Poster Session

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Consequences of lysosomal acid lipase deficiency on skeletal muscle functions, formation, and metabolism

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An important role of lysosomal proteolysis in skeletal muscle (SM) has been confirmed in many studies. However, studies on lipid hydrolysis in this case are limited. We hypothesize that lysosomal acid lipase (LAL), which hydrolyzes triglycerides (TGs) and cholesteryl esters (CEs) at acidic pH, may play an essential role in SM formation, function, and metabolism.

To study the consequences of LAL deficiency in SM, we used Lal-deficient (-/-) mice that showed lower running distance and workload in treadmill experiments, confirming increased fatigue due to impaired SM functions in these mice. Additionally, Lal-/- mice exhibited markedly reduced SM mass, cross-sectional area, and Feret diameter, which might be a sign of impaired muscle growth. Total cholesterol (TC) and CE concentrations were higher in the SMs of Lal-/- mice, whereas TG content was significantly decreased. The enhanced glucose uptake in the SM was more than compensated by the lack of fatty acids as substrate for energy production in Lal-/-. Nevertheless, the expression of MyHC, which is specific for slow oxidative fibers, was elevated in Lal-/- muscles.

In contrast to mice, C2C12 myoblasts treated with the LAL inhibitor Lalistat-2 had more lipid deposition and higher TG and CE concentrations. However, myofiber formation was comparable in Lalistat-2-treated and untreated C2C12 cells. These differences between the *in vivo* and *in vitro* models (particularly in TG concentrations) are likely attributable to the inaccessibility of fatty acids in Lal-/- mice due to the lack of white adipose tissue, lower plasma TG concentrations, and the increased glucose uptake in Lal-/- mice.

We conclude that LAL is critical in SM functions and metabolism, but not in the production of myofibers.

Metabolic alterations during aging in C57BL/6NRj mice

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Aging affects metabolism and is associated with the occurrence of metabolic disorders. Therefore, it is crucial to understand age-related alterations to develop -aging strategies.
In the current study, we have analyzed tissues, including the brain, skeletal muscle, liver, lung, and heart of female and male C57BL/6NRj mice, aged 3, 6, 12, and 24 months. Metabolite changes were determined by Nuclear Magnetic resonance (NMR) spectroscopy to identify metabolic features of aging and sex-specific differences. The use of multivariate data analysis techniques and chemometrics revealed significant separation between the age groups in the distinct tissues. Besides, we identified the most prominently changing metabolites in studied female and male tissues. A refined analysis of individual metabolite levels during aging revealed a biphasic pattern for various metabolites in the brain, heart, liver, and lung. In contrast, a linear decrease of amino acids was apparent in muscle tissues. Numerous metabolite changes already occurred at six months. Substantially sex-specific variations in regard to metabolites were identified in the liver.
We assume that age-related metabolic changes already occur at a comparably early aging state based on these results. Moreover, identified differences between female and male tissues stress the importance of distinguishing between sexes when studying age-related changes and developing new treatment approaches. Notably, age-related alterations in C57BL/6NRj mice were substantially different from previously studied C57BL/6J mice, stressing the importance of selecting the appropriate strain for studying aspects of aging.

Investigation of mitochondrial dysfunction and cellular senescence in different models and organisms

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Aging is a complex process that affects every living organism at the cellular level. The study of cellular senescence, the aging process of cells, has proven to be a valuable tool to elucidate molecular processes of aging. The eukaryotic protein fumarylacetoacetate hydrolase domaincontaining protein 1 (FAHD1) acts as a mitochondrial oxaloacetate decarboxylase and is involved in the regulation of the tricarboxylic acid cycle (TCA; citric acid cycle). Previous work in our lab has shown that reduction of FAHD1 levels or complete removal of the protein, either by knockout (KO) or knockdown (KD), ultimately leads to mitochondrial dysfunction and the onset of premature cellular senescence, in different human and murine cell types.
In this study we are exploring the effects of a Fahd1-KO in cells and mouse organs, as preliminary data suggested its possible implication in the regulation of the enzyme succinate dehydrogenase (SDH) (e.g., complex II of the ETC) and a direct link to the onset of premature senescence. For this purpose, we are using mouse embryonic fibroblasts (MEFs; wild type and Fahd1-KO) to study on one hand, the effects of Fahd1-KO on the growth and metabolism of these cells.
On the other hand, we are studying the effects of Fahd1-KO on mouse organ morphology and architecture, focusing on kidney, liver and heart. In parallel, we are developing a protocol for the reliable detection not only of FAHD1 but also of classical senescence markers in paraffin-fixed organ sections.

