Skeletal muscle abnormalities associated with heart failure were comparatively analyzed in both models.

Results: AOB and PAB animals at the compensated stage show a similar hypertrophy in the respective ventricle but maintained LV or RV function, no alterations in the expression of calcium-handling proteins, mitochondrial function and no increased fibrosis. Plasma BNP levels are moderately increased at this stage of disease, but show a massive increase at the stage of cardiac decompensation in both models. Furthermore, animals at the decompensated stage show reduced LV or RV function, septum hypertrophy and increased fibrosis. In addition, RV cardiomyocytes in the AOB model and LV cardiomyocytes in the PAB model are hypertrophied as well now. The typical change in NCX (↑) and SERCA (↓) protein expression was observed in the LV of AOB animals and in the RV of PAB animals at the decompensated stage. Similarly, a mitochondrial dysfunction was observed in both diseased ventricles. Enhanced inflammation, mitochondrial alterations and increased atrophy markers in skeletal muscle were more pronounced in the AOB model.

Conclusions: LV and RV appear to adapt to slowly developing pressure overload in a similar fashion. Genome-wide analyses may help to identify chamber-specific differences at the stage of compensatory hypertrophy and at the stage of heart failure.

We conclude that pathophysiological concentrations of MGO and GO affect mesenchymal stem cells increasing AGE levels and p21 expression, whereas adipogenic differentiation was not changed. Supported by DFG GRK 2155 ProMoAge

(15) Monitoring of methionine oxidation in living cells

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Methionine (Met) oxidation to methionine sulfoxide (Met-O) is a post-translational modification that can appear unspecifically during exposure of Met to oxidative stress, such as occurring in aged organisms and in various degenerative diseases and pathologies. A set of methionine sulfoxide reductases (MSRs), which catalyze the reduction of Met-O to Met, is present in virtually all organisms and play an important role in antioxidative defense. Met oxidation detection is typically done using biochemical techniques, excluding real-time measurements in living cells. Moreover, the absence of a specific antibody against Met-O disables western blotting or immunohistochemical detection.

We designed and optimized variants of superfolder green fluorescent protein (sfGFP), which provide excitation-ratiometric fluorescent signals reporting on the degree of Met oxidation. The recombinant fluorescent proteins were characterized under fully controlled conditions in a spectrofluorometer. Incubation of the indicator with Met oxidants showed robust changes in the fluorescent ratio F390/F480; full oxidation resulted in an about 2-fold decrease. Mass spectrometry analysis confirmed oxidation of a Met residue in the indicator responsible for the ratio changes. We determined the rate constant of Met oxidation for chloramine T to $615 \pm 24\ s^{-1}M^{-1}$. No changes in the fluorescent ratio after incubation of the sensor indicated that Met residue of the GFP variant is not accessible for MSRs; the indicator provides an accumulated signal for Met oxidation. In mammalian cell lines, expression of the indicator produced robust ratiometric signals suitable for single-cell photometry, live-cell imaging, and FACS analysis to monitor intracellular Met oxidation under various conditions. Similarly, Met oxidation in wild-type and genetically modified yeast strains with altered antioxidant systems was readily measurable in FACS analysis. In summary, the genetically encoded Met oxidation sensor promises to be a useful tool for studying Met oxidation in aged and diseased tissues and organs.

(16) Healthy ageing affects sperm DNA integrity, but not reproductive health

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Children of older fathers have higher risk for certain diseases. Nevertheless, how ageing specifically affects male germ cells is so far not completely understood. In a cohort of 197 healthy men (18-84 years), we found that semen, endocrine, and other reproductive parameters remained normal throughout life. With respect to sperm DNA, we found an age-dependent increase in telomere length in sperm ($r=0,41$, $p<0,001$) and an increase in DNA fragmentation, which was most prominent after the sixth decade, with around 60% of men older than 66 showing abnormal levels of DNA breaks. By whole genome bisulfite sequencing, we identified 236 sperm-specific differentially methylated regions (with a minimum methylation difference of 30%) between the youngest and oldest men, not present in blood. These changes were consistent enough to allow the derivation of an age-predictor for sperm based on six individual regions. Moreover, GO analyses of the DMRs revealed an association with nervous system development. Therefore, we propose that during ageing male germ cells are affected by an intrinsic and specific ageing process, distinguishable from the soma, and that these age-dependent changes might have consequences for fertility in older men.

