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Relating cellular size to function in therapy-induced senescence

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Inducing the senescent cancer cell (SenCC) fate has been considered a desired outcome during chemotherapeutic interventions. However, more recent studies show that SenCCs are able to escape senescence and develop strongly enhanced clonogenic growth potential thus mediating cancer relapse upon chemotherapy withdrawal (Milanovic et al. Nature, 2018). Although it is becoming clear that therapy-induced SenCCs are physiologically and clinically important, their basic cell biology and the ability to evade or modify classic senescence programs remain greatly understudied.

Treating various human epithelial cancer cell lines in vitro with one of the most commonly used in oncology chemotherapeutics Taxol, we noted that at clinically relevant concentrations this agent leads to the emergence of drug-tolerant persister cells. These cells possess molecular and morphological signatures of somatic cells undergoing senescence. However, unlike senescent normal somatic cells (Neurohr et al. Cell, 2019 and Lanz et al. Mol Cell, 2022), therapy-induced SenCCs display significantly increased nucleocytoplasmic ratio due to mitotic slippage-mediated polyploidization paralleled with increase in whole-cell size.

We surmised that polyploidization ($n > 2$) should increase protein translation by at least the factor of 1.5 in SenCCs. However, our measurements of total protein synthesis show that SenCCs decrease their protein production by ~ 30%. Given that SenCCs dramatically increase in size, their inability to scale-up protein production might lead to the dilution of some critical protein regulators. This is especially relevant for short-lived proteins such as DNA-binding proteins (Li et al. Mol Cell, 2021) including those responsible for DNA reparation. Such underscaling can lead to the accumulation of unrepaired DNA lesions (Gemble et al. Nature, 2022). Indeed, we found that SenCCs accumulate and tolerate excessive DNA damage. The latter might contribute to the acquirement of aggressive properties such as enhanced migration (Nader et al. Cell, 2021) and clonogenic survival potential (Larsen et al. Science, 2022), which we found to be more pronounced in large polyploid SenCCs than in their smaller-sized counterparts. We are currently dissecting the molecular mechanisms that lead to downscaling of protein production in therapy-induced SenCCs.

2

A cellular device to target cancer cells

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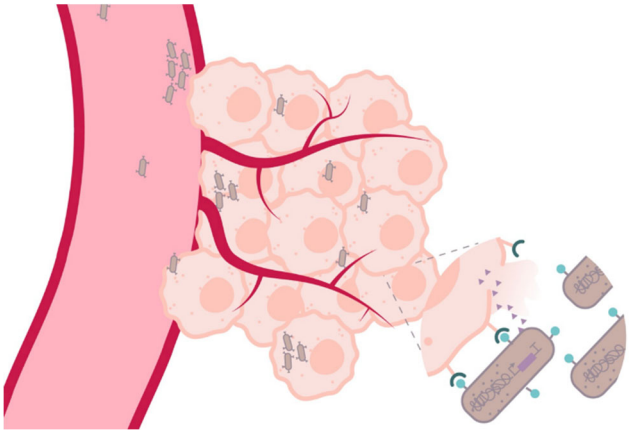
Cancer is a widespread and serious disease, impacting the lives of one in three individuals during their lifetime. Due to its high incidence, numerous treatment methods have been developed, with the Chimeric Antigen Receptor T-Cell (CAR-T) therapy remaining the most promising one. Chemotherapy, and radiation therapy are also widely used, however, they are not as effective as they cannot differentiate between healthy and cancerous tissues.

On the other hand, surgery remains an important treatment method, but it still is limited in terms of cancer types it can cure.

Therefore, there is an imperative need to create new cancer treatment methods that can provide accurate targeting and deliver therapeutics precisely.

In this thesis study, we have created an engineered bacterial device that can identify Jimt1 breast cancer cells, which are characterized by an excessive expression of human epidermal growth factor receptor 2 (HER2). The bacterial device contains 2Rs15d, a nanobody that binds to the HER2 receptor, expressed on its surface via Ag43 autotransporter protein. Upon localizing the tumor site, a therapeutic agent will be released into the medium.

