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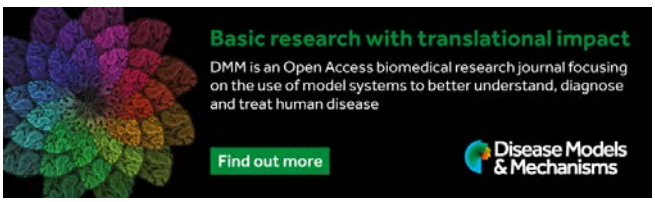

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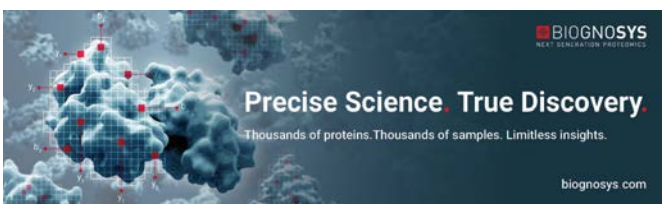

Poster presentations
G-JAM Meeting 2021
20 – 23 October

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Thursday, October 21st, 17:45-19:45 CET

Poster Session 2 (Even Poster Numbers)
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Abstract No. P01

The dynamic response of the 26S proteasome to paraquat challenge is lost with aging in Baboon cells

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Aging is associated with a progressive decline in the cellular homeostatic network including proteostasis. We previously characterized the effect of age and sex on cellular resilience using response and recovery of primary baboon fibroblasts to homeostatic challenges. We reported that donor age significantly reduced the ability of fibroblasts from male baboons to proliferate following exposure to paraquat (PQ). We hypothesized that this cellular resilience outcome is related to differences in the maintenance of proteostasis via activities of the proteosome complex in response to oxidative challenge. To test this, we used a fluorogenic substrate to measure chymotrypsin-like activities of the 26S and 20S proteasome in response to fibroblasts exposed to PQ (100 μM). We used an experimental design that involves no recovery (NR) and 24h recovery (24h R) from PQ challenge, with donor age and sex as variable factors. PQ decreased the chymotrypsin-like activities of the 26S proteasome in NR group in relation to untreated cells from young male and female donors, while 24h R restored 26S proteasome activities beyond baseline values in young males but no other group. Under basal and stress conditions, 26S proteasome activities were higher in young males than females. When compared with old males, fibroblasts from young males exhibited significantly higher activity of 26S proteasome at basal and after 24h recovery from PQ. Exposure to PQ caused no significant changes to 26S proteasome activities in old donors either in challenge or recovery. Surprisingly, we found 20S activities for all groups to be unaffected by PQ. Our results suggest that the dynamic responses of the 26S proteasome to PQ particularly in young males may facilitate their recovery from oxidative stress and resilience which is compromised by aging.

Contact

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Abstract No. P02

Role of macrophages in the loss of peripheral nerve homeostasis in ageing

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Macrophages are regarded as master regulator of tissue homeostasis, and alongside Schwann cells form the most important regulatory support system of the peripheral nervous system (PNS). The influence of ageing on the loss of tissue homeostasis and onset of age-related pathologies in tissues and organ systems is well documented. Peripheral neuropathies in old non-diseased individuals are often associated with elevated numbers of macrophages within the nerve microenvironment. Recent studies showed that monocyte-derived macrophages from the bone marrow constantly seed the sciatic nerve in steady state. However, the exact contribution of alterations in the functional characteristics of tissue-resident and/or bone marrow-derived macrophages on the peripheral nerve maintenance during ageing is not well studied.

Therefore, we performed transcriptome profiling of intact peripheral nerves of old and young mice. We detected an upregulation in pro-inflammatory gene expression, an increased expression of genes involved in Schwann cell de-differentiation and a decrease in myelination-associated genes. Additionally Cytokine profiling showed an increase in monocyte chemoattractant protein 1 (MCP1) and we observed an increased macrophage infiltration in the aged sciatic nerve. Our data shows a chronic inflammatory environment accompanied by an increased infiltration of macrophages in the intact sciatic nerves of old mice.

Furthermore, we observed a unique inflammatory signature in the RNAseq of steady state bone marrow-derived macrophages of old animals relative to their young counterparts.

Our aim is to characterise the (i) immune phenotype of bone marrow-derived macrophages to understand how their functional status might change during ageing, (ii) immune landscape of the sciatic nerve in old relative to young animals by using scRNA-seq and scATAC-seq techniques. We will also explore avenues through which nerve homeostasis can be restored by targeting macrophage cellular/molecular pathways identified.

Contact

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Abstract No. P03

ProteasomeID: quantitative mapping of proteasome interactomes for in vitro and in vivo studies

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Proximity labeling coupled to mass spectrometry enables in situ mapping of protein-protein interactions. Here, we have developed a strategy based on tagging of proteasomes with promiscuous biotin ligases and a newly generated mouse model to monitor the interactome of proteasomes in vivo. We demonstrate that the promiscuous biotin ligase BirA* can be incorporated in fully assembled proteasomes without negative impact on overall proteasome activity. Analysis of proteins labelled by tagged proteasomes retrieved more than half of the known proteasome-interacting proteins in a single mass spectrometry analysis, including assembly factors, activators and ubiquitin-cycle related proteins. We optimised the protocol for processing of proximity labelling samples to minimise contamination from streptavidin and implemented Data Independent Acquisition (DIA) mass spectrometry for label-free analysis of proximity labelling samples with high-throughput. We demonstrate the utility of our workflow by charting proteasome interactomes across mouse tissues, delineating common and tissue-specific interactors, and revealing a potential role of the proteasome in regulating endosome membrane dynamics by interaction with phospho inositol phosphatases.

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Abstract No. P04

4-Phenylbutyrate Slows Deficit Accumulation When Given to Aged Mice

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The rapid growth of the aging population and prevalence of age-associated chronic disease makes identification of treatments that can improve health in old age a critical goal of medical research. Previous studies have identified the FDA approved drug 4-Phenylbutyrate (PBA) as a promising candidate for combating many age-associated diseases.

Aims: In our research we sought to identify molecular effects of PBA both in *vitro* and in *vivo* that may contribute to these benefits as well as PBAs physiological effects in young and old mice.

Methods: To accomplish this, we used mouse cell lines from multiple tissue to evaluate changes to expression of stress response proteins, histone acetylation, and mitochondrial respiration. Additionally, we used genetically heterogenous UM-HET3 mice to evaluate effects of different doses in young healthy mice as well as aged mice, observing measurements such as food consumption, weight, body composition, frailty, and grip strength.

Results: We found that PBA alters mitochondrial respiration and appearance of mitochondrial networks in *vitro* and upregulates protein levels of mitochondrial dynamics proteins in muscle of mice in *vivo*. Remarkably, 26-month-old mice receiving PBA treatment for 3 months showed a significant slowing of deficit accumulation using a 28 measure frailty index.

Conclusions: These results are promising and suggest PBA may be beneficial to preventing or slowing age-related health declines through various molecular pathways including potential effects on mitochondria.

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Abstract No. P05

The effects of 1,3-1,6 ß-glucan in aging using Nothobranchius furzeri as animal model

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The short-lived annual fish Nothobranchius furzeri has an extremely short life span and accelerated expression of age markers. This model animal is particularly suited for investigating the effects of dietary interventions on longevity and age-related pathologies. ß-glucans are an ingredient of several animal feeds with actions on inflammation and mitochondrial functions. 1,3-1,6 ß-glucans modulate immune system by modifying phagocytic and autophagic activity. Also, 1,3-1,6 ß-glucans have antioxidant, anti-inflammatory, and antineoplastic activity. In this study, we used 1,3-1,6 ß-glucans from the cell wall of Saccharomyces cerevicae. We tested the effects of 1,3-1,6 ß-glucans on expression of age-related markers in Nothobranchius furzeri. Nothobranchius have been treated with 1,3-1,6 ß -glucans starting from 2 weeks post hatching until 27 weeks post hatching. 1,3-1,6 ß-glucans were included in a commercial feed in two different doses, a lower concentration (12,5 mg/Kg) and a higher concentration (125 mg/Kg).

1,3-1,6 ß-glucans induced a statistically significant decrease in lipofuscin accumulation in brain and liver and muscle fibrosis. Moreover, 1,3-1,6 ß-glucans increase autophagy in liver and brain. At the same time, the higher concentration of 1,3-1,6 ß-glucans induced renal toxicity visible as greater dilation of the renal tubules and an increase in the incidence of precipitations in tubules.

In conclusion, 1,3-1,6 ß-glucans seem to slow progression some age-related markers but also some toxicity. This suggests caution in promoting dietary use of ß-glucans to reduce age-related risk.

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Abstract No. P06

Regulation of Neuronal Cell Fate Determination by Endocytic Adaptor AP-2

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AP-2 is a heterotetrameric complex comprised of α, β, μ, and σ subunits that links clathrin and other endocytic proteins to sites of clathrin-mediated endocytosis. Full body knockout of AP-2(μ) in mice causes embryonic lethality before day 3.5 postcoitus (Mitsunari, T. et al., 2005). In contrast, depletion of AP-2(μ) in neurons results in postnatal neurodegeneration and defective synaptic vesicle recycling (Kononenko et al., 2014, Kononenko et al., 2017). However, it does not block plasma membrane retrieval during neuronal activity, questioning the canonical function of AP-2 in neurons and suggesting that AP-2 might perform different functions in mitotic versus postmitotic cells. Using a combination of biochemical, cell biology, and live imaging approaches, we show that AP-2 controls neuronal progenitor cells (NPCs) proliferation but is not required for neuronal differentiation. AP-2 can be found in wild-type NPCs at the centrosomes, where it colocalizes with gamma-tubulin complex protein 3 (GCP3) subunit of γ-tubulin small complex (γTuSC). Using mass spectrometry analysis, we identified GCP2, and GCP3, as novel interaction partners of AP-2 complex in neuronal cells, where the interaction between the γTuSC and AP-2 was confirmed in co-immunoprecipitation studies. Deletion of AP-2μ in NPCs leads to abnormal centrosome morphology, multinucleation, cell cycle arrest, and altered microtubule dynamics. This phenotype was not reproduced in NPCs treated with clathrin inhibitor PitStop2, suggesting that the role of AP-2 at centrosomes is independent of its function in clathrin-mediated endocytosis. Surprisingly, AP-2 was not required in NPCs committed to becoming neurons, suggesting that AP-2 is a positive regulator of symmetric cell division in neuronal cells. Despite no differences found in differentiation, AP-2 KO NPCs reveal defective migratory behavior, which results in an accumulation of doublecortin-positive cells in the lateral ventricle and causes the disorganization of cortical structure in AP-2μ KO brains. Since γTuSC comprises the part of the large γ-Tubulin organizing complex necessary for centrosome function during the cell cycle, our data suggest that AP-2 is required in neuronal mitotic cells for centrosome assembly during symmetric cell division. In contrast, AP-2 function in postmitotic neurons additionally includes the regulation of neuronal migration.

Contact

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Abstract No. P07

Aging impacts the inflammatory microenvironment guided by immunomodulatory mediators in an organ-specific manner

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Inflammaging represents chronic, low grade inflammation that accompanies aging and is a main driver of age-related diseases. The underlying sterile inflammation is characterized by elevated levels of inflammatory signaling mediators, including chemokines, cytokines and reactive oxygen species causing organ damage. Lipid mediators derived from polyunsaturated fatty acids are key regulators in the fine-tuning of both the promotion and the resolution of inflammation. Yet, it remains unclear how lipid mediators fit within the concept of inflammaging and how their biosynthesis and function is affected by aging. To date, there is a lack of studies describing inflammation as a concerted action between immunomodulatory mediators in an organ specific context. Here, we identify comprehensive signature profiles of inflammatory markers in organs of old C57BL/6 mice afflicted with inflammaging in comparison to organs of healthy adult mice. For the first time, we provide an extensive overview for multiple organs, integrating different aspects of inflammaging by combining chemokine and cytokine analysis with metabololipidomics and proteomic profiling. Thus, we reveal organ specific footprints regarding the distinct modulation of inflammation-associated cytokines, chemokines and lipid mediators by aging. In agreement with the concept of inflammaging, spleen and lung are defined by a pronounced pro-inflammatory microenvironment and impaired resolution of inflammation during aging, underlined by increased chemoattraction of immune cells and cytokine levels. Interestingly, brain displays increased levels of pro resolving mediators, while colon is characterized by an overall decrease in inflammatory signaling. Our results demonstrate that it proves difficult to establish one unifying concept for alterations of immunomodulatory mediators as consequence of aging but rather indicates the need for organ specific definitions. In this context, our data suggest that organ specificity should be translated to the understanding of homeostasis in healthy adult individuals as well. Moreover, our study implies the inclusion of lipid mediators into the concept of inflammaging as an additional tool for in depth characterization of the inflammatory microenvironment during aging. In particular combination of metabololipidomic and proteomic profiling approaches provide a broader and yet, more detailed understanding of processes involved in inflammaging offering new insights into potential organ specific therapeutical targets.