(17) Transcriptional and epigenetic landscape of the mouse intestine during aging

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SUMMARY

Aging is a complex multifactorial process leading to the loss of tissue/organ functionality and to an increase in disease risk. Aging-related intestinal dysfunctions include loss of barrier integrity, altered stress responses, nutrient malabsorption, and cancer formation. Many molecular mechanisms related to dysfunction and diseases are well-known (e.g. in cancer), however how the aging process impacts on them before the occurrence of dysfunctions and diseases is poorly understood.

In this study, we applied a multi-layered omics-approach to characterize the transcriptional and the epigenetic landscape of mouse intestinal epithelium during aging. We found gender and cell-type specific transcriptional and epigenetic alterations on key pathways and genes linked to intestinal dysfunctions, stem cell aging, organismal lifespan and cancer. Moreover, we identified a switch in the composition of the old intestinal stem cell subpopulations, represented by a drift towards a more secretory lineage committed (and less stem) state accompanied by functional epigenetic alterations.

KEYWORDS

Aging, intestinal dysfunction and cancer, transcriptome, DNA methylation, microRNAs, intestinal stem cells, single-cell RNAseq

(18) Tissue-Specific Expression Signatures of Aging

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Understanding the expression changes that come with age could be key in understanding the aging process itself. Previous meta-analyses and large-scale transcriptomics studies have revealed an aging signature involving the upregulation of genes involved in immune processes as well as a downregulation of genes involved in developmental and metabolic processes. We have conducted a meta-analysis of ageing microarray and RNA-Seq experiments in humans, mice and rats, aiming to identify common transcriptomic changes with age in the whole system, as well as specifically in the brain, heart, and muscle. To do this, 127 datasets were obtained from GEO, SRA and AgeMap and genes consistently differentially expressed with age across the datasets were identified. Across all tissues, 449 genes were consistently overexpressed and 162 genes were consistently underexpressed with age, these numbers are higher than those determined by a similar meta-analysis from 2009, and the results in both directions of expression overlap significantly with that previous analysis. Gene ontology enrichment analysis of these genes revealed a typical aging expression signature including upregulation of immune processes and downregulation of developmental and metabolic processes. In the brain, 147 genes were consistently overexpressed and 16 genes were consistently underexpressed with age, in the heart there were 35 overexpressed and only 5 underexpressed, while in the muscle there were 49 overexpressed and 73 underexpressed. Again, gene ontology enrichment analysis demonstrated typical responses to the ageing process including alterations in immune and stress responses as well as metabolic changes in all tissues, however there were some interesting differences between the tissues that hint at possible differences in how these tissues age. To complement the enrichment analyses, machine learning was employed. Using a Random Forest method, we identified which gene ontology terms were the best predictors of expression change with age for the analysis as a whole and for each individual tissue. These results provided a similar picture to that of the enrichment analysis, with immune and metabolic terms being the best predictors of expression change with age across tissues. These results provide a comprehensive transcriptomic signature of ageing, confirming the importance of immune, stress response and metabolic processes in the ageing organism, and also highlight some key transcriptomic differences between the brain, heart and muscle.

(19) Neural stem cells in ageing and disease and the impact of different APOE genotypes

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Aim: Stem cell ageing results in loss of organ function and onset of age-related diseases, like Alzheimer's disease (AD). AD is the most common form of dementia. The disease is characterized by complex molecular genetics, cognitive impairment, progressive neurodegeneration, and brain atrophy. The apoliporptein E (APOE) is one genetic risk factor for AD. Different APOE Isoforms are defined by two single nucleotide polymorphisms (SNP), leading to strong differences in protein function. The strongest association with AD is discribed for APOE4. Further, APOE is necessary for maintenance of adult neural stem cells (NSCs) in the adult brain. Underlying mechanisms for stem cell aging and age-related diseases are poorly understood. Therefore, the aim of the study was the characterization of aging markers including APOE in human NSC from young and old individuals, to establish a useful in vitro model for analyzing aging or disease-dependent alterations.