Through this platform, we aim to address the significant drawbacks of current cancer therapies.



A visual representation of the project's concept. Engineered bacteria move in the bloodstream and upon localizing themselves in the breast cancer site release a toxin that kills Jimt1 breast cancer cells.

Reference: Ostaku, J. (2021). A cellular device to target cancer cells. Bilkent University. Retrieved from http://repository.bilkent.edu.tr/handle/11693/76607

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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.

4

Tyrosine mediated nuclear import of CIRBP reveals a flexible NLS recognition by TNPO3

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Transportin 3 (TNPO3) is a nuclear transport receptor (NTR) binding to the cargo nuclear localization signal (NLS) containing a sequence of arginine-serine (RS) repeats. The binding is responsible for the nuclear import of proteins harbouring such NLS and requires serine phosphorylation. Previously, our group identified a tyrosine-rich region mediating the nuclear import of cold-inducible RNA binding protein (CIRBP), which is phosphorylation-independent. In this study, we aimed to investigate molecular details of this interaction.

Using crystallography with X-ray diffraction, the binding site on TNPO3 has been identified to overlap with the one for classical RS regions. The interaction is mediated by a short motif containing one arginine and three tyrosines at the C-terminal of CIRBP. Among these residues, R161 and Y164 are necessary for the interaction, whose mutations disrupt the TNPO3-CIRBP interaction and suppress the nuclear import of CIRBP.

In contrast to conventional RS regions, phosphorylation of serine and tyrosine in the NLS of CIRBP manifest suppressive effect to its interaction with TNPO3, showing a possible role as nuclear import inhibitor. From this study, we identified a new mechanism of nuclear import by TNPO3, with the binding through unmodified side chain of arginine and tyrosine.

This indicates the cargo-recognition of TNPO3 could be via flexible binding surface, which is possibly a common mechanism for its interactions with proteins not having classical RS-NLSs.

5

Multi-omics in one assay: qPRO-seq to decipher chromatin and transcriptional landscapes of fasting in adipose tissue

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Adipose tissue (AT) plays a central role in the regulation of systemic energy homeostasis through its endocrine function and storing excess lipids, that are released at the onset of fasting, upon which AT globally rewires its gene expression (GE) program. But the regulatory determinants are not well understood. Key players of the fasting response are specific transcription factors (TFs) that bind to promoter and enhancer regions. Enhancer RNAs (eRNAs) are short and unstable RNAs, that are generated from TF-bound enhancers. This study aimed to identify TF networks and eRNAs that coordinate the early response to nutrient withdrawal in mouse white AT over a fasting period of 3 hours.

Quick precision nuclear run-on sequencing (qPro-seq) utilizes active RNA polymerase II genome-wide, allowing detection of nascent RNA transcripts. Data were analyzed and visualized using several bioinformatics tools and R scripts.

Validating our method, we found genes involved in ß-oxidation to be upregulated after three hours of fasting, including Acads and lipid transporter slc25a20, while genes involved in lipid storage, including Gk and lipases, were downregulated after just one hour of fasting. Interestingly, GE analysis indicates immune cell infiltration as early as after three hours of fasting (e.g. Il1r1, Reg4). We detected more than 250 fasting activated enhancers that were annotated to the respective nearest genes, resulting in overrepresentation of various pathways depending on duration of fasting. Motif enrichment analysis of fasting selective enhancers identified several known adipose tissue specific TFs, such as GRE, CEBP, RXR, as well as novel ones, like golgi-associated olfactory signaling receptor (GFY).

In future, we will focus on analysis of super enhancer clusters, pathways and wet lab experiments to validate our findings. Since AT is very heterogenous and undergoes extensive remodeling upon fasting, cell-type specific TF network analysis and enhancer detection is envisioned.