A guide for an improved chemogenetic approach: Spatiotemporal control of H_2O_2 generation in single cells

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Hydrogen peroxide (H_2O_2), a prominent member of reactive oxygen species (ROS), serves as a signaling molecule at low concentrations and mediates physiological responses. However, at high concentrations, H_2O_2 becomes toxic and induces pathological oxidative stress. For many decades, the traditional method to study downstream effects of H_2O_2 has been through the exogenous application of H_2O_2 onto cultured cells and tissues. Yet this sort of application does not always truly mimic the physiological responses driven by endogenous sources.

As an alternative method, a chemogenetic tool based on D-amino acid oxidase (DAAO) was developed, which enabled on-demand intracellular H_2O_2 generation, through D-amino acid provision or withdrawal. However, this informative tool was poorly refined for living cells. In this study, we present insightful protocols for the comprehensive characterization of an evolved DAAO mutant (mDAAO) with enhanced catalytic activities. To detect mDAAO-sourced H_2O_2 generation, we employed an ultrasensitive H_2O_2 probe, HyPer7. Here were report correct localization, reversible functionality, and concentration-dependent kinetic activity of mDAAO at different subcellular locales such as mitochondrial matrix, nucleus, and cytosol. We identified distinct H_2O_2 generation profiles upon administration of different D-amino acids and observed a presumptive cross-competition between alanine stereoisomers at the level of amino acid transporters. We were the first to show H_2S production by mDAAO upon D-cysteine provision at single cell resolution by using genetically encoded H_2S biosensor, hsGFP. We also showed distinguished chemogenetic H_2O_2 generation profiles for different cell types.

We anticipate that the spatiotemporal control of mDAAO-driven H_2O_2 generation will serve as a valuable method to study downstream implications of redox perturbations in many areas of research such as aging, cancer and metabolomics where ROS are considered critical regulators of (patho-)physiological events.

Role of CLCA2 in stress-induced premature senescence

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The process of aging is characterized by a decreased ratio in the replacement of cells and an altered cellular function that leads to a loss of physiological conditions. In the context of skin aging, an increase in the number of senescent cells has been reported. These cells are characterized by different hallmarks that are subject of study such as cell cycle arrest, an increased expression of cell cycle inhibitors, accompanied with the secretion of a wide range of pro-inflammatory molecules, immunomodulators and proteases collectively known as SASP (senescence associated secretory phenotype), mitochondrial dysfunction and loss of proteostasis, among others. Recently, it has been published that senescent cells have decreased intracellular pH, and that the suppression of a mechanism counteracting this acidic pH could provoke senolysis. The origin of this pH alteration is unknown, but it could be related with the oxidation of macromolecules, dysregulated electrochemical gradients, metabolic changes and/or lysosomal damage.

Our study is focused on stress-induced premature senescence of dermal skin fibroblasts, and several models to study these processes were previously stablished in our group. Bioinformatics analysis of RNAseq and microarray data obtained from fibroblasts submitted to UVB- and tBHP-induced senescence was performed, looking for common differently expressed genes. From our candidates, CLCA2 was upregulated in both models. Upregulation of this gene was validated by qPCR, western blot and immunofluorescence. The protein CLCA2 was described as a regulator of chloride channels, which might be important for pH regulation in the senescence process.

We are currently investigating the role of CLCA2 and its participation in intracellular pH regulation during stress-induced premature senescence.

Is *LPA* a longevity gene?