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Abstract No. P08

Using Caenorhabditis elegans to investigate amyloid diseases

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Millions of people worldwide suffer from amyloid diseases; the prevalence is predicted to increase due to factors such as rising life expectancy and pervasiveness of obesity. The therapies that are available are sparse or only ameliorate symptoms, and there is thus an urgent need to understand how and why proteins aggregate into amyloid in vitro and in vivo. Of particular importance, the factors influencing the aggregation of amyloid proteins in a natural biological setting need to be better understood, so that new therapies targeting this process can be developed.

This project investigates two amyloid-forming proteins: β2-microglobulin (β2m) and α-synuclein. Whilst β2m aggregates extracellularly and is associated with dialysis-related amyloidosis (DRA), α-synuclein aggregates intracellularly and is associated with Parkinson's disease (PD), multiple system atrophy (MSA) and dementia with Lewy bodies (DLB). Specifically, these proteins are being investigated in the context of their aggregation propensity and toxicity in Caenorhabditis elegans (C. elegans). This is an important model organism that is often used to bridge the gap between in vitro studies and investigations in higher organisms.

We have investigated the biological relevance of known amyloidogenic human β2m variants expressed in C. elegans and their secretion into the extracellular space, to test whether the human physiology and pathology of aggregation is recapitulated in the nematode model. In addition, we have used C. elegans to determine how point mutations in the N-terminal 'master controller' region of α-synuclein affect C. elegans. Ultimately, the work conducted in this project is intended to lead to a greater understanding of the molecular mechanisms underlying amyloid diseases and help identify new targets in the development of therapeutics to prevent and treat such diseases.

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Abstract No. P09

Spatial and solubility characterization of the aging brain proteome in a short-lived vertebrate

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Aging is characterized by a progressive loss of functionalities in cells and tissues. Loss of proteostasis is a hallmark of aging characterized by the formation of protein aggregates and loss of stoichiometry of macromolecular complexes, including ribosomes (Kelmer Sacramento et al. 2020). Aging has been also associated with an imbalance of cellular functions responsible for the maintenance of protein localization, such as nucleus-cytoplasmic transport (D'Angelo et al. 2009), and changes in general organelles composition (Reviewed in (Bouska et al. 2019)). This project aims to understand general changes in the composition of cellular organelles and protein complexes by applying biochemical fractionation techniques coupled with quantitative mass spectrometry (LOPIT-DC, (Geladaki et al. 2019)). This technique allows the characterization of subcellular localization for thousands of proteins in a single experiment, allowing the creation of the so-called “Organellar Map”. Parallel to this, protocols for addressing proteome solubility propensity were also implemented, creating “solubility profiles” for the proteome of young and old animals. These combined efforts allowed the generation of the first organellar map of the Nothobranchius furzeri brain proteome that enabled the identification of different protein re-localization events that occur during the aging process. Our LOPIT-DC proteome map achieved a good separation of the main cellular compartments (including organelles such as lysosomes), allowed the reliable and reproducible localization of nearly 6000 proteins, and displayed a good concordance in classification between adult (12 weeks post-hatching) and old (39 weeks post-hatching) samples. The preliminary results show major re-localization events occurring in different subcellular compartments with proteins related to lysosome, autophagy, and nuclear envelope, being some of the most prominent examples. We also observe a general decrease in solubility of the proteome, associated in particular with an increased presence of ribosomes in the most-detergent resistant fraction of the proteome. Together these results describe a first attempt to characterize the spatial complexity of the brain proteome during the process of aging.

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Abstract No. P10

Uncoupling of ribosomal proteins and their transcripts in diapause and aging in the short-lived killifish Nothobranchius furzeri

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Nothobranchius furzeri is the shortest-lived vertebrate under laboratory conditions, shows many typical phenotypes of vertebrate and human aging and represents an experimental model for aging research. This annual killifish species inhabits ephemeral habitat with seasonal dryings, which led to evolution of adaptive developmental traits to enable embryo survival during the dry season. One important feature is the possibility to arrest embryonic developmental arrest, called diapause, which delays development and hatching to match the seasonal inundations. Diapause is coupled to metabolic rate reduction and depression of protein synthesis and it can occur in three developmental stages: during gastrulation (I), at mid-somitogenesis (II) and before hatching (III). Diapause II embryos have muscles, heart, primordial germ cells, a brain containing stem and differentiated cells, and neuromuscular junctions. Diapause is a facultative state and embryos can proceed with direct development. Even if the development is arrested, recent studies showed that Diapause II is a dynamic state with active transcription of some genes such as transcripts coding for ribosomal proteins. This finding is puzzling, given the great depression of protein synthesis and it is unclear whether higher transcript levels also result in higher protein expression. Interestingly, there is an overlap between the transcript regulation observed in diapause and aging (Reichwald et al., 2015), indicating that molecular adaptations that enable diapause also influence aging.

With this study, we aim to determine differences in protein regulation of embryos in diapause and during direct development by use of peptide mass-spectrometry based proteomics. In particular, we optimized conditions to obtain the proteome of single embryos and compared diapause II (DII) embryos with direct developing (DD) embryos. Differential expression analysis between two 7 dpf groups showed, several protein biosynthesis components were downregulated during diapause II. All detected ribosomal subunits were downregulated despite up-regulation of their transcripts. The same uncoupling in the regulation of ribosomal proteins and their transcripts was previously reported in the aging brain (Kelmer et al., 2020), which indicates further link between the molecular mechanisms activated during aging and diapause.

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Abstract No. P11

Protein CoAlation and the antioxidant function of coenzyme A: a renaissance of a key metabolic cofactor

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Coenzyme A (CoA) was discovered by Fritz Lipmann in the middle of last century and this discovery earned him a Nobel prize which he co-shared with Hans Krebs who deciphered the citric acid cycle at the same time. CoA and its thioester derivatives (acetyl-CoA, malonyl-CoA, HMG-CoA etc.) participate in diverse anabolic and catabolic pathways, allosteric regulatory interactions and the regulation of gene expression. Dysregulation of CoA/CoA derivatives biosynthesis and homeostasis has been associated with various human pathologies, including cancer and neurodegeneration and metabolic disorders.

We have recently uncovered a novel unconventional function of CoA in redox regulation, involving covalent protein modification by this cofactor and termed it CoAlation. Cell-based and animal models were employed to demonstrate that protein CoAlation is a reversible and widespread post-translational modification induced by oxidizing agents and metabolic stress in prokaryotic and eukaryotic cells. We developed a robust mass spectrometry-based methodology for the identification of CoA-modified proteins in cells and tissues which allowed us to identify over 2100 CoAlated proteins. Protein CoAlation alters the molecular mass, charge, and activity of modified proteins, and protects them from irreversible sulfhydryl overoxidation. We have employed biochemical, biophysical, crystallographic and cellular approaches to study the mode of CoA binding to a panel of selected metabolic enzymes and signalling proteins. Based on these findings, we propose that under physiological conditions CoA functions to produce metabolically active thioester derivatives but has a potential to act as an antioxidant in cellular response to oxidative or metabolic stress. The progress on molecular dissection of the CoAlation/deCoAlation cycle and the antioxidant function of CoA in health and disease will be discussed.

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Abstract No. P12

Exporting protein aggregates from cells to study aging and disease

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Aging is still one of the biggest mysteries of science. What causes aging is still not understood. A time-dependent accumulation of cellular damages like DNA damage, protein aggregates, defective mitochondria etc. is one of the main hypotheses for what causes aging. To identify if protein aggregates are a causal factor in the aging process we engineered a system to export protein aggregates from cells. We applied the budding yeast Saccharomyces cerevisiae as a model organism. It can be easily manipulated genetically, exhibits an aging phenotype, which shares many properties with more complex organisms and is used as a model for aging of adult stem cells or post-mitotic cells. Yeast cells divide asymmetrically and aging factors are actively retained in the mother cell, so that the daughter cell is born rejuvenated with a full replicative lifespan potential. The engineered aggregate transport system (ATS) transports protein aggregates from the mother cell into the daughter cell. Endogenous protein aggregates are recognized and exported by the ATS as well as model aggregates like mutant Huntingtin -the disease causing agent in Huntington's disease. The ATS consists of Hsp104 fused to a daughter-targeting factor, and was integrated into the genome. Hsp104 is a disaggregase and forms inclusions in the presence of protein aggregates. Surprisingly, the ATS always formed highly concentrated inclusions and sequestered even the endogenous Hsp104 entirely into the ATS, suggesting that a seed is necessary for Hsp104 inclusions to form. This seed is provided by the daughter-targeting factor, which organizes into concentrate patterns. We identified that Hsp104 oligomerization, presence of nucleotide binding domains in Hsp104 and Hsp70s are essential for ATS formation. Interestingly, we discovered that Huntingtin aggregates can not only be targeted artificially to the daughter cell, but also to other structures in the cell, like the endosome, eisosomes or metabolic bodies, provided that a seed is pre-formed. Finally, we performed a genome-wide screen using synthetic genetic array (SGA) technology to search for other proteins than Hsp104 that can organize inclusions. We identified 72 proteins to have such an ability, among them the thioredoxin peroxidase Tsa1 and the small heat shock protein Hsp42.

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Abstract No. P13

GMPPA defects result in a progressive neuromuscular disorder with α-Dystroglycan hyperglycosylation

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Glycosylation is the most common post-translational modification of proteins and lipids. The glycosylation status can affect protein stability and conformation. It plays a prominent role in cell-to-cell communication, cell matrix interaction, adhesion, protein targeting and folding, viral or bacterial infection, progression of cancer and aging. Abnormal glycosylation of proteins can induce deleterious effects as observed in congenital disorders of glycosylation (CDGs). CDGs often manifest as myopathies because of hypoglycosylation of the sarcolemma-associated protein α-Dystroglycan (α-DG). Typical examples are GDP-mannose-pyrophosphorylase-B (GMPPB) defects which cause different muscle disorders. GMPPB generates the sugar donor GDP-mannose for glycosylation. Although its homolog GMPPA is catalytically inactive, GMPPA defects cause AAMR-syndrome, which is characterized by achalasia, alacrima, mental retardation and progressive muscle weakness.

To elucidate the function of GMPPA we generated a GMPPA knockout (KO) mouse model. Importantly, these mice recapitulate many features of human AAMR syndrome. Homozygous GMPPA KO mice show a progressive gait disorder with muscle weakness. Furthermore, KO mice show cognitive impairments, progressive neurodegeneration and structural brain alterations, such as cortical layering defects. With co-immunoprecipitation and enzyme activity assays, we identified GMPPA as an allosteric feedback inhibitor of GMPPB. Depletion of GMPPA thus results in increased GDP-mannose levels and increases incorporation of mannose into glycochains of various proteins, including α-DG. GMPPA knockdown in myoblasts recapitulates these findings and reveals that the hyperglycosylation accelerates α-dystroglycan turnover and thereby reduces its overall abundance, which is likely a central event in the pathophysiology of the myopathy. Importantly, dietary mannose depletion corrected α-DG glycosylation, prevented neurodegeneration and normalized motor functions as well as skeletal muscle morphology in mice. We thus identified GMPPA defects as the first CDG characterized by α-DG hyperglycosylation, unveiled the underlying disease mechanisms and identified potential dietary treatment options.