Methods: Induced pluripotent stem (iPS) cell lines were generated from blood of young and old donors. Further, iPS cells were obtained from LOAD patients carrying APOE4. IPS cells were differentiated into NSCs using differentiation media. NSC was caracterized by evidence of certain NSC markers like SOX1, Nestin or SOX2 with FACS or qPCR. Afterwards we analyzed transcript and protein expression in these NSCs. Telomere length was investigated in iPS cells and NSCs using a qPCR-based method. We induced the expression of APOE3 using expression plasmids to recover APOE expression in NSCs from APOE4 carriers.

Results: The successful differentiation of NSCs from iPS cells was shown by the expression of crucial marker genes including SOX1, SOX2 or Nestin. We also analyzed the gene expression of aging marker genes according to the literature and found that aging markers were differently expressed in young and old NSCs. We found significant changes in the expression of aging markes, like ATG7 or FGF2, by the comparison of healthy and LOAD derived NSC. There are also aging markers that showed an APOE4-dependent expression pattern like ATG7 and PTEN. Telomere length was analyzed as an aging marker and revealed shortening after differentiation of iPS cells into NSCs.

Conclusion: We successfully established a stem cell model, which can be used to understand common and rare molecular and cellular mechanism of aging. These findings provide a tool for understanding the gentic backround of age related disease like AD. We demonstrated that telomere length and the expression of certain markers represent powerful aging markers in NSCs.

(20) The GID Ubiquitin Ligase Complex is a Regulator of AMPK Activity and Organismal Lifespan

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The AMP-activated protein kinase (AMPK) regulates cellular energy homeostasis by sensing the metabolic status of the cell. AMPK is regulated by phosphorylation and dephosphorylation as a result of changing AMP/ATP levels and by removal of inhibitory ubiquitin residues by USP10. In this context, we identified the GID-complex, an evolutionarily conserved ubiquitin-ligase-complex (E3), as a negative regulator of AMPK activity. Our data show that the GID-complex targets AMPK for ubiquitination thereby altering its activity. Cells depleted of GID-subunits mimic a state of starvation as shown by increased AMPK activity and autophagic flux as well as reduced MTOR activation. Consistently, *gid*-genes knockdown in *C. elegans* results in increased organismal lifespan. This study may contribute to understand metabolic disorders such as type 2 diabetes mellitus and morbid obesity and implements alternative therapeutic approaches to alter AMPK activity.

Keywords: AMPK; autophagy; GID; longevity; MTOR; primary cilium; ubiquitination

(21) MicroRNA-496 – A new, potentially aging-relevant regulator of mTOR

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Recent findings strongly support a role for small regulatory RNAs in the regulation of human lifespan yet little information exists about the precise underlying mechanisms. Although extensive studies on model organisms have indicated that reduced activity of the nutrient response pathway, for example as a result of dietary restriction, can extend lifespan through the suppression of the protein kinase mechanistic target of rapamycin (mTOR), it still is subject of debate whether this mechanism is operative in humans as well.

Here, we present findings indicating that human microRNA (miR)-496 targets 2 sites within the human mTOR 30UTR. Coexpression of miR-496 with different fusion transcripts, consisting of the luciferase transcript and either wild-type mTOR 30UTR or mTOR 30UTR transcript with the miR-496 binding sites singly or combined mutated, confirmed this prediction and revealed cooperativity between the 2 binding sites. miR-496 reduced the mTOR protein level in HeLa-K cells, and the levels of miR-496 and mTOR protein were inversely correlated in Peripheral Blood Mononuclear Cells (PBMC), with old individuals (nD40) harbouring high levels of miR-496 relative to young individuals (nD40).