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Purification and cryo-EM of TRPC3

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Calcium permeable transient receptor potential channel 3 (TRPC3) is a lipid-sensitive channel mainly expressed in brain and heart cells. Dysfunction of TRPC3 leads to diseases such as cardiac hypertrophy and dementia. In order to understand the role of TRPC3 in pathology and to develop suitable pharmacological strategies to control this channel, the protein structure as well as possible ligand binding sites need to be discovered. Previous studies revealed the structure of TRPC3 without specific ligands and only in one functional state – the closed conformation. Knowledge about the protein structure in other functional states will allow deeper understanding of TRPC3 activity and conformational changes in ligand binding sites during channel gating.

We hypothesize that the lipid environment around TRPC3 is an important key feature in channel function and activity. We assume that previous purification procedures resulted in dramatic changes in the lipid environment leading to a non-functional channel.

The aim of this study is to purify TRPC3 under near-native conditions and to resolve the structure in the active conformation in order to identify the determinants of the channel's activity and its binding sites.

To achieve this goal, we used new lipid nanodiscs systems which retain the natural environment of the protein. These nanodiscs are stabilized by new amphiphilic copolymers, as well as one small-molecule glycoamphiphile. We successfully extracted TRPC3 from Pichia pastoris and HEK293 cells. Then, we used the most promising compounds to purify TRPC3 from both cell types.

In summary, two new amphiphilic copolymers and the small-molecule-amphiphile can extract TRPC3 from both cell types and are therefore used for protein purification. So far, we successfully purified TRPC3 from both cell types, by using the small-molecule amphiphile.

7

Influence of age on the tumor microenvironment in a cohort of non-small cell lung cancer

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Immune Checkpoint Blockade (ICB) led to better outcomes in non-small cell lung cancer (NSCLC) but only a subset of patients benefits from current treatment regimens. Different molecular subtypes show diverse responses to treatment. Additionally, age is suggested to be an important factor in ICB response. It was reported that the survival advantage of ICB in comparison to conventional therapy was only modest in patients aged over 75. To evaluate the influence of age on the immune environment (IE) in lung cancer, a thorough characterization was performed in patients with untreated NSCLC.

To characterize the immune environment, flow cytometry and multiplex immunohistochemistry were used. Additionally, TCR sequencing and RNA sequencing were performed. The findings were validated in public datasets.

Higher infiltration of T cells in older patients was found, which could be mainly attributed to CD4 T cells. Validation in the TCGA-LUAD cohort additionally revealed an upregulation of regulatory T cells. No difference was found regarding T cell clonality and Tumor Mutational Burden (TMB)

Higher regulatory T-cell infiltration is associated with treatment failure of ICB. Further research on the biochemical mechanisms of regulatory T cells in ICB response elderly lung cancer patients is warranted.

8

The sex-specific metabolic signature of C57BL/6NRj mice during aging

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Aging goes along with alterations in metabolism, which are currently discussed as potential targets for anti-aging strategies. Due to intact reactive oxygen species homeostasis and glucose metabolism, C57BL/6NRj mice are especially suitable to study cellular alterations in metabolism. We applied Nuclear Magnetic resonance spectroscopy to analyze five different tissues of this mouse strain during aging and included female and male mice aged 3, 6, 12, and 24 months. Metabolite signatures allowed separation between the age groups in all tissues, and we identified the most prominently changing metabolites in female and male tissues.

A refined analysis of individual metabolite levels during aging revealed an early onset of age-related changes at 6 months, sex-specific differences in the liver, and 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.

Based on these results, we assume that age-related metabolic alterations happen at a comparably early aging state and are potentially associated with a metabolic switch. 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.

Besides, metabolomic features seem to be highly dependent on the genetic background of mouse strains.

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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.