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Abstract No. P14

Ageing clocks, measuring biological time using lipidomics

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Complexity is a fundamental feature of biological systems. Omics- techniques, such as transcriptomics, metabolomics, proteomics and lipidomics can now routinely track many thousands of molecules in parallel. Longitudinal data tracks changes across such species and captures complex biological dynamics. However, this approach also transfers the original biological complexity to the resulting datasets, posing new challenges in data integration and analysis. Ageing is an example of a biological process that exhibits complex dynamics across multiple molecular species and scales of organisation. Ageing is characterised by slow, cumulative, and detrimental change, driven by intrinsic biological stochasticity. This dynamic is mediated by complex interactions and feedback within and between different levels of biological organization (ranging from macromolecules, organelles and cells to tissue and organs). Ageing dynamics, in contrast to physiology and extrinsic stochasticity, are characterized by a slow change yet a defining property of biological ageing is an exponential (rapid) increase in morbidity and mortality with time. These features distinguish ageing from the aetiologies of specific diseases and may provide key insights into the underlying biology. I will give an overview on some of our work on ageing clocks based on longitudinal datasets and their utility as biomarkers for drug discovery.

Contact

Jan Gruber

Abstract No. P15

tba

Sanjib Guah

Background: A defining pathological feature of the progressive neurodegenerative disorder Alzheimer's Disease (AD) is the accumulation of misfolded, hyperphosphorylated tau (Phoshpho-tau) into intraneuronal neurofibrillary tangles (NFT), and specific phospho-epitopes such as Thr(T231) are among the earliest hallmarks of AD. Mitochondrial dysfunction is an early feature of AD, and abnormal, toxic tau PTMs contribute to disease pathogenesis. A major bottleneck in understanding the mechanisms behind the neurotoxicity of pathological forms of Tau is the lack of genetically tractable models that can recapitulate the effects of Tau PTMs in a short time frame.

Method: Human 0N4R Tau (wild type) was expressed in neurons through single-copy gene insertion. Mutation was introduced through CRISPR-Cas9 genome editing, including T231E (Thr→Glutamic acid), to mimic phosphorylation of a commonly observed pathological epitope.

Aims: 1. To determine the impact of AD relevant tau PTM on mitochondrial stress responses and how this influence healthy aging of neurons using a novel, single copy human tau transgenic C. elegans strain. 2. To test whether tau with AD relevant PTM alters mitophagy and whether changes in mitophagy contribute to phenotypic severity with age. 3. To address whether enhancing Mitochondrial Quality Control (MQC) is a viable therapeutic avenue.

Results: C. elegans single-copy expression of wild type human tau did not elicit overt pathological phenotypes at baseline. However, strain expressing disease associated PTM-mimetic (T231E) exhibited reduced touch sensation as a parameter of neuronal function and neuronal morphological abnormalities that increased with age. Remarkably, the PTM-mimetic selectively impaired mitophagy following mitochondrial oxidative stress, but had no effect on autophagy, and furthermore reduced mitolysosomal trafficking.

Conclusion: Limiting the expression of tau results in a genetic model where modifications that mimic pathologic tauopathy-associated PTMs contribute to cryptic, stress-inducible phenotypes that evolve with age. Our studies highlight a selective mechanism through which disease-associated Tau PTM may suppress compensatory responses to mitochondrial stress that occur with age and provide a new perspective into the pathogenic mechanisms underlying AD. Overall, our findings provide important new insights that impaired clearance of defective mitochondria is a key event in AD pathogenesis and that stimulated mitophagy represents a potential therapeutic intervention.

Abstract No. P16

Modifiers of TDP-43 Toxicity

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Protein toxicity is thought to underlie several, yet incurable, age-related neurodegenerative diseases, including Parkinson's disease and Amyotrophic Lateral Sclerosis (ALS). TDP-43 aggregation is the major pathological hallmark of ALS and present in 97% of all cases, suggesting that TDP-43 contributes to the disease mechanism. How protein toxicity triggers cell-and physical dysfunction and leads to degeneration is still not understood.

This project aims to find disease mechanisms and uncover targets to suppress ALS-related TDP-43 toxicity. For this aim, a combination of genetic- and phenotypic screens in a Caenorhabditis elegans model for disease are being used. We make use of a C.elegans strain with overexpressed human TDP-43, which shows several cellular- and behavioral ALS disease phenotypes, including age-related motor impairment is used as a model. We performed a genetic screen, which identified 22 mutant animals that show a suppression of this impairment. The strongest suppressor mutant, called MOTT-22 (Modifier of TDP-43 Toxicity 22), was selected for further experiments. We are currently verifying and characterizing a candidate gene that may be responsible for the suppression of motor impairment in MOTT-22. After finding a candidate gene for MOTT-22, gene functions in the cell will be studied to find new mechanisms involved in protein toxicity.

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Abstract No. P17

Composition of lysosomes in brain cells

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Lysosomes are subcellular compartments that degrade macromolecules and damaged organelles, and integrate cell signaling in response to changes in nutrient availability. Despite the essential roles of the lysosome across tissues, little is known about their proteomic composition (1). Here, we focus on the cell-type specific analysis of the brain to further understand the role of the lysosome in the central nervous system.

Lysosomes constitute 1-3% of the total cell volume and only 0.2% of total cell protein mass (2). Thus, measuring changes in lysosomal composition at the tissue-level is challenging. To address these challenges, we used an innovative approach to purify intact lysosomes from cells (3). Tagging the lysosome with a lysosomally-localized Tmem192-3xHA fusion protein, called LysoTag, allows the rapid immunopurification of lysosomes from cells to analyze the protein content using state-of-the-art liquid chromatography and mass spectrometry. We introduced LysoTag in mice and took advantage of existing Cre lines to selectively purify lysosomes from neurons, astrocytes, oligodendrocytes and microglia.

Proteomic characterization of isolated lysosomes revealed core lysosomal proteins that are shared across the four different cell types of the brain, but also identified novel cell-type specific lysosomal proteins, indicating unique functions of lysosomes in different cell types. Our work demonstrates that it is possible to quantitatively monitor the composition of lysosomes in vivo and reveals previously unappreciated lysosomal localization of disease-relevant proteins in specific cell types of the brain.

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Abstract No. P18

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The aging research community is making great progress towards understanding the myriad biological processes that contribute individually and inter-dependently to the aging of cells, organs, and organisms. Based upon these efforts there is signficantly increasing activity within the clinical research community, and in biotech and pharma, to translate discoveries related to the fundamental biology of aging into therapies that could benefit mankind by addressing current unmet medical need and increasing healthspan.

I would like to introduce you to Rejuveron Life Sciences AG, an integrated biotechnology platform company that develops and improves therapies to promote healthy aging and prolong lifespan for everyone. We support healthy aging pioneers to transform their discoveries into medicines, by offering a comprehensive package including funding, start-up help, and guidance from our experienced drug discovery and development team, together with a specialist scientific advisory board. In addition, we can offer a fully equipped incubator laboratory space in the Bio-Technopark Schlieren-Zurich if required. I will provide a brief overview of the scientific approaches we are taking in two of our daughter companies to (i) improve the brain vasculature and to (ii) remove senescent cells during cancer therapy.

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Abstract No. P19

Nucleolar TFIIE plays a role in ribosomal biogenesis and performance

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Ribosome biogenesis is a highly energy-demanding process in eukaryotes which requires the concerted action of all three RNA polymerases. In RNA polymerase II transcription, the general transcription factor TFIIH is recruited by TFIIE to the initiation site of protein-coding genes. Distinct mutations in TFIIH or TFIIE lead to the rare autosomal recessive disorder trichothiodystrophy (TTD) characterized by a variety of symptoms including brittle hair, ichthyosis, and premature aging symptoms. While TFIIH is known to play an additional role in the production of ribosomal RNA (rRNA) by RNA polymerase I, an involvement of TFIIE in RNA polymerase I transcription has not been described yet. Here we uncovered an unexpected role of TFIIE in rRNA synthesis by RNA polymerase I. With high resolution microscopy we detected TFIIE in the nucleolus, at the site of active rRNA synthesis. With specific inhibition of RNA polymerase I transcription, TFIIE de-localizes from the nucleolus, implying RNA polymerase I transcription-dependent localization of TFIIE to the nucleolus. Mutations in TFIIE affects gene-occupancy of RNA polymerase I, rRNA maturation, ribosomal assembly and performance. In consequence, the elevated translational error rate at the ribosome with imbalanced protein synthesis and turnover results in an increase in heat-sensitive proteins. The results of our study unravel a novel role of TFIIE in RNA polymerase I transcription and identify loss of proteostasis as a possible pathomechanism in TTD.

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Abstract No. P21

Increased fidelity of protein synthesis extends lifespan

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presenter: Ivana Bjedov
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Loss of proteostasis is a fundamental process driving aging. Proteostasis is affected by the accuracy of translation, yet the physiological consequence of having fewer protein synthesis errors during multi-cellular organismal aging is poorly understood.

AIM: We aim to understand biological significance of reducing errors in protein synthesis with special emphasis to ageing.

METHODS: Lifespan, heat shock resistance, translation measurements, translation accuracy measurements by dual luciferase reporters, phylogenetic analyses, developmental delay measurements

RESULTS: Our phylogenetic analysis of RPS23, a key protein in the ribosomal decoding center, uncovered a lysine residue almost universally conserved across all domains of life, which is replaced by an arginine in a small number of hyperthermophilic archaea. When introduced into eukaryotic RPS23 homologs, this mutation leads to accurate translation, as well as heat shock resistance and longer life, in yeast, worms, and flies. Furthermore, we show that anti-aging drugs such as rapamycin, Torin1, and trametinib reduce translation errors, and that rapamycin extends further organismal longevity in RPS23 hyperaccuracy mutants. This implies a unified mode of action for diverse pharmacological anti-aging therapies. These findings pave the way for identifying novel translation accuracy interventions to improve aging.

CONCLUSIONS: Our findings demonstrate for the first time in a metazoan organisms that improving translation accuracy by a single point mutation in a ribosome decoding centre promotes health and longevity in yeast, worms and flies.

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Abstract No. P22

Inactivation of histone chaperone HIRA unmasks a link between normal embryonic development of melanoblasts and maintenance of adult melanocyte stem cells.

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Histone chaperone Hira, which deposits histone variant H3.3 into chromatin in a DNA replication-independent manner, has been implicated in epigenetic memory and is thought to play a role in both early development and aging. However, there are only sparse data on connections between integrity of embryonic development and healthy aging. The pigmentary system, consisting of differentiated melanocytes and melanocyte stem cells (McSCs) of the adult hair follicle and their precursor melanoblasts in embryos, has been valuable in understanding mechanisms of development, aging and disease. Here, we describe a conditional knockout mouse model, Tyr::Cre Hira$^{fl/fl}$, in which McSCs, melanocytes and their embryonic melanoblast precursors are specifically deficient for Hira and chromatin deposition of histone H3.3. We find that Hira is required for establishment of normal embryonic melanoblast numbers in vivo, supported by single cell RNA sequencing data, and melanoblast identity in vitro. Despite this, by birth, Tyr::Cre Hira$^{fl/fl}$ mice contain a comparable number of melanocytes as wild type mice, and young adults have normal functioning McSCs and only very mildly hypopigmented hair coat. However, neonate melanoblasts from Tyr::Cre Hira$^{fl/fl}$ mice are sensitive to stress both in vitro and in vivo and exhibit more telomere-associated DNA damage foci, a marker of premature aging, than do those from wild type mice. In line with this, knock out of Hira during embryogenesis in Tyr::Cre Hira$^{fl/fl}$ mice caused a premature defect in adult McSC maintenance and premature hair greying, while inducible knock out of Hira in young adult Tyr::Cre-ERT2 Hira$^{fl/fl}$ mice resulted in no observable defect. These studies of the Hira histone chaperone show that perturbations of in utero embryogenesis can cause only modest phenotypic variations at birth and in young adulthood, but profound abnormalities and features of unhealthy aging in later life.

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Abstract No. P23

Transcriptional repression of NFKBIA triggers constitutive IKK- and proteasome-independent p65/RelA activation in senescence

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The IκB kinase (IKK)-NF-κB pathway is activated as part of the DNA damage response and controls both inflammation and resistance to apoptosis. How these distinct functions are achieved remained unknown. We demonstrate here that DNA double-strand breaks elicit two subsequent phases of NF-κB activation in vivo and in vitro, which are mechanistically and functionally distinct. RNA-sequencing reveals that the first-phase controls anti-apoptotic gene expression, while the second drives expression of senescence-associated secretory phenotype (SASP) genes. The rapidly activated first phase is driven by the ATM-PARP1-TRAF6-IKK cascade, which triggers proteasomal destruction of inhibitory IκBα, and is terminated through IκBα re-expression from the NFKBIA gene. The second phase, which is activated days later in senescent cells, is on the other hand independent of IKK and the proteasome. An altered phosphorylation status of NF-κB family member p65/RelA, in part mediated by GSK3β, results in transcriptional silencing of NFKBIA and IKK-independent, constitutive activation of NF-κB in senescence. Collectively, our study reveals a novel physiological mechanism of NF-κB activation with important implications for genotoxic cancer treatment.