Together, these findings point to the possibility that miR-496 is involved in the regulation of human aging through the control of mTOR.

Rubie *et al.,* Cell Cycle 2016

(22) MicroRNA-496 and Mechanistic Target of Rapamycin Expression are Associated with Type 2 Diabetes Mellitus and Obesity in Elderly People

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Background: Mechanistic target of rapamycin (mTOR) regulates lipid and glucose metabolism thus playing a key role in metabolic diseases like type 2 diabetes mellitus (T2DM). Recently, we demonstrated a functional interaction of microRNA-496 (miR-496) with mTOR and its impact on the regulation of human ageing.

Objectives: As T2DM is most prevalent in older adults, we hypothesized that miR-496 may also have an impact on mTOR regulation in T2DM.

Methods: Based on real-time PCR and enzyme-linked immunosorbent assay, mTOR gene and protein expression as well as miR-496 expression were monitored in peripheral blood mononuclear cells (PBMC) from T2DM patients (median age: 71) and healthy age- and BMI matched controls (median age: 69).

Results: We demonstrated significant upregulation of phospho-mTOR and P70S6 Kinase (P70S6K) levels and significant downregulation of miR-496 in PBMC from elderly T2DM patients in comparison to a BMI and age-matched control cohort. Moreover, significant upregulation of phospho-mTOR protein and significant downregulation of miR-496 were observed in advanced stages of obesity.

Conclusions: BMI-dependent upregulation of mTOR and the inverse expression profile of miR-496 observed in elderly T2DM patients suggest a correlation with T2DM. Hence, our results indicate a potential association of miR-496 with mTOR expression in elderly T2DM patients and obesity. Since phosphorylation of P70S6K was also elevated in T2DM patients, we conclude that mTOR signaling through TORC1 may be affected in the regulation of T2DM..

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(23) The relationship of DNA-methylation and Transposable Elements: Why do some instances break out of control?

Robert Schwarz

DNA methylation (5mC) is a known repressor of Transposable Elements (TEs) in vertebrates. Reduced 5mC levels can revoke the repression and in consequence lead to TE expression. In a former survey we found evidence of activity during aging of a retrotransposon family of the order LINE in the turquoise killifish *Nothobranchius furzeri*. Hence, we are interested in the expression of TEs in concordance with 5mC levels to understand drivers and mechanisms of TE control by methylation during aging.

Evidence of DNA methylation change and the resulting expression of single TE instances can be difficult to ascertain due to the sheer mass of instances scattered across the genome to which a given transcript aligns (multi-mapping reads). A better resolution, i.e., the exact identification of a locus of origin and thus the expressed TE instance, is needed to assess DNA methylation and other influences on TE expression and control.

We used TETools, which can handle multi mapping reads on TE family level, to analyze existing data along an age gradient of *N. furzeri* (JenAge Database). We additionally adapted TETools to analyze the data set on instance level. With this approach we are able to identify differentially expressed instances of TEs with aging or in other comparable conditions.

In the next step, we aim to add 5mC data in a locus-specific way to explore the relationship of 5mC-levels, TE expression, and aging. We furthermore intend to analyze human and mouse data sets (JenAge Database) to get an overview of TE expression during aging in different species.

With this comprehensive approach, the analysis of instance-specific circumstances of control and loss of control will come within reach.

(24) Pregnancy risks by hyperglycaemia: Metabolites like methylglyoxal and glyoxal act as histone modifiers in early embryos

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In the endocrine-metabolic disorder diabetes mellitus, blood glucose concentrations and its toxic metabolites, methylglyoxal (MGO) and glyoxal (GO), are increased in cells and tissues also during pregnancy.

The aim of this study was to determine specific histone modifications and the expression of histone modifying enzymes in early mammalian embryos exposed to a diabetic environment *in vivo* and components of a diabetic environment *in vitro*. Therefore, six-day-old blastocysts were cultured with MGO and GO. *In vivo* blastocysts were obtained from rabbits with an Alloxan induced diabetes mellitus. Histone modifications of blastocysts were examined by LC-MS/MS. Expression of histone modifying genes was analysed by quantitative Real-Time PCR *in vivo* and *in vitro*.