The curious case of JTV-519 as an inhibitor of MICU1-dependent mitochondrial Ca^{2+} uptake

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The mitochondrial Ca^{2+} uptake (mtCU) is a complex mechanism that is established by multiple proteins. The mitochondrial Ca^{2+} uniporter (MCU) together with the essential MCU regulator (EMRE) forms a multimer thereby creating a Ca^{2+}-permeable pore, which is regulated by mitochondrial Ca^{2+} uptake 1 (MICU1). As pivotal powerhouse of the cell, mitochondria are involved in many pathologies and the protein complex involved in mtCU has got a lot of attention as promising target for pharmacological interventions.

In the present study, we used genetically encoded Ca^{2+} indicators targeted to different cellular and subcellular compartments to test the effect of JTV-519 on the mtCU in HeLa and EA.hy926, a human umbilical vein endothelial cell-derived cell line. Notably, JTV-519 is in clinical use because of its cardioprotective effect by inhibiting the channel activity of ryanodine receptor 2 (RyR2) and the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA). In our study, JTV-519 selectively prevents the mtCU in these cell types with an approximately 10-fold higher potency than its other reported effects. Application of siRNA against MICU1 prevented the inhibitory effect of JTV-519 on mtCU, indicating that MICU1 is a target of JTV-519.

Hence, applying an already established MICU1-CFP – MICU1-YFP FRET interaction assay in single cells, JTV-519 was found to inhibit Ca^{2+}-triggered dimerization of MICU1. Further findings, in which the siRNA-mediated knockdown of protein arginine N-methyltransferase 1 (PRMT1), which methylates MICU1 at R455, abolished the inhibition of mtCU by JTV-519 suggests that methylated MICU1 is a prerequisite for the interaction with JTV-519. Additional analyses reveal an impact of JTV-519 on mitochondrial bioenergetics, morphology, mobility, fragmentation and ER – mitochondria colocalization.

We herein report that JTV-519 is more potent to prevent mtCU than all its other reported effects. However, to inhibit mtCU PRMT1-methylated MICU1 has to be present, thus, presenting JTV-519 as potential compound to selectively act on cancer and senescent cells with their high PRMT1 activity.

11

Exploring sleep deprivation induced deficits in AD fruit fly model

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Alzheimer's disease (AD) is a severe aging-associated neurodegenerative disorder, characterized by memory loss, paranoia, sudden changes in behaviour as well as sleep alterations. It has been postulated that sleep disturbance triggers a feedback process accelerating AD progression, by impairing the clearance of amyloid beta, one of the main pathological hallmarks associated with AD.

Here, we use the fruit fly as a model system to investigate amyloid beta induced pathology prompted by sleep deprivation. After utilizing The Sleep Nullifying Apparatus (SNAP), we allow flies to regenerate, after which we assess their general health status by using a novel automated climbing assay apparatus. Using our fly tracking algorithm analysis, we observe sleep deprivation induced motoric deficits in an AD fruit fly model. This system may be used for evaluation of compounds which improve sleep quality, thereby counteracting amyloid toxicity.

Thus, we present a simple approach for the identification of possible therapeutic interventions targeting sleep deficits in AD, which might provide a strategy for the identification of new potential AD therapies.

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Novel insights into the metabolic and autophagic control of starvation-induced lifespan extension in yeast

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Autophagy is a versatile and conserved recycling process that serves essential roles in maintaining cellular and organismal homeostasis. During starvation, autophagy is increased and degrades cargo, providing the means to endure nutrient scarcity. The natural and body-endogenous polyamine spermidine can induce autophagy when exogenously administered to model organisms and extends lifespan in an autophagy-dependent fashion. Similar to autophagic routines, the polyamine pathway is highly conserved and essential for cellular survival and function.

Here, we report that starvation drives polyamine biosynthesis in yeast cells in an enzymatic manner. Genetic or pharmacological perturbations of polyamine biosynthesis drastically alter the metabolic and proteomic responses to starvation. Consequently, starvation-induced autophagy is diminished in yeast cells deprived of polyamines, alongside a loss of survival benefits elicited by starvation during chronological aging.