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Abstract No. P24

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Hematopoietic stem cells (HSCs) produce all blood cells, including cells of the innate and adaptive immune system in addition, they have the ability to self-renew throughout life. However, during aging, HSCs lose their self-renewal capacity and become more biased towards the myeloid lineage.

Several transcription factors have been shown to regulate lineage potential and self-renewal capacity. The proto-oncogene Myc is a transcription factor maintaining HSC homeostasis by controlling the proliferation, differentiation and migration, here Myc mainly serves as a transcriptional activator. On the other hand, binding of Myc to the `Myc-interacting zinc-finger protein 1´ (Miz-1) leads to the repression of several target genes involved in proliferation like the negative cell cycle regulators p21 or p15 and thereby inducing cell proliferation. However, in a Myc-independent way Miz-1 is essential for lymphocyte development by regulating cytokine signaling and apoptosis in lymphoid progenitor cells. Little is known about Miz-1 function in HSCs. We found that in young Miz-1-deficient mice, HSCs and progenitor cells accumulation in the bone marrow. This is accompanied by an increase in cell cycle activity and a loss of quiescence for most long term HSCs. In addition, we observed an accumulation of myeloid progenitors to the expense of cells of the lymphoid lineage in the bone marrow and peripheral organs. Similar effects have been described in aged mice. To obtain further insights in the function of Miz-1 in aging of the hematopoietic system we analyzed young (3 month) and old (24 month) wt and Miz-1-deficent mice. We performed RNA-seq experiments on cell-sorted HSCs and progenitors from young and aged cells. Here, we identified altered transcriptional profiles that underpin these changes. Among these factors are several genes of the interferon signaling pathway, which were upregulated in Miz-1-deficient mice. As expected, young Miz1-deficent mice somehow resembled aged wt mice, whereas very little changes were detectable in 24-month-old wt and Miz-1-deficient mice. Taken together, these results point to a critical role of the Miz-1 transcription factor in maintaining HSC function and regulating age-related processes of the hematopoietic system.

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Abstract No. P25

scAgeCom: a murine atlas of age-related changes in intercellular communication

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Dysregulation of intercellular communication is a well-established hallmark of aging. To better understand how this process contributes to the aging phenotype, we built scAgeCom, a large-scale atlas presenting how cell-type interactions vary with age in 23 mouse tissues. We first created an R package, scDiffCom, designed to perform differential intercellular communication analysis between two conditions of interest in any mouse or human single-cell RNA-seq dataset. The package relies on its own list of curated ligand-receptor interactions compiled from established studies. We applied this tool to single-cell transcriptomics data from the Tabula Muris Senis consortium and the Calico murine aging cell atlas. Our atlas can be accessed online from an interactive web application (https://scagecom.org). It provides both tissue-specific and cross-tissue results that can be explored from different angles and levels of detail (gene-centric, cell-type-centric, ontology-centric, etc). The most widespread age-related changes we observed include upregulation of immune system processes, inflammation and lipid metabolism, as well as downregulation of extracellular matrix organization, growth, development and angiogenesis. Due to its generality and to the large number of tissues considered, scAgeCom contains a large amount of generated data, waiting to be interpreted, which might provide the community with new therapeutic targets and new hypotheses regarding the relationship between aging and intercellular communication.

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Abstract No. P26

Neutrophils induce paracrine telomere dysfunction and senescence in ROS-dependent manner

Anthony Lagnado

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Aims: Senescence, the state of irreversible arrest observed in somatic cells is characterised by a Senescent Associated Secretory Phenotype (SASP) which includes pro-inflammatory cytokines, chemokines and extracellular matrix proteases. The SASP is believed to play a role in the recruitment and activation of immune cells, including macrophages, CD4 T and NK cells which have been shown to play a role in clearance of senescent cells. However, the underlying mechanisms driving senescence in the liver are not completely understood. Here, we investigated the hypothesis that neutrophils recruited from the circulation in response to damage can act as drivers of cellular senescence and as such contribute to organ dysfunction during aging and disease.

Methods: Culture of human fibroblasts, co-culture with neutrophils, analysis of telomere length by FISH, DNA damage response assays (Immunocytochemistry, COMET), Immuno-FISH. Wild-type mice were injected with CCl4. Neutrophils infiltration was blocked by Ly6G neutralising antibody delivered using a mini-pump. INK-ATTAC transgenic mice. RNA in situ hybridization, human and murine precision-cut liver slices, RNA sequencing, Mass cytometry by time of flight (CyTOF).

Results and Conclusions: Here, we show that telomeres in non-immune cells are highly susceptible to oxidative damage caused by neighboring neutrophils. Neutrophils cause telomere dysfunction both in vitro and ex vivo in a ROS-dependent manner. In a mouse model of acute liver injury, depletion of neutrophils reduces telomere dysfunction and senescence. Finally, we show that senescent cells mediate the recruitment of neutrophils to the aged liver and propose that this may be a mechanism by which senescence spreads to surrounding cells. Our results suggest that interventions that counteract neutrophil-induced senescence may be beneficial during aging and age-related disease.

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Abstract No. P27

An accurate transcriptome-based senescence classifier

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Cellular senescence is a state of irreversible growth arrest associated to morphological, metabolic and functional changes. Senescence programs are highly heterogeneous as influenced by induction stimuli, cell lineages and organismal developmental stage. Diversity of various senescence programs makes the accurate detection of senescent cells a difficult task, despite recently a handful of 'core' senescence-associated genes has been suggested. Building up on this strategy, we aim to use machine learning models to develop better and more universal classifiers of senescence. Here, we describe the preliminary development of such senescence classifier. We generated numerous whole-transcriptome datasets of different subtypes of senescent cells. These datasets were then used to develop a senescence classifier based on a set of machine learning models, including RF (random forest), SVM (support vector machine), NB (naive bayes), LDA (linear discriminant analysis), LR (logistic regression), kNN (k nearest neighbors), and an ensemble learner. Among these models, the LR gave the best performance (with an average accuracy of 0.94) and was selected for later analysis. 97 genes were selected by the LR to predict the senescence status. Apart from the cross validation, we also used a publicly available dataset of senescent dermal fibroblast (GSE78128), only one out of six samples was misclassified by our method.

In conclusion, based on the transcriptional profiles of different senescence-associated phenotypes, we have the preliminary generation of an accurate senescence classifier based on machine learning methods.

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Abstract No. P28

The specification of quiescent neural stem cells in the vertebrate zebrafish brain

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In the adult vertebrate brain, neural stem cells (NSCs) constitute astroglial cells that generate a limited number of neuronal and glial cell types. They are crucial for brain homeostasis and repair. Essential parameters of NSC maintenance include stemness (capacity to self-renew) and quiescence (undergo cell division rarely). Previously, it was found that the adult NSCs emerge during embryonic neurogenesis, where a slowly dividing subpopulation of embryonic NSCs was identified that can later give rise to young adult NSCs. These progenitor cells that later become adult NSCs slow down the cell cycle, and are set aside as a reserve pool early in brain development. However, it is not fully understood how and why this subset of cells is selected as adult NSCs.

In order to solve this problem, we decided to use zebrafish as model organism. We will propose to study how embryonic neural stem cells in the developing zebrafish telencephalon are selected to enter quiescence and remain as the adult NSCs. First, we will determine whether embryonic NSCs in the telencephalon uniformly slow their cell cycle or whether some progenitor cells begin to divide more slowly than others during embryogenesis. We apply a H2B-label retention analysis method combined with the TetOn induction system as used previously in zebrafish. The main idea is that Histone-labeled retention assay can be efficiently used to study stem cell divisions through time-lapse imaging which used in chased experiments. Embryonic NSCs maybe retain high levels of H2b label that gets diluted through each round of cell division, thus showing their slow proliferation/few cell cycles.

According to the model we built up in here, it can clearly to describe the subset of quiescent/slow-cycling NSCs. Because of quiescence means gradually exit the cell cycle, it could have relevance or similar (reverse) mechanisms with ageing. We intend to start with single-cell transcriptome analysis to find the candidate genes which involved in regulation, this will promote clarify the cell biological properties of selected quiescent NSCs. The project will hopefully answer the questions which from this perspective that involve in some developmental mechanism on aging.

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Abstract No. P29

Long intergenic non-coding RNA LINC01021 promotes survival of senescent cells and plays a regulatory role in SASP expression

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Introduction: Cellular senescence is a state of irreversible cell cycle arrest that can occur as a consequence of telomeric shortening or excessive genotoxic stresses. Cells that enter this state function abnormally and contribute to a deleterious microenvironment through secretion of pro-inflammatory and tissue remodeling factors. The senescence-associated “program” is characterized by a heterogeneous transcriptional signature of differentially expressed protein coding genes that can be used to identify senescent cells or exploited for therapeutic interventions. In contrast to protein-coding RNAs, the senescence-associated non-coding transcriptome remains poorly understood. LINC01021 is a p53-responsive long intergenic non-coding RNA (lincRNA) that is positively associated with senescence, however its function in senescence remains unknown.

Aims: Determine the functional role of long non-coding RNA LINC01021 in 1) survival of stress-induced senescent cells and 2) regulation of secreted inflammatory factors known as senescence-associated secretory phenotype (SASP).

Methods: To study the potential survival role of LINC01021 in senescent cells, siRNA transfection is used in cell culture to knockdown LINC01021 and survival measured by MTS and CellTox Green cytotoxicity assays. SASP mRNA expression is determined by RT-qPCR and secreted SASP proteins assayed by ELISA. NF-kB signaling is assayed using a luciferase NF-kB reporter BJ cell line.

Results: Using RT-qPCR we find a non-coding RNA called LINC01021 to be significantly upregulated in DNA damage-induced senescent cells compared to quiescent and proliferating cells. Using in vitro cell models of cellular senescence, we show that elevated LINC01021 expression is maintained over time and exerts a pro-survival function in senescent IMR-90 cells. In accordance, siRNA-mediated knockdown of LINC01021 can result in cell death of some senescent cell types, possibly through a calpain and/or cathepsin mediated mechanism. Interestingly, while some cell types appear resistant to the senolytic effect of LINC01021 knockdown, a reduction in NF-kB signaling and reduced expression of some pro-inflammatory secretory proteins is observed.

Conclusions: Combined, the data indicate LINC01021 may serve as a lincRNA target to ablate senescent cells and reduce the senescence burden associated with cancer treatment and aging.

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Abstract No. P30

Landscape of protein post-translational modifications in the aging brain: dysregulation, cross-talk and site competition

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Cell proteostasis can be defined as the dynamic process that ensures correct folding and assembly of newly synthesized polypeptide chains, and the rapid degradation of damaged, mislocalized, or unfolded proteins. The main pathways involved in the maintenance of proteostasis are the ubiquitin-proteasome system, lysosome/autophagy, and several chaperons collectively defined as the quality control system. All of these processes are regulated by protein posttranslational modifications (PTMs). Therefore, perturbation of PTMs dynamics can be an indication of proteostasis maintenance decline. How the whole PTMs landscape changes during aging and how this can contribute to the increased vulnerability of aged cells to disease is still not completely elucidated. To investigate age-related changes of PTMs, we initially focused on the mice brain ubiquitinylome, since accumulation of ubiquitin positive protein aggregates is an established hallmark of aging and neurodegeneration. Using a K-GG remnant motif enrichment coupled with mass spectrometry, we were able to quantify more than 3000 modified ubiquitination sites in young and old brains. By normalizing the changes in ubiquitin modified peptides levels with the changes in the whole proteome abundance, we identified several proteins that are targeted by altered ubiquitination in aging without being affected in their total protein level. Moreover, in a previous study, we identified carboxymethyl-lysine (CML), an advanced glycation end-product, to occur on ubiquitin and other components of the UPS. We found in our data that CML modifies several lysines on ubiquitin, including the residues K6, K27, K33, and K63 that are generally used for the assembly of poly-ubiquitin chains. This suggests a potential interference of non-enzymatic PTMs on ubiquitin chain assembly by direct competition for the same lysine residues. In the future, we plan to expand our analysis to other PTMs including phosphorylation and acetylation, in order to gain further insights into how different PTMs interact and compete with each other in the context of aging.