High levels of MGO and GO led to a reduction of H3 acetylation at lysine 18 and lysine 23 in the embryoblast and to an increase of di-methylation on lysine 27 (H3K27) in the trophoblast. EZH2 (*enhancer of zeste homolog 2*), a H3K27 methyl transferase, was transcriptionally downregulated in the embryoblast of MGO/GO exposed blastocysts and in the embryoblast from diabetic rabbits.

Our data indicate that blastocysts challenged by diabetic components (MGO and GO), changed their epigenetic code and regulate their modifying enzymes. The embryo can adapt to an altered uterine environment in an epigenetic manner by alterations in histone modifications.

(25) Autoantibodies against advanced glycation end-products in probands of the Carla Cohort Study

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Advanced glycation end-products (AGEs) are formed in a series of non-enzymatic reactions between reducing sugars or dicarbonyls and proteins, peptides, nucleic acids or lipids. Structural alterations due to this glycation can form new epitopes on the affected proteins, triggering an immune response and formation of antibodies.

We hypothesize that these autoantibodies can correlate with certain chronic diseases like diabetes mellitus (DM) and Alzheimer's disease and their change over time can predict the disease state and progress.

Plasma from probands (n=1779) of the CARLA Cohort Study (Cardiovascular diseases, Living and Aging in Halle) will be tested for the presence of anti-AGE autoantibodies to evaluate antiAGE-autoantibody levels in health and diseases.

The CARLA Study collected data at baseline (2002-2006) with three follow-ups through self-administered and computerized interviews as well as physical examination and various diagnostic procedures.

In a pilot study on antiAGE-autoantibodies we previously realized detection through an in-house developed ELISA protocol, which measured the antibody binding against modified bovine albumin (BSA) in comparison to native BSA in patients undergoing CABG(coronary artery bypass grafting).

However, the method was limited by the detected auto-reactivity against the unmodified bait for some patients leading to false negative results.

To overcome this problem, human albumins (HSAs) were tested for baseline-AGEs by different methods. The least modified albumin was then exogenously modified using different conditions and time spans. Subsequently, successful modification of HSA was confirmed by various methods. A modified ELISA protocol will now be established and then measurement of the samples will be conducted.

(26) Epigenetic Markers of Aging in Diploid Fibroblasts of Patients with Seckel Syndrome

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Syndromes having features of accelerated aging are called progeroid. These include Seckelsyndrome, Cockkeyn syndrome, ataxia-telangiectasia, and some others. At the same time,diploid fibroblasts obtained from such patients may differ in the number of observed markers ofaging. Study of panel of aging markers (SA-β-gal, γ-H2AX, 53BP1, HP1-γ, SIRT1, SIRT6,3meH3K9, 3meH3K27) helps to detect similarities and differences in progeroid syndromes.Seckel syndrome type 1 (microcephalic primordial microsomia) is a severe autosomal recessivehereditary disease characterized by advanced neurodegeneration, increased risk of tumordevelopment, and sharply reduced life expectancy. Mutation in the atr gene, encoding ATRprotein kinase (Ataxia telangiectasia, and Rad3-related kinase), one of effector kinases of globalcellular DNA damage response (DDR), serves as the cause of Seckel syndrome type 1. DDR is a signaling mechanism that coordinates cell cycle transitions, DNA replication, DNA repair, andapoptosis. When describing primary fibroblasts from patients with Seckel syndrome, significantdifferences from other progeroid syndromes, i.e. Hutchinson-Gilford syndrome, Cockaynesyndrome, Louis-Barr syndrome (ataxia-telangiectasia) were found. Study of panel of agingmarkers showed that in patients with Seckel syndrome only part of these, associated with theaccumulation of DNA damage, and DNA repair defects, demonstrates accelerated aging, while,on the contrary, the other part, associated with epigenetic changes, corresponds to a ‘younger’ or,even tumor phenotype. Data obtained corroborated our hypothesis that there existed an arrange of several independent patterns of aging markers. Consequently, Seckel syndrome cell lines maybe applied as a model for studying aging processes and carcinogenesis, and for the purpose of testing geroprotectors, and anticancer drugs.