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There are several plasma lipids and apolipoproteins implicated with longevity. The most cited is HDL-cholesterol or apoAI. This has been explained by their anti-atherogenic, anti-oxidative and immune modulating properties. Lipoprotein(a) whose characteristic protein is coded by the apo(a) gene LPA, however, is considered as one of the most atherogenic plasma lipoprotein and in fact individuals with elevated Lp(a) suffer from myocardial infarction, stroke, heart failure and eventually from early death. Apo(a) is a rather complex glycoprotein with numerous genetic isoforms that strongly mediate gene expression and plasma levels. Although the physiological function of apo(a) is merely unknown it has been speculated that nature hardly creates such a complicated protein without any reason.

We have measured plasma Lp(a) in three generations in a family kindred and found that levels increase significantly with age. We also found that the Lp(a) plasma concentrations in octo-nonagenarians were significantly higher as compared to a Caucasian population with an average age of 54 years – the opposite that we had expected. We speculate that individuals with high Lp(a) are prone to heart diseases only if they suffer from additional risk factors. In the absence of additional risk factors, apo(a) might be beneficial and life extending under certain conditions. The physiological basis for this might be manifold and mostly relates to protection from cancer. There are several such pathways discussed relating among others to angiogenesis, Ox-Phos and secretory phospholipases that will be discussed during the poster presentation.

EGCG promotes condensate formation of fused-in-sarcoma in a methylation-dependent manner

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Millions of people worldwide are affected by neurodegenerative diseases (NDs), and to date, no effective treatment has been reported. The hallmark of these diseases is the formation of pathological aggregates and fibrils in neural cells. Many studies have reported that catechins, polyphenolic compounds found in a variety of plants, can directly interact with amyloidogenic proteins, prevent the formation of toxic aggregates, and in turn play neuroprotective roles. Besides harboring amyloidogenic domains, several proteins involved in NDs possess arginine-glycine/arginine-glycine-glycine (RG/RGG) regions that contribute to the formation of protein condensates.

Here, we aimed to assess whether epigallocatechin gallate (EGCG) can play a role in neuroprotection via direct interaction with such RG/RGG regions. We show that EGCG directly binds to the RG/RGG region of fused in sarcoma (FUS) and that arginine methylation enhances this interaction. Unexpectedly, we found that low micromolar amounts of EGCG were sufficient to restore RNA-dependent condensate formation of methylated FUS, whereas, in the absence of EGCG, no phase separation could be observed.

Our data provide new mechanistic roles of EGCG in the regulation of phase separation of RG/RGG-containing proteins, which will promote understanding of the intricate function of EGCG in cells.

The role of melanocytes in skin pigmentation, senescence, and skin aging induced by exposure to environmental stressors

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Extrinsic aging of human skin is mainly a result of exposition to environmental factors such as sunlight, air pollution, and cigarette smoke. Melanocytes become less active and senescent with aging and environmental factors can lead to premature aging and pigmentation disorders. However, the mechanisms involved under exposition of melanocytes to environmental stressors are not fully understood.

We treat melanocytes with UV (UVA+UVB) or urban particulate matter (UPM) or a combination of these two stressors (UV+UPM), in order to understand how these environmental stressors affect melanocytes, but also the skin as a whole by *ex vivo* experiments. To investigate morphological and physiological parameters we examined proliferation, senescence status, apoptosis, pigmentation, and DNA damage.

Preliminary results using UV or UPM alone as well as the combination of both stresses have demonstrated that melanocytes respond diversely to each different type of stress in terms of senescence markers, cell survival and pigmentation. Accordingly, all three types of treatments induced different changes in *ex vivo* skin biopsies, indicating that an understanding of the underlying molecular processes activated in response to these treatments are vital to estimate the impact of exposure to such environmental factors on the progression of skin aging and health in general.

Taken together, this new experimental setup will allow us to perform further research on mechanisms of extrinsic skin aging, including the role of melanocytes in this process and could give rise to the development of new therapeutical targets for pigmentation disorders and premature skin aging.