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Abstract No. P31

ATR maintains mitochondrial integrity and functionality

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DNA damage response (DDR), including DNA repair, cell cycle checkpoints, apoptosis, and gene transcription, is essential for the maintenance of genomic stability. The protein kinase ATR is a key DDR molecule found to be mutated in the human chromosomal instability syndrome ATR-Seckel. ATR is primarily activated by DNA single-strand breaks and stalled replication forks. Although ATR is well studied for its DDR function, yet there are observations, including our recent findings in neuropathies, hinting its involvement in other cellular functions.

We found that ATR locates at mitochondria to maintain their health and if deleted, mitochondrial structures and functions are disrupted. Biochemical analyses reveal that ATR deletion impairs electron transfer chain (ETC) functionality leading to a defective oxygen consumption and overproduction of reactive oxygen species (ROS) in mitochondria, which in turn elicits nuclear DDR. Interestingly, our co-IP studies detect a direct interaction of ATR with the mitochondrial protein PINK1 necessary to initiate mitophagy. When ATR is deleted, both PINK1 and Parkin protein levels are reduced rendering cells refractory to mitophagy and thus, leading to an accumulation of abnormal mitochondria and defective mitochondrial networks in murine fibroblast, human cancer and importantly in ATR-Seckel cells. Mitochondrial metabolic dysfunctions are particularly pronounced in ATR knockout mouse neural cells and brain tissues, connecting the metabolic function of ATR with neuronal homeostasis. Our study uncovers an unexpected function of ATR, beyond its classical role in DDR, in mitochondrial metabolism, which protects cells from metabolic malfunctions and oxidative damage.

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Abstract No. P32

Differential vulnerabilities of primary and secondary senescent cells to first-generation senolytic compounds in the diseased kidney

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Acute kidney injury (AKI) and chronic kidney disease (CKD) are rising health problems contributing to an increased morbidity, mortality and global economic burden (Hoste et al. 2018). It has been shown that the preclinical senolytic treatment in particular with the drug cocktail dasatinib + quercetin (D+Q) is therapeutic post damage in both AKI and CKD (Wang, 2021). However, the mechanism of action and/or cell specificities *in vivo* within the diseased kidney are still lacking (Serrano and Barzilai 2018; van Deursen 2019). Here, we compare the two best described first-generation senolytic drugs, namely D+Q and navitoclax, in fibrotic acute and chronic renal disease. The analysis of the acute kidney injury model (one intraperitoneal injection of folic acid, 250mg/kg) indicates opposing therapeutic effects of navitoclax versus DQ. Both senolytic treatments showed a reduction in senescent epithelial and interstitial cells based on quantitative assessment of the senescence marker senescence-associated beta-galactosidase (SA-ß-GAL) as well as p21. However, navitoclax did not change the disease outcome whereas D+Q remarkably improved renal morphology, fibrosis and function upon AKI induction. *In vitro,* we observe that navitoclax but not D+Q robustly kills different mouse and human renal cell types that have been damaged directly by different senescence inducing compounds, here referred to as “primary senescence”. In contrast, we saw that D+Q but not navitoclax kills cells that have been treated with conditioned medium of primary senescent epithelial cells, here referred to as “secondary senescence”. We are considering a different vulnerability of primary and secondary senescent cells to the different senolytic compounds that in turn impacts on renal disease outcomes. These findings indicate that primary and secondary senescent cells play different roles within fibrotic diseases and that selectively targeting secondary senescent cells might be superior to targeting primary senescent cells in some scenarios. These findings open the possibility of novel drug screening approaches differentiating between primary and secondary senescent cells.

Abstract No. P33

The Optical Stem Cell Activity Reporter (OSCAR): a new tool to understand dormant cell biology

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Dormant somatic stem cells lie at the core of tissue homeostasis and response to injury.

This stem subpopulation is characterized by greatly reduced metabolic activity thus protecting from genetic damage and prolonging survival, moreover, have great potential to be used in regenerative medicine and plays a critical role in therapy resistance and cancer relapse. However, even if dormant cells have been detected in several tissues, their identification and isolation remain elusive, thus complicating their study.
Here we show a new reporter mouse line that allows to isolate and characterize dormant stem cells irrespective of tissue of origin and surface markers, but only depending on their transcriptional state. Based on the observation that phosphorylation of RNA Polymerase II (RNApII), a hallmark of active transcription elongation is largely absent in dormant stem cells from multiple lineages, we have developed the Optical Stem Cell Activity Reporter (OSCAR), a live cell, ratiometric reporter of dormancy that owes its activity to the insertion of a short RNA Polymerase II (RNApII) kinase target peptide directly into the backbone of the yellow fluorescent protein Venus.By using small intestinal organoids cultures derived from mice ubiquitously expressing OSCAR, we showed dynamics of dormancy induction and cellular differentiation in real time. Moreover, we showed several populations of OSCARhigh and OSCARlow cells in the small intestine; and in microscopy, RNA-Seq and single cell culture analysis we further confirmed that OSCAR enables direct identification and isolation of dormant cells from heterogeneous populations, thus confirming the reliability of this tool to understand dormant cell biology.

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Abstract No. P34

The mouse metallomic landscape of aging and metabolism

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Organic elements make up 99% of an organism but without the remaining 1% of inorganic bioessential elements, termed the metallome, no life could be possible. The metallome is involved in all aspects of life, including charge balance and electrolytic activity, structure and conformation, signaling, acid-base buffering, electron and chemical group transfer, redox catalysis energy storage and biomineralization.

Aims: In this collaboration between geochemists and systems geneticists, we reveal the potential of an overlooked “omic” layer: the metallome. By analyzing the metallome in tandem with the phenome, metabolome and proteome, we show networks of interactions that are organ-specific, age-dependent, isotopically-typified and that are associated with a wealth of clinical and molecular traits.

Methods: We describe the evolution with age of the metallome and copper and zinc isotope compositions in five mouse organs. 16 mice per group aged 6, 16 and 24 months underwent metabolic phenotyping, then liver, kidney, brain, quadriceps and hearts were collected and 20 metals and 2 stable isotopes were quantified by ICP-MS. In parallel, the metabolome and proteome of the liver were measured.

Results and Conclusions: We show a highly conserved and reproducible age- and organ-specific metallome fingerprint across different studies, making this omic layer remarkably reproducible . The copper isotope composition in liver is strongly age-dependent, extending the existence of aging isotopic clocks beyond bulk organic elements. Furthermore, iron concentration and copper isotope composition relate to predictors of metabolic health, such as body fat percentage and maximum running capacity at the physiological level, and adipogenesis and the OXPHOS pathway at the biochemical level. Our results shed light on the metallome as an overlooked omic layer and open perspectives for potentially modulating cellular processes using careful and selective metallome manipulation.

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Abstract No. P35

SIRT7 in FLT3 ITD driven cell aging

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Young hematopoietic stem cells (HSC) have a quiescent cell cycle state and an unbiased differentiation capacity. Aging of HSC results in enhanced proliferation and stimulated myeloid differentiation. Cell intrinsic alterations underlie HSC aging: Loss of immune function and an increased incidence of myeloid leukaemia are two of the most clinically significant consequences of aging of the hematopoietic system. SIRT7 was recently identified as key regulator of HSC aging: A reduced SIRT7-expression in aged murine hematopoietic stem cells results in reduced longevity and increased proliferation.

Molecular alterations within the hematopoietic system influence HSC longevity and development of age-related myeloid stem-cell disorders like acute myeloid leukemia (AML). Defining differentiation pathways is central to understand pathogenesis of this hematopoietic disorder. Mutations in receptor tyrosine kinases (RTK), particularly FLT3 ITD mutations (Fms-like tyrosine kinase with internal tandem duplications) represent one of the important classes of driver mutations in a subset of 25 - 30% of AML patients. We demonstrated that hematopoietic cell lines expressing constitutive active FLT3 ITD showed decreased SIRT7, which could be abolished by inhibiting its oncogenic kinase activity. By using our established in vivo mouse models, we could demonstrate that bone marrow cells of mice expressing FLT3 ITD carried suppressed SIRT7. Important, low SIRT7 levels were detected in AML patients. With positive treatment response, SIRT7-expression increased, but showed reduction when patients progressed or relapsed. Pharmacologic inhibition of driver mutations in AML (FLT3-ITD) restored SIRT7 levels in cell lines and patient samples. Furthermore, SIRT7-expression increased with time during phorbol ester PMA-mediated monocyte differentiation of THP-1 cells. SIRT7-overexpression in THP-1 cells resulted in increased expression of differentiation markers.

Taken together, our data reveal a molecular correlation of FLT3 ITD-based development of myeloid neoplasms to the expression of SIRT7 as factor controlling HSC quiescence and establish changes of SIRT7-expression as relevant pathomechanism in AML. SIRT7 might act as tumor suppressor and could potentially serve as general biomarker for monitoring treatment response in myeloid stem-cell disorders.

Abstract No. P36

Health- and lifespan regulation by C/EBPbeta comes of age

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Few conditions are known that attenuate the adverse effects of ageing, including calorie restriction (CR) and reduced signalling through the mechanistic target of rapamycin complex 1 (mTORC1) pathway. We showed before that the expression of the inhibitory C/EBPbeta-LIP transcription factor is controlled by mTORC1 and that ablation of C/EBPbeta-LIP, creating a C/EBPbeta super-phenotype, results in CR-type metabolic improvements in mice (Zidek et al., 2015 DOI 10.15252/embr.201439837). Recently, we showed that these C/EBPbeta super-mice display improvements in a broad spectrum of ageing parameters, including cancer incidence, motor coordination, glucose tolerance and memory/naïve T-cell ratio, and less age-related inter-individual variation in gene expression. Finally, we showed that female C/EBPbeta super-mice display an extended median lifespan of 20.6% and an increase in maximum lifespan of 9% (Müller et al. 2018 DOI: 10.7554/eLife.34985). Our new data show that on a high-fat diet (HFD) C/EBPbeta super-mice have smaller adipocytes that express less of the CD68 inflammation marker, do not develop a fatty liver (steatosis), and have improved insulin sensitivity and glucose tolerance compared to wt mice. Importantly, a recent study by the Niehrs lab showed that preventing binding of C/EBPbeta to enhancer sites by experimental impairment of DNA-demethylation causes premature aging in mice (Schäfer et al. 2018 DOI 10.1101/gad.311969.118). Altogether, these studies demonstrate a crucial role of C/EBPbeta in the aging process and suggest that restriction of the inhibitory C/EBPbeta-LIP sustains health and fitness during ageing. Furthermore, we discuss therapeutic strategies inhibiting the translation into C/EBPβ-LIP may offer new possibilities to treat age-related diseases and to prolong healthspan (Zaini et al. 2017 DOI: 10.1038/srep42603).

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Abstract No. P38

Precise machine learning suggest that brain cells facing a neurodegenerative insult are the subject of molecular decompensation and aging

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Loss of cellular homeostasis has been implicated in the etiology of several neurodegenerative diseases (ND). However, the molecular mechanisms that underlie this loss remain poorly understood on a systems level in each case. More largely, acceleration of neuronal aging is suspected to be involved in ND pathogenesis, but the molecular features (e.g. size and strength) of this acceleration remain poorly understood on a systems level and across brain cell types.

To investigate these questions, we used a novel computational approach (Geomic) based on the application of shape deformation concepts to the analysis of complex omics data. We used Geomic to analyze and integrate dimensional RNA-seq and in vivo neuron survival data obtained in the striatum of Huntington's disease (HD) model mice. We mapped the temporal dynamics of homeostatic and pathogenic responses in four striatal cell types of HD model mice.

We found that most pathogenic responses are mitigated and most homeostatic responses are decreased over time, suggesting that neuronal death in HD is primarily driven by the loss of homeostatic responses. Moreover, we found that different cell types may lose similar homeostatic processes, e.g. endosome biogenesis and mitochondrial quality-control in Drd1-expressing-neurons and astrocytes. The relevance of these data to HD was validated by human stem cell, GWAS and post-mortem brain data.