Keywords: primary fibroblasts, premature aging, ATR, Seckel syndrome, aging markers.

(27) The chalcone cardamonin activates Nrf2 in HepG2 human hepatoma cells independent of the cellular selenium status

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Many secondary plant metabolites as well as the micronutrient selenium (Se) are thought to be beneficial for human health, such as for prevention of oxidative stress-related disorders. In this regard, the chalcone cardamonin has recently been identified as inducer of Nrf2-regulated antioxidant enzymes, two of them being selenoenzymes.

We investigated a potential interplay between the cellular selenium (Se) status and the Nrf2 signaling pathway, using HepG2 cells cultured under Se-deficient (0 µM), Se-adequate (0,1 µM) and Se-supranutritional (1 µM) conditions. Expression of selected Nrf2 target genes was measured by qRT-PCR and immunoblotting. Cellular Nrf2 localization was detected by immunoblotting after cytoplasmic/nuclear fractionation. Sulforaphane (SFN), a well-known plant-derived Nrf2-activator, and diethyl maleate (DEM), a thiol-modulating chemical, served as controls.

Treatment with the three substances resulted in rapid induction of Nrf2 and its enrichment in the nucleus, independent of the cellular Se status. All three compounds caused an up-regulation of Nrf2 target genes, although with differences regarding extent and time course of their induction. The most pronounced induction of the selected Nrf2 target genes, with up to 20 fold increase in gene expression, was observed for HMOX1/HO-1. The Se status did not significantly affect gene or protein expression of the Nrf2 target genes.

(28) The impact of maternal age on target gene expression in embryos and reproductive organs

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Women approaching advanced maternal age have poor fertility outcome. It is highly likely that metabolic stress modulates the age-related decline in fertility and influences embryo development. Our working hypothesis is that maternal age modulates the expression of key factors for embryo-maternal communication in embryos and supplying uterine tissue, the endometrium, during the preimplantation period.

To study effects of maternal age on early embryonic development, we obtained preimplantation blastocysts and endometrium from young (16 weeks) and old rabbits (over 108 weeks) on day six post coitum. We investigated the expression of metabolic (e.g. PPAR□ and PPAR□), main signal transduction (e.g. CREB, mTOR and Drosha) and developmental target genes (e.g. Brachyury, IGFs and FGF2) as well as target genes directly associated with cellular age (SIRT1, ATG7 and Klotho) and stress (NRF2 and ATF3). The expression was quantified on mRNA level by quantitative RT-PCR and on protein level by Western Blot.

In the endometrium from old rabbits stress and age-associated markers like Nrf2 and SIRT1 were downregulated, related due to a reduced phosphorylation CREB and ATF3 amount. Contrary, in day 6 blastocysts the phosphorylation of CREB was increased. Furthermore, genes were affected which are responsible for gastrulation (Brachyury), miRNA processing (Drosha) and histone methylation (EZH2) as well as protein synthesis and processing (eIF4E and SIRT1). Metabolic targets, like pACC and PPAR, were altered only on protein level of blastocysts.

Our results show that maternal age influences endometrium and embryo in different ways. In the endometrium of old rabbits the signal transduction of cellular stress response and ageing were decreased. In embryos growth factor signalling and corresponding developmental pathways were impaired. The results indicate that age-related changes have consequences for the embryo-maternal cross talk during (pre)implantation and may be crucial for decreased fertility at higher maternal age.