In sum, we identified the internal polyamine pathway as a critical regulator of the metabolic, proteomic and autophagic responses to starvation, which ultimately confer starvation-associated lifespan extension during chronological aging in yeast.

microRNA as predictive molecule for in vitro fertilization process

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Infertility currently affects a significant part of the population and due to its high prevalence is classified by the WHO as a population disease. In regard of the increasing trend of infertility in the world, more and more couples are seeking for the help of assisted reproduction and, at the same time, the number of performed *in vitro* fertilizations (IVF) is increasing. Under physiological circumstances the key processes determining the IVF success are the window of implantation, the endometrial receptivity and the selection of a competent embryo. These processes are regulated at the gene level. The main factors that influence the regulation of gene expression are short non-coding RNAs (sncRNAs), which modify gene expression, especially at the post-transcriptional level. The elucidation of the action mechanism of specific microRNAs in the context of infertility proves the possibility of their use in clinical diagnostics.

The aim of this work was to differentiate microRNAs molecules using next-generation sequencing (NGS) and bioinformatic analyses, which would predict the patient's readiness for implantation of the embryo into the uterus in the in vitro fertilization process. Sequencing was performed using a NextSeq 500 (Illumina). The evaluation of the selection of biomarker molecules was based on at least one of the methods with the determination of the correlation of the molecules and the machine learning methods (e.g. Random Forest Classifier, SVC, SVM, SGD Classifier).

Of the total number of miRNAs identified 6 specific molecules were selected (miR-26a-2-3p, miR-874-5p, miR-6734-5p, miR-4767, miR-4775, miR-18b-3p) showing a high predictive specificity of the patient´s successful outcome in the IVF process.

Selected miRNA molecules in the presented study appear to be promising prognostic-predictive molecules that could play a key role in predicting the readiness of women for the IVF process as well as in predicting of successful IVF outcome.

AnnexinV-VDAC1 Interaction Regulates Mitochondrial Ca^{2+} Access Capacity

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Mitochondrial Ca^{2+} homeostasis plays a key role in shaping cytosolic Ca^{2+} signaling, governing energy production and apoptosis. While the movement of Ca^{2+} ions across the inner mitochondrial membrane (IMM) is strictly determined by the activity of Mitochondrial Calcium Uniporter Complex, in the outer mitochondrial membrane (OMM) the permeability to Ca^{2+} ions are achieved via the Voltage-dependent Anion Channel 1 (VDAC1).

Here, we report the role of the Ca^{2+}-binding and phospholipid-binding protein, Annexin V (AnxV) in the Ca^{2+} access capacity of the mitochondria via its interaction with VDAC1 in HeLa cells. Strikingly, immunoblotting of isolated mitochondria with proteinase-K digestion as well as electron microscopy images revealed the localization of AnxV at the cytosolic face of the OMM and within the mitochondria. Using proximity ligation assay, we confirmed the intermolecular interaction between AnxV and VDAC1.

Hence, by using various genetically encoded Ca^{2+} sensors targeted either to the cytosol, the intermembrane space (IMS), the mitochondrial matrix, or cristae lumen (CL) we showed that AnxV knockout (KO) severely decreased mitochondrial Ca^{2+} elevations in IMS, the CL, and the matrix upon stimulation with an IP3-generating agonist. Notably, mitochondrial-associated membranes (MAMs), mitochondrial membrane potential, cytosolic Ca^{2+} levels, and Ca^{2+} release kinetics of the endoplasmic reticulum (ER) were not affected by AnxV-KO.

Hence, AnxV was found to be fundamental for mitochondrial Ca^{2+} uptake of high Ca^{2+} elevations with the mitochondria - ER junction but does not play a role in mitochondrial Ca^{2+} sequestration of global moderate Ca^{2+} elevations due to store-operated Ca^{2+} entry (SOCE). Our present data highlight the Ca^{2+} source-dependent involvement of AnxV in mitochondrial Ca^{2+} uptake in OMM via interacting with VDAC1.