These findings provide a new paradigm and a database of future targets to probe for therapeutic discovery in HD and other NDs based on remodeling stress response to re-instate neuronal homeostasis and resilience.

With regard to interrogating neuronal aging in NDs, these findings provide suggestive yet systems-level evidence for molecular aging to be a dynamic phenomenon that may significantly affect several brain cell types in neurodegenerative conditions, particularly in the early phases of ND processes.

Reference: https://elifesciences.org/articles/64984

Database: http://www.broca.inserm.fr/geomic/index.php; see also the Brain-C knowledge base at http://www.broca.inserm.fr/BrainC_database/gene_info.php?q=Alg9

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Abstract No. P39

Mutagenic, mitochondrial motifs and species lifespan

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The “theory of resistant biomolecules” posits that long-lived species show resistance to molecular damage at the level of their biomolecules. Here, we test this hypothesis in the context of mitochondrial DNA (mtDNA) as it implies that predicted mutagenic DNA motifs should be inversely correlated with species maximum lifespan (MLS).

First, we confirmed that guanine-quadruplex and direct repeat (DR) motifs are mutagenic, as they associate with mtDNA deletions in the human major arc of mtDNA, while also adding mirror repeat (MR) and intramolecular triplex motifs to a growing list of potentially mutagenic features. What is more, triplex motifs showed disease-specific associations with deletions and an apparent interaction with guanine-quadruplex motifs.

Surprisingly, even though DR, MR and guanine-quadruplex motifs were associated with mtDNA deletions, their correlation with MLS was explained by the biased base composition of mtDNA. Only triplex motifs negatively correlated with MLS even after adjusting for body mass, phylogeny, mtDNA base composition and effective number of codons.

Taken together, our work highlights the importance of base composition for the comparative biogerontology of mtDNA and suggests that future research on mitochondrial triplex motifs is warranted.

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Abstract No. P40

H3K9me selectively blocks transcription factor activity to ensure differentiated tissue integrity.

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Histone H3 lysine 9 methylation (H3K9me) characterizes heterochromatin, which silences repetitive elements and tissue-specific genes. Here we show that in C. elegans loss of H3K9me leads to a highly divergent upregulation of genes with tissue- and developmental stage-specificity. During development H3K9me is lost from differentiated cell type-specific genes and is gained at genes expressed in earlier developmental stages or other tissues. The continuous deposition of H3K9me2 after terminal differentiation is necessary to maintain repression. H3K9me ensures silencing by restricting the binding of a defined set of transcription factors (TFs) at promoters and enhancers that drive gene expression. However, increased DNA accessibility upon loss of H3K9me is neither sufficient nor necessary to drive derepression. Instead increased accessibility correlates with derepression for a subset of genes that are positioned away from the nuclear envelope in wild-type nuclei, while perinuclear genes that are derepressed by loss of H3K9me retain low accessibility despite being transcriptionally active. Overall, muscle-specific H3K9me deposition is shown to restrict TF binding in a muscle-specific manner, leading to subtle defects in tissue development.

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Abstract No. P42

LncRNAs in hybrid DNA/RNA in patients with psoriasis.

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Keywords: Psoriasis, Telomere length, RNA-DNA hybrid, TERRA, RNase HII, centromeres
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Abstract: Aging is widespread across the tree of life, but is it ubiquitous? Here we explore the fundamental limits of longevity in metazoans and revisit claims of biological immortality in highly-regenerative fissioning organisms.

AIMS: Previous arguments have stated that damage to the adult stem cells is passed to offspring in fissioning species,which violates basic assumptions of the evolutionary theories of aging and may allow for selection of agelessness.In Aim 1, we modify mathematical models of evolutionary aging to explore differences in expected damage repair in fissioning and non-fissioning organisms. However, even if agelessness is evolutionarily favored, deleteriousmutations may inevitably push an individual to age through similar mechanisms which push asexual populations to extinction. The expected rate of decay due to DNA mutations are analyzed in Aim 2.

METHODS: For Aim 1, we consider cellular (passed on to the next generation in fissiparous reproduction) and extracellular(rejuvenated during fission) damage and repair. We use both analytic methods which seek to maximize the intrinsicrate of population growth to find the optimal repair rate and subsequent lifespan for fissioning and non-fissioning organisms. For Aim 2, we use agent based simulations of mutations and competition in stem cells and individualsto estimate rates of genetic decline.

RESULTS: According to the Aim 1 models, repair for both cellular and extracellular damage is higher in fissioning organismswhen compared to non-fissioning organisms, and lifespan is subsequently higher as well. However, invoking somedegree of positive pleiotropy is necessary for agelessness to be evolutionarily selected. The Aim 2 models suggest inevitable genetic decline which may occur at quite a rapid pace. Although certain parameter sets allow for a slowdecline, the likelihood of such parameters being realized in natural populations is suspect.

CONCLUSION: While agelessness might be evolutionary and mechanistically achievable, our models suggest senescence in fissioning organisms may not only be possible but likely. We plan on measuring damage accumulation of multiple forms in fissioning and non-fissioning flatworms to test our model predictions. Ultimately, the phenomenon of biological immortality in fissioning organisms may in fact be found to be extremely slow aging.

Abstract No. P43

Macrophage Dysfunction in Neurofibromatosis Type 2 (NF2)

Michael Reuter, Annemarie Carlstedt, Johanna Schleep, Helen Morrison

Peripheral nerves (PN) in the mammalian adult must be functionally maintained throughout life and have remarkable regenerative capacity. In the disease Neurofibromatosis Typ 2 (NF2) a mutagenic loss of the NF2 gene encoding for the tumor suppressor Merlin is causative for a faulty nerve repair program. The consequence of the faulty maintenance and repair system, is neurodegeneration leading to neuropathies including the formation of Schwann-cell derived tumors (Schwannomas).Macrophages play a crucial role in NF2 tumorigenesis. Since first, histological analysis revealed the presence high macrophage numbers in NF2 related Schwannomas. Second, anti-inflammatory treatment with aspirin lead to reduced macrophage number and a reduced tumor size. However, the precise effect merlin loss in macrophages and its consequences for the NF2 disease are still unknown. Here we show that loss of merlin in macrophages lead to reduced phagocytic capacity. Both heterozygous as well as homozygous merlin loss in bone-marrow-derived-macrophages (BMDM), isolated from corresponding mouse models, showed a decreased phagocytic capacity for E.coli particles in vitro. Interestingly, both NF2-knock-out macrophages have an accelerated cell-proliferation. To investigate the effect of merlin loss in macrophages in the context of nerve repair, we performed nerve injury experiments in-vivo, using several NF2 disease mouse models including a macrophage specific NF2 knock-out mouse line. Merlin loss in macrophages led to a decreased/delayed myelin-debris phagocytosis three days after neve injury. Since the debris clearance is a prerequisite for proper nerve repair, this finding illustrates the contribution of macrophages to the malfunctioning repair system in NF2. Together with the altered microenvironment this will promote tumorigenesis.These results may help to develop new therapeutic interventions in NF2 treatment where macrophages represent as a promising target cell for drug treatments.

Abstract No. P44

Lztr1 dependent mitochondrial dysfunction and metabolic aging

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Leucine zipper-like transcriptional regulator 1 (LZTR1) is a protein that belongs to the BTB-Kelch superfamily and interacts with the Cullin3 (CUL3)-based E3 ubiquitin ligase complex. Mutations in LZTR1 have been linked to several diseases as Noonan syndrome, glioblastoma, and schwannomatosis. While in the past Lztr1 subcellular localization has been reported to the Golgi apparatus and autophagosomes, recent work in the Morrison group identified a previously undescribed localization to mitochondria and/or mitochondria-associated membranes (MAMs). Further supporting a potential link to mitochondrial functions, depletion of Lztr1 in cultured murine Schwann cells (mSCs) resulted in increased mitochondrial respiration and ATP synthesis. To explore this energetic phenotype and to evaluate how Lztr1 loss may contribute to organism-wide (metabolic) aging, a pilot study has been conducted to test the energy expenditure of global Lztr1 +/- (HET) compared to wild type (WT) mice with the Oxymax/CLAMS – an indirect calorimetry-based system. Our analysis indicates that loss of Lztr1 triggers a specific remodeling of the metabolic flux at a young age that fails during aging and leads to dramatic changes in body weight and composition with a pronounced depletion of body fat. Comparison of the liver proteome by mass spectrometry (LC-MS) further revealed major differences between old HET and WT mice with a strong focus in the mitochondrial compartment. From these preliminary results, we conclude that Lztr1 influences overall metabolism and that Lztr1-depletion is associated with specific metabolic shifts both in mitochondrial functionality and energy expenditure profiles.

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Abstract No. P45

The Limits of Longevity

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Abstract: Aging is widespread across the tree of life, but is it ubiquitous? Here we explore the fundamental limits of longevity in metazoans and revisit claims of biological immortality in highly-regenerative fissioning organisms.

AIMS: Previous arguments have stated that damage to the adult stem cells is passed to offspring in fissioning species,which violates basic assumptions of the evolutionary theories of aging and may allow for selection of agelessness.In Aim 1, we modify mathematical models of evolutionary aging to explore differences in expected damage repair in fissioning and non-fissioning organisms. However, even if agelessness is evolutionarily favored, deleteriousmutations may inevitably push an individual to age through similar mechanisms which push asexual populations to extinction. The expected rate of decay due to DNA mutations are analyzed in Aim 2.

METHODS: For Aim 1, we consider cellular (passed on to the next generation in fissiparous reproduction) and extracellular(rejuvenated during fission) damage and repair. We use both analytic methods which seek to maximize the intrinsicrate of population growth to find the optimal repair rate and subsequent lifespan for fissioning and non-fissioning organisms. For Aim 2, we use agent based simulations of mutations and competition in stem cells and individualsto estimate rates of genetic decline.

RESULTS: According to the Aim 1 models, repair for both cellular and extracellular damage is higher in fissioning organismswhen compared to non-fissioning organisms, and lifespan is subsequently higher as well. However, invoking somedegree of positive pleiotropy is necessary for agelessness to be evolutionarily selected. The Aim 2 models suggest inevitable genetic decline which may occur at quite a rapid pace. Although certain parameter sets allow for a slowdecline, the likelihood of such parameters being realized in natural populations is suspect.

CONCLUSION: While agelessness might be evolutionary and mechanistically achievable, our models suggest senescence in fissioning organisms may not only be possible but likely. We plan on measuring damage accumulation of multiple forms in fissioning and non-fissioning flatworms to test our model predictions. Ultimately, the phenomenon of biological immortality in fissioning organisms may in fact be found to be extremely slow aging.

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Abstract No. P46

Impaired M2 macrophage polarization in aging shifts the lipidome to pro-inflammatory mediators driving chronic inflammation and impairing tissue maintenance

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Inflammation is the natural defensive response of the immune system to an injury or infection and is regulated by small molecule mediators that trigger different phases of the inflammatory process. In particular, lipid mediators (LM) and cytokines exhibit crucial regulatory functions in the progression and resolution of inflammation. Macrophages play a central role in this process and can adopt distinct phenotypes with specialized functions depending on their microenvironment: inflammatory M1 macrophages drive inflammation through the release of pro-inflammatory cytokines and LMs, like prostaglandins (PG) and leukotrienes (LT), while resolving M2 macrophages promote inflammation resolution and tissue regeneration by production of anti-inflammatory cytokines and specialized pro-resolving mediators (SPM). Age-related dysfunction of macrophages in the resolution of inflammation and tissue maintenance due to chronic and unresolved, low-grade inflammation (“inflammaging”) has been reported. Yet, the underlying molecular mechanisms and functional consequences of those processes remain poorly understood. Here, we show that polarization of peritoneal macrophages (PM) from old mice towards an M2-like phenotype is impaired versus adult mice, resulting in aberrant LM formation and cytokine secretion. In PMs isolated from adult mice (PM-A) we observed a shift in LM formation from PGs and LTs to SPMs already after 4 h of M2 polarization with interleukin-4. In contrast, PMs from old mice (PM-O) produced mainly LTs and PGs in substantial amounts upon polarization. This pattern persists over the course of 48 h of polarization. Proteomic profiling revealed that polarization of PM-A towards M2 yields a more distinct phenotype when compared to PM-O, clearly separated from M1. We observed similar aging-related changes in the lipidome and cytokine profile within several tissue from mice [1]. Hence, we hypothesize that during aging macrophage polarization towards M2 is impaired, which in turn drives chronic inflammation and disturbs tissue maintenance. By combining state-of-the art lipidomic and proteomic profiling, we aim to uncover new molecular targets for pharmaceutical interventions to improve therapeutic strategies for elderly patients with chronic inflammatory diseases.