No death without sweets?
Hexokinase 1 prevents mitochondrial fission during energy stress

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Dysregulation of cell death due to mitochondrial malfunction is increasingly associated to aging and age-related diseases. In normal aging, increased cell death contributes to the loss of non-dividing cells, such as neurons, causing neurodegenerative diseases. In contrast, resistance to cell death in cancer cells and senescent cells, increases their abundance during aging. Therefore, mechanisms are needed to regulated cell death. A promising mitochondrial bound enzyme, Hexokinase 1 (HK1), is described to prevent cell death. However, it is not entirely clear how HK1 prevents cell death.

Here we reveal that HK1 forms astonishing ring structures during energy stress. HK1-rings are localized at mitochondrial fission sites, characterized by the contact with endoplasmic reticulum membrane. Finally, we demonstrate that HK1-rings prevent mitochondrial fission during energy stress, thereby most likely preventing cell death pathways.

Given the high expression levels of HK1 in neurons, this novel function of HK1 have multiple implications in aging of the brain.

Combining oxidative phosphorylation inhibitors with nutrient restriction to target metabolic flexibility in hepatocellular carcinoma

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Cancer cells utilize both oxidative phosphorylation (OxPhos) and aerobic glycolysis for energy production, and can also tune these metabolic pathways to support their proliferation and biomass production. Hence, inhibiting one of the two pathways can lead to the compensatory upregulation of the respective pathway, leading to resistance to primary metabolic therapies. We previously showed that if we therapeutically inhibit OxPhos and simultaneously reduce glycolysis through glucose restriction, this can lead to significant reduction in cancer cell viability and tumor growth.

Here, we hypothesized that, in general, OxPhos inhibitors in combination with nutrient restriction can reduce cancer cell viability, comprising a broad treatment strategy for hepatocellular carcinoma (HCC), the predominant form of liver cancer. To test this hypothesis, we used three HCC-derived cell lines, which were treated with six different OxPhos inhibitors in standard growth medium and in low glucose or glucose-free starvation media. Based on the initial viability screen, we selected two inhibitors, gboxin and atovaquone, for further analyses in HepG2 cells. The effect of atovaquone on cell viability was significantly enhanced already in low glucose medium, while gboxin was effective only in combination with glucose-free medium. Metabolic profiling demonstrated that HepG2 cells highly rely on OxPhos, which was significantly inhibited by both drugs. However, the cells seemed to be able to overcome this inhibition by increasing their glycolytic activity in the presence of glucose in the medium. Finally, the glucose depletion was necessary to inhibit both pathways and completely block cell growth.

Together, our results shed light on the synergistic effect of glucose depletion and OxPhos inhibition in HCC-derived cell lines and prompt further investigation of nutrient deprivation as adjunct to OxPhos inhibitors for HCC therapy.

The disordered p53 transactivation domain is the target of FOXO4 and FOXO4-DRI

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A central process contributing to the phenotype of aging is cellular senescence, which is characterized by a stable cell cycle arrest associated with macromolecular alterations and secretion of proinflammatory cytokines and immune modulators. The FOXO4 – p53 axis has been identified as pivotal in maintaining the viability of senescent cells. Even though reports have identified the binding sites, structural information of the complexes and the regulatory mechanisms controlling this interaction are elusive.

Here, we unraveled the disordered p53 transactivation domain as the main target of FOXO4 Forkhead binding. In addition to this interface, we identified binding between the DNA binding domain of p53 and the disordered N-terminal and C-terminal regions of FOXO4. NMR-based structural models of the FOXO4 Forkhead Domain in complex with the transactivation domain 2 of p53 revealed that the transactivation domain 2 folds upon binding to a positively charged surface provided by the FOXO4 Forkhead Domain. FOXO4-DRI is a potent senolytic peptide, which was previously described to interfere the FOXO4-p53 interaction. We show that FOXO4-DRI binds to the p53 transactivation domain 2 thereby competing with FOXO4 Forkhead binding. Both FOXO4-DRI and the p53 transactivation domain 2 are disordered in solution, but fold synergistically upon binding. Furthermore, we show that phosphorylation of p53 transactivation domain 2 enhances the binding affinity for FOXO4 Forkhead and FOXO4-DRI. Taken together these data provide a detailed biophysical characterization of the FOXO4 – p53 interaction and the interaction between p53 and FOXO4-DRI, which is fundamental to understand regulation of cellular senescence and to develop potent inhibitors to alleviate age-related diseases.