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The spatial and temporal relationship between cellular senescence and the process of skin healing

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The term "cellular senescence" refers to an irreversible cell cycle arrest that can be caused by a variety of stressors and is accompanied by an accumulation of damage, changes in cell shape and secretory profile, and epigenetic abnormalities in primary cells. There is an accumulation of senescent cells in development, aging and tissue regeneration; all of which are conditions with increased damage and pro-growth stimuli. Importantly, while the reason for this accumulation is unclear, the senescent cells which persist in the tissue directly contribute to the pathology of age-related diseases, chronic wounds and tumor growth.

This poster is aimed at sharing unpublished results on the kinetics of senescent cell accumulation at the sites of wounds in mice and pigs, particularly in acute mechanical wounds. BALB/c mice were wounded on the mid-back with 1 cm diameter full-thickness wounds, while minipigs were injured using a standard 6 mm biopsy punch. Wound samples were taken at various time points to characterize the kinetics of healing and senescence induction.

In addition to elaborating on the temporal relationship between senescence and wound healing, we will also reveal our novel results on the spatial relationship between the site of wounding and senescence induction. Moreover, we demonstrate a clear correlation between senescence markers and features of healing such as reepithelialization and trans-differentiation of fibroblasts.

Finally, we will describe the candidate proteins and pathways involved in the induction of cellular senescence at wounds.

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Presenilin-1 controls pancreatic beta-cell metabolism by regulating mitochondrial Ca^{2+} sensitive NADH shuttles

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Intracellular Ca^{2+} ions are known to regulate many metabolic processes in a cell. We have recently shown that presenilin-1 establishes an endoplasmic reticulum (ER) Ca^{2+} leak directed towards mitochondria in pancreatic beta cells (PMID: 30790505). This leak was found to be indispensable for glucose-stimulated insulin secretion of beta cells (PMID: 31529929).

The aim of current work was to elucidate the mechanism of presenilin-1 mediated regulation of pancreatic beta-cell metabolism. Silencing of presenilin-1 in pancreatic beta cells showed delayed and reduced cytosolic Ca^{2+} oscillations in response to glucose, but not pyruvate, pointing at possible importance of presenilin-1 for glycolysis. Additionally, presenilin-1 knockdown (KD) showed reduced pyruvate production upon glucose elevation, while lactate production was increased, indicating a metabolic switch. In support of the metabolic switch, we detected increased glutamine reliance of presenilin-1 KD cells. Furthermore, cellular redox state was altered by presenilin-1 KD with reduced cytosolic NAD+/NADH ratio, a possible reason for observed metabolic alterations.

Analysis of subcellular Ca^{2+} concentrations revealed reduced matrix and mitochondrial intermembrane space Ca^{2+}, while global cytosolic and ER Ca^{2+} levels were not altered by presenilin-1 KD. Detailed analysis of glycolytic intermediates showed an accumulation of metabolites preceding and including glyceraldehyde-3 phosphate (GAP) in presenilin-1 KD cells, pointing to a bottleneck at the reaction catalyzed by GAP dehydrogenase that requires NAD+. These results led us to hypothesize that presenilin-1 mediated ER Ca^{2+} leak is important for mitochondrial intermembrane space residing Ca^{2+} sensitive NADH shuttles that recycle cytosolic NADH and provide NAD+ for glycolysis. Overexpression of Ca^{2+} insensitive mutants of these shuttles rescued the effects of presenilin-1 KD.

Thus, we have identified presenilin-1 as a regulator of pancreatic beta-cell metabolism, which acts by establishing an ER Ca^{2+} leak that controls mitochondrial Ca^{2+} sensitive NADH shuttles that recycle cytosolic NADH to maintain glycolysis and supply mitochondria with the substrate.