[1] Schädel, P. et al. Aging drives organ-specific alterations of the inflammatory microenvironment guided by immunomodulatory mediators in mice. FASEB Journal 35, (2021).

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Abstract No. P47

Impact of glycosylation on the ageing heart

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Aging is associated with the progressive deterioration of the structure and function of the heart and is a dominant risk factor for cardiovascular diseases, which is one of the leading causes of death in developed countries. Even though characteristics of the aged heart such as cardiac hypertrophy, fibrosis and inflammation are well described, the underlying molecular mechanisms remain largely elusive. Since glycosylation is the most-common post-translational modification and plays a prominent role in various biological processes, we hypothesized that changes in the glycoproteome might also contribute to the age-related functional decline of the cardiovascular system.

We here provide the first analysis of the cardiac glycoproteome of mice at different ages. Our Western Blot analyses as well as MALDI-TOF based glycome analysis clearly demonstrate an increase in glycans carrying mannose residues in the aged heart, while complex-type *N*-glycans decrease over age. Notably, measurements of blood sugar levels showed an increase in mannose levels in aged mice. Elevated free mannose levels in aged mice may reflect increased release from glycans. In accordance with increased mannosylation, the abundance of the enzyme facilitating the supply of the sugar donor GDP-mannose, GDP-mannose-pyrophosphorylase-B (GMPPB), increases with age. Our MS analysis of glycoprotein pull-downs further suggests changes of proteins relevant for pathways that have previously been reported to be aging relevant, such as ER stress, sirtuin signaling, ECM remodeling, cytoskeleton network, and the immune system.

In summary, we propose that alterations of the cardiac glycoproteome might play a role in age-related functional decline of the heart.

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Abstract No. P48

Mitochondrial ROS production may not explain differences in life expectancy in a rat model of genetically determined high or low exercise capacity

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High exercise capacity is associated with lower risk for cardiovascular diseases and better outcome in disease and surgical interventions. Aging is associated with decreasing exercise capacity and increasing oxidative stress, originating to a great part from mitochondrial ROS production. In the model of rats with inherited high (HCR) or low (LCR) intrinsic exercise capacity, LCR present with 1/3 lower life expectancy and we aimed to assess, if this is related to increased oxidative stress.

Female adult (15 weeks) and old (100 weeks) HCR and LCR were tested for exercise capacity and cardiac function. ROS production from cardiac and skeletal muscle mitochondria was assessed and oxidative stress determined.

Cardiac contractile function decreased with age and was higher in old LCR compared to old HCR. Exercise capacity decreased with age in HCR only, but remained at a higher level than in LCR. Both, cardiac and skeletal muscle showed a decrease of mitochondrial ROS production with age. In parallel, antioxidative capacity of catalase was increased with age which was more pronounced in LCR in the heart and in HCR in skeletal muscle. However, oxidative protein damage increased to the same extent in old HCR and LCR.

The Increase in oxidative stress with aging was found independent of genetically determined exercise capacity. It seems to be associated with non-mitochondrial ROS production.

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Abstract No. P49

Transposons are double edged sword for inflammaging and cancer resistance

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Age-related activation of LINE1 transposable elements contributes to age-related inflammation and senescnece by promoting age-related pathologies. However, there transposons also have a bright side where they serve as tumor suppressors.

Blind mole rats (BMRs) are small rodents, characterized by exceptionally long lifespan (> 21 years) and resistance to both spontaneous and induced tumorigenesis. Here we report that cancer resistance in the BMR is mediated by retrotransposable elements (RTEs). BMR cells and tissues express very low levels of DNA methyltransferase 1 (DNMT1). Upon cell hyperplasia, the BMR genome DNA loses methylation, resulting in activation of RTEs. Up-regulated RTEs form cytoplasmic RNA/DNA hybrids, which activate cGAS-STING pathway to induce cell death. Although this mechanism is enhanced in the BMR, we show that it functions in mice and human. We propose that RTEs were coopted to serve as tumor suppressors that monitor cell proliferation and are activated in premalignant cells to trigger cell death via activation of innate immune response. RTEs activation is a double-edged sword, serving as a tumor suppressor but in late life contributing to aging via induction of sterile inflammation.

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Abstract No. P50

Signalling profile of proteasome inhibition-induced and stress-induced senescence in human lung and dermal fibroblasts

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Cellular senescence is described as an irreversible cell cycle arrest and constitutes one of the hallmarks of aging. Senescence exerts its effects via Senescence Associated Secretory Phenotype, and by altering the signalling profile and producing intra- and extra-cellular inflammatory moieties. We have developed two in vitro senescence models to study senescence-induced signalling alterations by utilizing a multiplex bead-based phosphoproteomic and secretome profiling.

Cell viability was assessed with CellTiter Glo assay. HFL1 and BJ cells were exposed to sub-lethal doses of bortezomib or H2O2 to induce senescence. Senescence phenotype was evaluated by cell morphology, inhibition of cell proliferation and senescence-associated β-galactosidase staining. ROS levels were assessed using CM-H2DCF-DA. Custom multiplex assay panels were developed to evaluate the phosphoproteomic and secretome profile of senescence models.

Our results showed that HFL1 and BJ cells exposed to 9.7nM and 10.5nM bortezomib, respectively, acquired a senescence like phenotype after 10 days of continuous exposure. Stress-induced premature senescence was induced after a 2-hour exposure of HFL1 and BJ cells to 75 and 125uM H2O2, respectively, followed by a 72-hour recovery in culture media. Multiplex analysis demonstrated significant upregulations of p21, c-JUN, MEK1 and p38 in bortezomib-treated HFL1 cells compared to control, while CREB1, SMAD3 and STAT3 were significantly downregulated, indicating activation of anti-apoptotic and stress signalling pathways, as well as cell cycle arrest. BJ cells treated with bortezomib followed a similar pattern, demonstrating significant upregulation of p21, c-JUN and MEK1. For H2O2 treated HFL1 cells, p53, p21, AKT and c-JUN were overexpressed, as a response to DNA damage, cell cycle arrest and inhibition of apoptosis. Results from H2O2-treated BJ cells agreed with HFL1 cells, with p53 and p21 showing the most significant alterations. The secretome profiling analysis is under development.

Proteasome inhibition with bortezomib and oxidative stress with H2O2 induce a stable cell cycle arrest, an increase in SA-β-gal positive cells and upregulation of known senescence associated markers. Multiplex analysis revealed differences in the signalling profile between proteasome inhibition and stress induced senescent cells. These differences along with secretomic alterations will be further investigated in accordance with transcriptomic data to decipher the diverse senescence programs.

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Abstract No. P51

Proteome dynamics during myogenesis identify the cytoskeletal protein Leiomodin 1 as a promoter of muscle stem cell differentiation

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The cytoskeleton of the skeletal muscle plays a fundamental role in cell morphology, adhesion, growth, and signaling and ultimately regeneration of skeletal muscle. It is known that the cytoskeleton is highly remodeled during myogenic differentiation, a process that muscle stem cells (MuSCs) undergo following activation in response, e.g., to injury. However, the dynamics of this process remain only partially characterized and understood. In order to investigate proteome dynamics during myogenesis, we analyzed a five days differentiation time-course experiment of primary myoblasts using mass spectrometry. We used these data to identify clusters of protein displaying distinct changes in abundance during myogenic differentiation. Using this approach, we identified the cytoskeletal protein Leiomodin 1 (Lmod1) among a group of proteins that increases abundance during early phases of myoblast differentiation. Therefore, we hypothesized that Lmod1 might play a role in the organization and rearrangement of the MuSC cytoskeleton. Lmod1 is a powerful actin filament nucleator, which is preferentially expressed in smooth muscle cells, while the related Lmod2 is highly abundant in fully differentiated skeletal muscle cells. We analyzed the effect of Lmod1 knockdown on primary myoblasts and found that depletion of Lmod1, but not Lmod2, severely affected the terminal myogenic differentiation and amount of myonuclei per myotube. Conversely, overexpression of Lmod1 improved myogenic differentiation, it led to an accelerated myotube formation and improvement in myoblast fusion without depletion of reserve cells. Interestingly, we found the levels of Lmod1 to be increased in MuSC freshly isolated from old mice, suggesting that Lmod1 might be a contributing factor to the impaired MuSC functionality in the aged causing reduced regenerative capacity of old skeletal muscle. In the future, interventions targeting Lmod1 or its interaction partners could be designed to improve skeletal muscle regeneration in the elderly.

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Abstract No. P52

Peripheral Nerve Ageing - Maintenance, Repair, and CCl11 as a potential ageing factor

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The peripheral nervous system has a striking ability for regeneration and functional recovery post-injury - even in adulthood. However, as we age repair processes become slower and incomplete. To develop therapies that target this inefficient repair, we first need to acquire a better understanding of when ageing changes begin within the peripheral nerve. This includes identifying the cell types first affected by ageing, and the underlying molecular mechanisms (both cell intrinsic and extrinsic). Previous work from our group compared young and old mice, showing that advanced age presents a low-grade chronic inflammation. Additionally, Schwann cells dedifferentiate to a repair cell phenotype, despite a lack of injury (Büttner et al., 2018). In particular, two pro-inflammatory cytokines are upregulated in old nerves: CCL2 and CCL11. However, the extent to which CCL11 is an ageing factor in the peripheral nervous system is unknown. We aim to determine the temporal and spatial origin of detrimental ageing changes, and the contribution of systemic factors, in particular CCL11.

To determine the impact of CCL11 in peripheral nerve ageing, we are investigating its role in both intact and injured sciatic nerves. Cohorts of wild-type mice received i.p. injections of CCL11 at differing dosages.

Results after nerve injury indicate that CCL11 application leads to repair deficiencies such as a delay in remyelination. For the intact nerve, immunostainings and qPCR results show no obvious differences in selected targets within the nerve. However, a proteomic screen indicated significant metabolic changes within the CCL11-treated intact nerve, showing an upregulation of key glycolytic and TCA cycle enzymes. We hypothesise that this suggests a potential “pre-priming effect”, which ultimately contributes to the delay in remyelination observed in injury experiments. Currently, we are using RNAscope® HiPlex in situ hybridisation to investigate RNA distributions and relationships within the ageing intact nerve, with the aim of determining the local source of CCL11, and its receptors CCR2, CCR3, and CCR5. In addition to this, we are employing the use of a CCL11-KO mouse model to gain further knowledge on the potential benefits, or indeed detriments, a lack of CCL11 has on both peripheral nerve maintenance and repair.

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Abstract No. P53

Too little to die, too much to survive – Error catastrophe in human cells and its relevance for therapy

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Cancer cells often show defects in DNA repair leading to either high numbers of base substitutions or chromosomal aberrations. For instance, the loss of DNA mismatch repair (MMR) underlying many colorectal carcinomas, increases mutation rates considerably. More recently, a new class of tumors has been identified with mutations in the exonuclease domain of the DNA polymerases (pol) ε or δ. These cancers show extremely high numbers of base substitutions. However, excessive error rates result in an accumulation of mutations in essential genes, which progressively impair (cancer) cell fitness. This phenomenon, called error catastrophe, may lead to subsequent extinction of a cell population. Although this effect has already been discussed excessively, it has been formally demonstrated only for simple model organisms such as yeast. Even though conventional cancer chemotherapy is largely based on the introduction of DNA damage, the upper limits of mutation rates are still unknown.

Here, we introduced different exonuclease mutations of pol δ/ε in an isogenic human cell models. Using fluctuation analysis, we could demonstrate strong mutator phenotypes. Cell phenotypes and the observed mutation rates may indicate that they are not far from a tolerable upper limit. To confirm this we aim to achieve synthetic lethality by combined functional knockout of different replication fidelity caretakers . To our surprise human cells seem to be more resilient than expected to extremely high error rates and tolerate even better compared to budding yeast. Some mutator phenotypes we demonstrated in human HAP1 cells one wouldn't believed to be tolerable. Based on these, we started to characterize molecular mechanisms allowing cells to deal with the accumulation of such unexpected high rates of errors. Finally we discuss our findings in terms of their possible meaning for cancer therapy aging of (stem) cells.