T3-induced enhancement of mitochondrial Ca^{2+} uptake as a boost for cellular metabolism

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Thyroid hormones are the main regulators of cellular metabolism, conveying their action via regulation of expression changes. Thereby, the biologically active triiodothyronine (T3) induces the expression of genes to enhance mitochondrial metabolic function. Notably, mitochondrial Ca^{2+} is essential to the function of Ca^{2+}-dependent matrix dehydrogenases and, thus, mitochondrial respiration. However, just few genes are controlled in their expression by thyroid hormones, among others the uncoupling proteins 2 and 3 (UCP2/3). The biologically T3 induces upregulation of UCP2/3 in various cell types.

In the current study, we studied the impact of T3 on $[Ca^{2+}]_{mito}$ homeostasis. T3 induced a significant upregulation in mRNA expression of UCP2 and UCP3 and of protein arginine methyltransferase 1 (PRMT1) in HeLa cells after 3 h. Live-cell imaging in HeLa cells expressing mitochondrial-targeted Ca^{2+} biosensors revealed that short-time incubation (3 h) with T3 elevates basal $[Ca^{2+}]_{mito}$ and causes increased $[Ca^{2+}]_{mito}$ uptake upon Ca^{2+} depletion of the endoplasmic reticulum (ER), while cytosolic Ca^{2+} levels remained unchanged. Also, T3-induced enhancement of mitochondrial Ca^{2+} uptake depends on the mitochondrial Ca^{2+} uniporter (MCU), UCP2, and PRMT1 that are essential for increased mitochondrial ATP ($[ATP]_{mito}$) production after T3 treatment. T3's impact on $[Ca^{2+}]_{mito}$ correlates with the expression and activity of UCP2, MCU und PRMT1 and translates into increased $[ATP]_{mito}$. Increases in mitochondrial ATP and $[Ca^{2+}]_{mito}$ supply the production of reactive oxygen species (ROS).

We revealed that enhanced mitochondrial Ca^{2+} uptake is essential to elevate mitochondrial ROS production after 3 h of T3 incubation. These results suggest that mitochondrial Ca^{2+} homeostasis is essential for the role of T3 in controlling metabolic activity.

p53 transactivation domain mediates binding and phase separation with poly-PR/GR

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The most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) is the presence of poly-PR/GR dipeptide repeats, which are encoded by the chromosome 9 open reading frame 72 (C9orf72) gene. Recently, it was shown that poly-PR/GR alters chromatin accessibility, which results in the stabilization and enhancement of transcriptional activity of the tumor suppressor p53 in several neurodegenerative disease models. A reduction in p53 protein levels protects against poly-PR and partially against poly-GR neurotoxicity in cells. Moreover, in model organisms, a reduction of p53 protein levels protects against neurotoxicity of poly-PR. The mechanistic details leading to poly-PR mediated stabilization and activation of p53 remain enigmatic.

Here, we aimed to study the detailed molecular mechanisms of how p53 contributes to poly-PR/GR-mediated neurodegeneration. Using a combination of biophysical techniques such as nuclear magnetic resonance (NMR) spectroscopy, fluorescence polarization, turbidity assays, and differential interference contrast (DIC) microscopy, we found that p53 physically interacts with poly-PR/GR and triggers liquid–liquid phase separation of p53. We identified the p53 transactivation domain 2 (TAD2) as the main binding site for PR25/GR25 and showed that binding of poly-PR/GR to p53 is mediated by a network of electrostatic and/or hydrophobic interactions.

Our findings might help to understand the mechanistic role of p53 in poly-PR/GR-associated neurodegeneration.