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(29) Histones as a target for glycation

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Glycation is a non-enzymatic reaction between reducing sugars and amino groups of proteins resulting in production of Advanced Glycated End products (AGEs). AGEs accumulate during aging and have been implicated in the pathophysiology of age-related diseases. Glycation of nuclear proteins at lysines or arginines may alter their function and may have an impact on epigenetic regulation of gene transcription. Nuclei from human embryonic kidney cells HEK293A and human kidney carcinoma cell line Caki-2, as well as from young and replicative senescent fibroblasts (Wi-38), were isolated and analyzed via liquid chromatography-tandem mass spectrometry (LC-MS/MS). Most of the AGE-modifications were identified on the core histones. In order to investigate the glycated histones in more details, chromatin from young and replicative-senescent endothelial cells (HUVEC) and fibroblasts (Wi-38), as well as from primary renal and mammary cells with corresponding tumor cell lines (Caki-2 and MCF-7) was extracted and analized by LC-MS/MS. In average 7,3% from all core histone PSMs contained AGE-modifications. Carboxymethyl-lysine (CML) was the most abundant AGE-modification in all cases. Thirty modified sites on the core histones were identified (K96, K119, K120 on H2A; K44, K47, K109, K117 on H2B; K32 and K80 on H4), 10 of them were present in the most of the samples, indicating that the histones have hotspots, which are more prone to be glycated. Surprisingly, all identified modified sites are located inside the histone fold domains, although the majority of described histone post-translational modifications (PTMs) occur on the tail domains. Since PTMs on the histone fold domain influence on DNA-binding and control structure and stability of a nucleosome, glycation of histones may have an impact on gene transcription, which is planned to be investigated.

(30) The effect of glycation on the permeability of human blood-brain barrier

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The human blood-brain barrier (BBB) seperates the blood and everything that circulates in it from the brain parenchyma. The function of the barrier is to protect the brain and to regulate its metabolism strictly. It is built up of the basement membrane and various cells of which the microvascular endothelia cells are the most important ones. They are connected by tight- and adherens junctions resulting in the characteristic low permeability and the high transendothelial electric resistance. Dysfunction of the BBB are linked to several pathologies including postoperative delirium as a complication after cardiac surgery. Postoperative delirium is a life threatening acute complication and the incidence increases dramatically in elderly and diabetic patients. Another example for BBB dysfunction is the microbial transversal, which can cause meningitis. Although the mechanisms of microbial transversal are still not completely understood, morbidity and mortality caused by meningitis can be correlated with increased age and diabetic. Both, age and diabetes are associated with increased levels of advanced glycation endproducts (AGEs). AGEs are the endproducts of glycation, a non-enzymatic posttranslational modification. The aim of this project is to investigate the connection between age-dependent glycation of endothelial cells of the BBB and increased incidence of postoperative delirium and meningitis.

(31) A putative role of Nit1 in oxidative stress

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The mammalian Nit1 (nitrilase-like protein 1) has been identified as a potential tumor suppressor in forestomach [1] and colorectal [2, 3] cancer. Previous studies in our lab have demonstrated that Nit1 inhibits Wnt/β-catenin signaling by competing β-catenin away from LEF-1 [2]. Moreover, just recently Nit1 enzymatic function was unravelled and defined as a deaminated glutathione (dGSH) amidase [4]. Considering the functional interaction between β-catenin and FoxO [5], as well as the role of glutathione in oxidative stress, we here assess whether Nit1 is associated with oxidative stress response. In the present work, we observed that Nit1 was downregulated in response to H2O2- and L-BSO-induced oxidative stress. Next, mutual regulation between FoxO3a and Nit1 was investigated. In this context, we observed that FoxO3a overexpression upreguated Nit1, while Nit1 overexpression attenuated expression of FoxO target genes. Interestingly, this inhibitory effect was independent of Nit1 enzymatic activity. Moreover, we could show that FoxO3a and Nit1 interact by co- immunoprecipitation both after overexpression and at endogenous level. Notably, β-catenin, as well as hydrogen peroxide treatment, interfered with the Nit1-FoxO3a interaction. Ultimately, knock-out of the Nit1 homologue nft-1 in C. elegans resulted in healthy ageing. Taken together, we hypothesize that Nit1 serves as a modulator of FoxO3a activity and is involved in oxidative stress response.