Contact

Abstract No. P54

Transient adenine base editing treats premature aging in mice

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Hutchinson-Gilford progeria syndrome (HGPS) is a rare premature ageing disorder caused by a point mutation in the *LMNA* gene (*LMNA* c.1824 C>T), resulting in the production of a detrimental protein called progerin. Adenine base editors (ABEs) recently emerged with a promising potential for HGPS gene therapy. However adeno-associated viral (AAV) delivery systems currently used for gene editing have raised concerns, and the long-term effects of heterogeneous mutation correction in highly proliferative tissues like the skin are unknown. In this study we use a non-integrative transient lentiviral vector system, expressing an ABE to correct the HGPS point mutation in the skin of HGPS mice. Transient ABE expression from a non-integrative vector system in a skin-specific humanized HGPS mouse model, corrected the mutation in 20.8-24.1% of the skin cells. Four weeks post-ABE delivery, the HGPS skin phenotype was improved and clusters of progerin-negative keratinocytes were detected, indicating that the mutation was corrected in both progenitor and differentiated skin cells. Our results demonstrate that transient non-integrative viral vector mediated ABE expression is a plausible approach for future gene-editing therapies.

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Abstract No. P55

Combined fiber atrophy and decreased muscle regeneration capacity driven by mitochondrial DNA alterations underlie the development of sarcopenia

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Aims: Mitochondrial dysfunction caused by mitochondrial DNA (mtDNA) deletions have been associated with skeletal muscle atrophy and myofiber loss. However, whether these defects at the myofiber level cause sarcopenia, the aging related loss of muscle mass and strength, remains unclear. Also, the contribution of mtDNA alterations in muscle stem cells (MuSCs) to sarcopenia remain to be investigated.

Methods: We expressed a dominant-negative variant of the mitochondrial helicase TWINKLE (Baris et al., Cell Metab 2015; Holzer et al., J Cell Biol 2019) specifically in differentiated myofibers (K320E-Twinkleskm mice) or MuScs (K320E-Twinklemsc mice), respectively.

Results: K320E-Twinkleskm mice at 24 months of age had significantly higher levels of mtDNA deletions compared to controls and revealed a progressive increase in the proportion of cytochrome c oxidase deficient fibers (COX-), reaching a maximum of 3.03%, 4.36%, 13.58% and 17.08% in EDL, SOL, TA and GAS muscles, respectively. However, they did not show accelerated loss of muscle mass, muscle strength or impaired physical performance in vivo, compared to controls. Histological analyses revealed ragged red fibers and stimulated regeneration in K320E-Twinkleskm, indicating activation of MuSCs. RNAseq demonstrated significant enrichment of genes associated with protein synthesis, but also protein degradation, as well as muscle fiber differentiation and cell proliferation. In contrast, K320E-TwinkleMuSc mice showed 30% of COX- fibers in regenerating muscles at 7 days following cardiotoxin destruction. Notably, regenerated muscles showed dystrophic changes, increased fibrosis, increased fat cells and reduced muscle mass (regenerated TA: 40.0mg vs 60.2mg, p=0.0171). In contrast to muscles from K320E-Twinkleskm mice, freshly isolated MuSCs from old K320E-TwinkleMuSc mice were completely devoid of mtDNA alterations. However, after passaging in vitro, mtDNA copy number as well as respiratory chain subunits decreased.

Conclusions: Taken together, accumulation of large-scale mtDNA alterations in myofibers alone is not sufficient to cause sarcopenia. Expression of K320E-Twinkle in MuSCs is tolerated in quiescent MuSCs, but leads to mtDNA and respiratory chain depletion upon activation, in vitro as well as in vivo. Altogether, our results suggest that the accumulation of mtDNA alterations in myofibers activates regeneration from MuSCs during aging, which leads to sarcopenia if such alterations have expanded in MuSCs as well. However, MuSCs have efficient quality control mechanisms to counteract their accumulation in the quiescent state.

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Abstract No. P56

Protein O-GlcNAcylation in senescence models of primary endothelial cells

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Aim: O-GlcNAcylation, the enzymatic addition of O-linked β-N-acetylglucosamine (O-GlcNAc) to serine/threonine residues of proteins, is a posttranslational modification that regulates protein and cellular functions. O-GlcNAcylation is altered in age-related diseases, including vascular abnormalities developed in the course of metabolic disorders as a consequence of endothelial dysfunctions. However, the mechanisms linking aging, O-GlcNAcylation and metabolically triggered endothelial dysfunction remain unclear. To further our understanding of potential regulatory mechanisms, we here investigate the role of O-GlcNAcylation in senescent endothelial cells.

Methods: We induced senescence of primary human endothelial cells using a replicative model (culturing up to passage 20) and, alternatively, an oxidative stress-induced model (treatment with H2O2 for 7 days). O-GlcNAcylation patterns and levels of key enzymes in metabolic pathways were investigated by immunoblotting. To monitor global changes in the proteome of senescent cells, we performed mass spectrometry (MS)-based proteomics.

Results: In both models we observed a significant decrease of O-GlcNAcylated proteins in senescent cells compared to control. Further, in the replicative senescence model, the levels of glutamine:fructose-6-phosphate amidotransferase 1 (GFAT1), a rate-limiting enzyme of the O-GlcNAc machinery, as well as of O-GlcNAc transferase (OGT), an O-GlcNAc-attaching enzyme, were significantly reduced. In contrast, we observed a trend towards increased levels of O-GlcNAcase (OGA), an O-GlcNAc-cleaving enzyme. Reassuringly, these results were in line with our MS-based proteomics data. In the oxidative stress-induced premature senescence model, immunoblotting showed an even more pronounced reduction of GFAT1, however, the levels of OGT and OGA remained largely unchanged. Based on these initial findings on key overall O-GlcNAcylation and key enzymes, we hypothesize extensive remodeling of the proteome and cellular function driven by O-GlcNAcylation during endothelial cell senescence. To gain further insight, we are currently developing a streamlined MS-based workflow for the unbiased and site-specific quantitative analysis of the O-GlcNAcome.

Conclusions: We observed decreased O-GlcNAc levels in senescent endothelial cells, which could be explained by alterations detected in protein levels of key regulatory components (GFAT1, OGT, and OGA), thus hinting towards an important functional role of O-GlcNAcylation during endothelial cell aging.

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Abstract No. P57

Lifespan extension and neuroprotection by dietary restriction requires OXR1-mediated retromer function

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Dietary restriction (DR) is the most robust method to delay aging and the onset of neurodegeneration, though the mechanisms behind this phenomenon remain unclear. Further, it remains unknown which factors influence why different individuals will respond to dietary interventions to different degrees. We reared over 150 fully sequenced fly strains from the *Drosophila* Genetic Reference Panel under *ad libitum* feeding or diet-restricted conditions and measured lifespan as well as healthspan. Through genome-wide association study, we identified genetic variants associated with influencing these traits under each dietary condition. A variant in one gene, *mustard* (*mtd*, called *Oxidation resistance 1, OXR1*, in humans), significantly associated with DR-specific lifespan. We demonstrate that *mtd/OXR1* in neurons is necessary for DR-mediated lifespan extension and that neuronal overexpression of human *OXR1* is sufficient to extend lifespan upon DR in flies. Neuronal knockdown of *mtd* also inhibits dietary restriction-associated slowing of age-related visual decline, arguing for a specific role of mtd/OXR1 in DR-mediated neuroprotection. We additionally identified that natural variants of OXR1 in the promoter are associated with enhanced longevity upon DR and are regulated by the transcription factor *Traffic jam* (*TJ*). We further show that mtd is essential for stabilizing the retromer complex, which is necessary for trafficking transmembrane proteins for reuse. As a result of OXR1 deficiency, the retromer destabilizes and lysosomes become overused. Overexpression of retromer proteins or supplementation with chaperone compound R55 rescues the lifespan defects and neurodegeneration seen in mtd-deficient flies, and R55 is capable of rescuing lysosomal aggregation in cells from humans with OXR1 deficiency. Thus, *mtd*/*OXR1* enhances protein recycling in response to DR through the retromer, thus improving neuronal health and lifespan.

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Abstract No. P58

Proliferative effect of HGF on HepG2 cells is associated with upregulation of autophagy

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Background:

Age is one of the key risk factors to develop malignant diseases leading to a high incidence of hepatic tumors in the elderly population. The only curative treatment for hepatic tumors is surgical removal, which initiates liver regeneration. However, liver regeneration is impaired with aging, leading to an increased surgical risk for the elderly patient. Due to the increased risk, those patients are potentially excluded from curative surgery.

Hepatocyte growth factor (HGF) is a strong mitogen for hepatocytes. However, the specific mechanism promoting hepatocyte proliferation is not well understood.

Hepatocytes require abundant ATP for cellular division and growth. Accumulating evidence demonstrated that autophagy promotes cellular ATP synthesis by degrading dysfunctional organelles or superfluous bulk material. We raise the hypothesis that the effect of HGF in promoting hepatocyte proliferation may be related to the activation of autophagy.

Methods:

We used a hepatocyte cell line (HepG2) as cell culture model. In contrast to the control groups, experimental groups were either treated with 40ng/ml Hepatocyte growth factor (HGF), 50 µM Chloroquine (CQ) (autophagy inhibitor) and 40ng/ml HGF + 50 µM Chloroquine (Combi-group) for 24h respectively. Cell number (CCK-8) and ATP-content were investigated using spectrophotometric assays. Autophagy related proteins (AMPK, ULK1, LC3B and p62) were examined using western blot.

Results:

HGF induced about 20% increase in the number of HepG2 cells after 24h in culture compared to control, indicated by a stronger CCK8 signal. In contrast; treatment with CQ resulted in a significantly lower cell number after the same time in culture. Combi-treatment leads to partial recovery of cell number. These results were associated with a parallel modulation of ATP-content. WB revealed weak bands for LC3B-II and p62 in control and HGF-treated groups, but strong bands in CQ and even stronger bands in combi-group. In contrast, WB did not reveal a difference in the signal intensity for AMPK and ULK1.

Conclusion and perspective:

Our results suggest that induction of autophagy may play a positive role in in HGF-induced hepG2 proliferation by providing the needed energy via an increased ATP synthesis. To further elucidate the pathway involved, the ongoing experiments are designed to detect the key markers to mTOR and S6K.

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Abstract No. P59

Characterization of age-related changes in the proteome and metabolism of adipose-derived stem cells

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In midlife, obesity and an increase in adipose tissue correlate with a higher risk of age-related diseases such as cardiovascular and metabolic disorders. Adipose tissue is a multicellular, metabolic and endocrine organ. Adipocytes maintain and regenerate from stromal mesenchymal stem cells (MSCs) throughout life. We suppose an influence of adipose tissue-specific MSCs ageing on pathology of adipose tissue function in elderly. To investigate this stem cell ageing in vivo and in vitro, we isolated adipose tissue-derived stem cells (ASCs) and established a primary ASC culture from young (16 weeks) and old rabbits (>108 weeks) and visceral (VASCs) and subcutaneous adipose tissue (SASCs), respectively (Jung et al. 2019). The ASCs from old animals showed an age- and origin-depended decrease of stem cell function and a reduction in their adipogenic differentiation capacity (Jung et al. 2019).

In this study, we compared the metabolic properties of living young and old ASCs by using the Seahorse Mito Cell Stress Test (XF96, Agilent) to compare the mitochondrial respiration. Furthermore, the proteome of six SASC and VASC lines from old and young rabbits was analysed using a nano-flow reversed-phase liquid chromatography coupled mass-spectrometry based label-free quantification approach.

We found that the overall protein expression and metabolic capacity (mitochondrial respiration) of adipose-derived mesenchymal stem cells differed according to the tissue origin and age of the donor. The expression of mitochondrial proteins such as the voltage-dependent channels (VDAC1-3) and proteins involved in cholesterol metabolism was altered in SASCs from old donors. In addition, VASCs showed changes in caveolae-associated proteins and plasma membrane rafts (CAV1, CAVIN1, FLOT2) depending on the age of the donor. Our data suggest that disturbances in cell metabolism and associated protein expression may be one reason for the ageing of mesenchymal stem cells in adipose tissue.

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References

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