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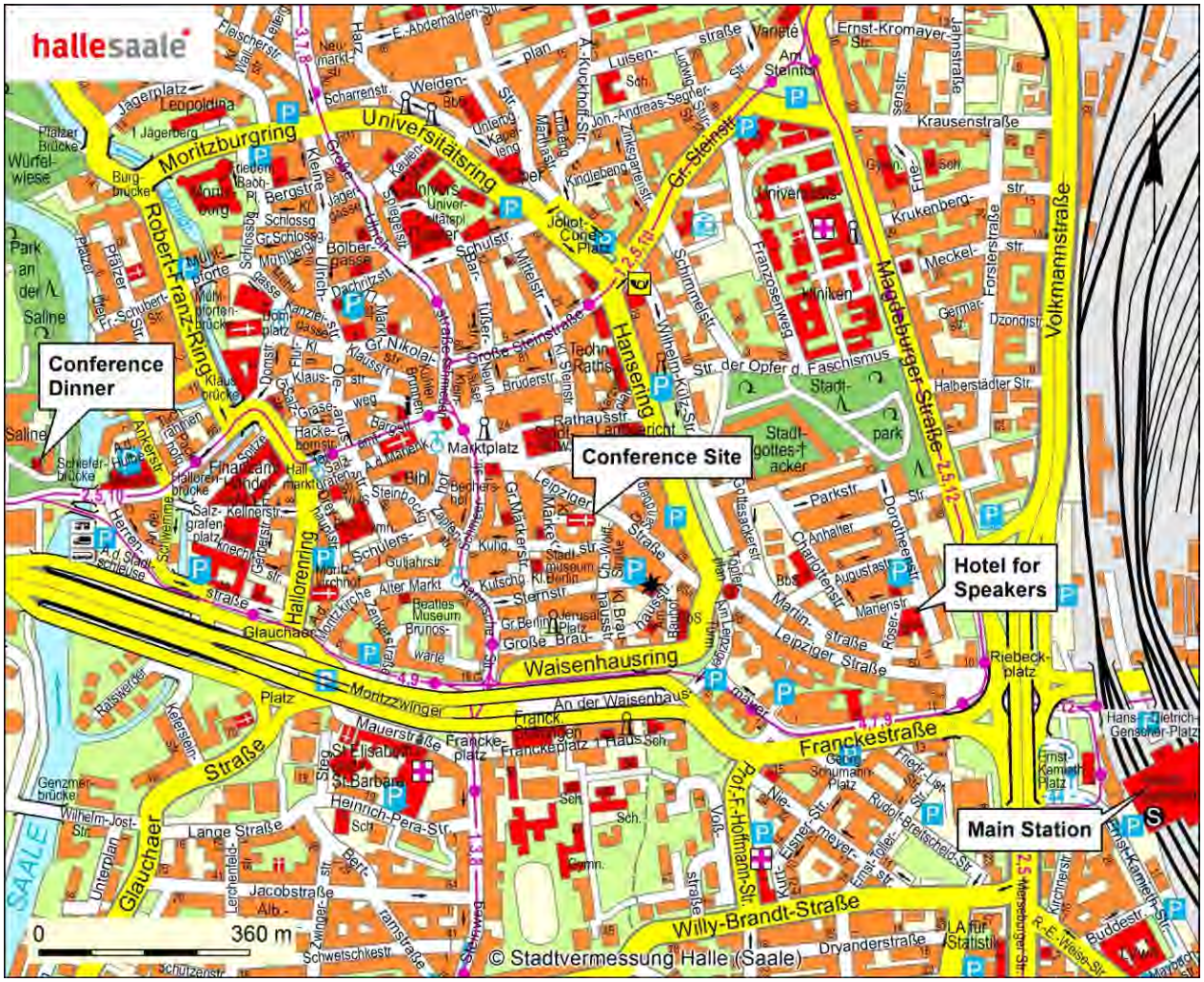

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