FINAL REPORT

1 General Information

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Project title: Telomerase reverse transcriptase in motion - dynamics of subcellular localization and functional consequences in aging and senescence

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2 Zusammenfassung / Summary

Telomerase ist bekannt als das Enzym, das im Zellkern der Telomerverkürzung entgegenwirkt. Seine katalytische Untereinheit Telomerase Reverse Transkriptase (TERT) findet sich auch in Mitochondrien post-mitotischer Zellen, z.B. im kardiovaskulären System, wo sie eine Rolle in der Erhaltung der Atmungskettenaktivität spielt. Unter bestimmten Bedingungen wird sie aus dem Zellkern exportiert während gleichzeitig die Menge in den Mitochondrien ansteigt. Es ist nicht klar ob die gleichen TERT-Moleküle, die den Zellkern verlassen, auch in Mitochondrien gelangen oder nur neu synthetisierte TERT in diese Organellen importiert wird. Als Voraussetzung für Studien zum intrazellulären TERT-Transport mit fotokonvertierbaren Proteinen haben wir gezeigt, dass die Mikroinjektion solcher Moleküle die Morphologie der Mitochondrien und den Import in diese nicht beeinträchtigt. Zudem haben wir ein exogen exprimiertes fluoreszierendes Protein lokal in lebenden Zellen fotokonvertiert. Um zu untersuchen, welche Mitochondrienfunktionen von TERT beeinflusst werden haben wir das mitochondriale TERT-Interaktom mit BioID charakterisiert. In einer ersten Validierung haben wir die Interaktion mit dem mitochondrialen Transkriptionsfaktor A (TFAM) mit *proximity ligation assays* in murinen kardialen Fibroblasten und primären humanen Endothelzellen verifiziert. Weitere interessante TERT-Interaktionspartner waren mehrere Assemblyfaktoren für Komplex I der Atmungskette und Komponenten des *mitochondrial contact site and cristae organizing system* (MICOS). Die Interaktionen mit den Assemblyfaktoren können als Erklärung für den positive Einfluss von TERT auf die Komplex I-Aktivität dienen. Der MICOS-Komplex ist wichtig für die Ausbildung und Dynamik der Cristae, in denen die Atmungskette lokalisiert ist, und für den Aufbau der Atmungsketten-Superkomplexe, was wiederum die Verbindung zu TERT unterstreicht. Experimente zum Verständnis der Rolle von mitochondrialer TERT in einem physiologischen Kontext zeigten, dass sie nicht nur die mitochondriale Respiration verbessert, sondern auch Endothelzellmigration und Myofibroblastendifferenzierung. Zudem milderte sie die Folgen eines experimentellen Herzinfarkts ab. Schlussendlich konnten wir zeigen, dass der Telomerase-Aktivator 65 (TA-65) den Mitochondrienimport von TERT verstärkt und die gleichen Effekte wie mitochondriale TERT auf Respiration, Endothelzellmigration, Myofibroblastendifferenzierung, und Infarktgröße hat. Überdies verhinderte TA-65 die Induktion von Endothelzellseneszenz. Basierend auf diesen Arbeiten könnte eine detaillierte Charakterisierung der molekularen Mechanismen wie TERT die mitochondriale Respiration verbessert und wie TA-65 den Mitochondrienimport von TERT induziert langfristig dazu beitragen, Strategien zur Verbesserung der kardiovaskulären Gesundheit zu entwerfen.

Telomerase is known as the enzyme counteracting telomere shortening in the nucleus. Its catalytic subunit Telomerase Reverse Transcriptase (TERT) is also present in mitochondria of post-mitotic cells, e.g.in the cardiovascular system, where it plays a role in maintaining respiratory chain activity. Under certain conditions, it is exported from the nucleus with a concomitant increase in the mitochondria. It is unclear, if the same TERT molecules that leave the nucleus enter the mitochondria, or if only newly synthesized TERT is imported into these organelles.

As a prerequisite to study intracellular TERT-trafficking with photoconvertible proteins we showed that microinjection of such molecules does not disturb mitochondrial morphology and protein import. Moreover, we successfully converted an exogenously expressed fluorescent protein locally inside living cells. To study, which aspects of mitochondrial function are affected by TERT we characterized the mitochondrial TERT interactome with BioID. As first validation, we verified the interactions of TERT with the mitochondrial transcription factor A (TFAM) by proximity ligation assays in murine cardiac fibroblasts and in primary human endothelial cells. Other interesting TERT interaction partners were several assembly factors for respiratory chain complex I and components of the mitochondrial contact site and cristae organizing system (MICOS). The interaction with the aforementioned assembly factors could directly explain the positive impact of TERT on complex I activity. The MICOS complex is critical for formation and dynamics of cristae, where the respiratory chain is located and which is also important for respiratory chain supercomplex assembly, again connecting TERT to mitochondrial respiration. Experiments aimed at understanding the role of mitochondrial TERT in a physiological context showed that it not only enhances mitochondrial respiration, but also improves endothelial cell migration and myofibroblast differentiation. Moreover, it improved the outcomes after experimental myocardial infarction. Finally, we showed that the Telomerase Activator 65 (TA-65) increases mitochondrial import of TERT and has the same effects as mitochondrial TERT on mitochondrial respiration, endothelial cell migration, myofibroblast differentiation and infarct size. Moreover, it prevented the induction of endothelial senescence. Based on this work, an in-depth characterization of the molecular mechanisms of how TERT improves mitochondrial respiration and how TA-65 induces mitochondrial import of TERT could in the long run help to devise strategies to improve cardiovascular health.

3 Progress Report

Telomerase Reverse Transcriptase (TERT) has, besides its task in telomere-preservation, also non-telomeric functions in the nucleus, but also in the mitochondria, e.g. the maintenance of respiratory chain function. Under short-term oxidative stress, TERT is exported from the nucleus with a concomitant increase of its level in the mitochondria, potentially to protect these cellular powerhouses and signaling organelles. Upon higher or prolonged stress, also mitochondrial TERT is lost. However, it is not clear, whether under short-term stress TERT “travels” from the nucleus to the mitochondria or if only newly synthesized TERT protein is imported in these organelles in these conditions.

To address this question, we had proposed the project *Telomerase reverse transcriptase in motion - dynamics of subcellular localization and functional consequences in aging and senescence* within the framework of the *DFG-RFBR Cooperation: Joint German-Russian Project Proposals in all fields of science*. Within the original project proposal, we had set the following aims:

1. Does TERT "travel" from the nucleus to the mitochondria?
2. Can TERT directly go from the nucleus to the mitochondria?

3. Which aspects of mitochondrial functions are affected by TERT?
4. Do stimuli relevant for aging, age-related diseases and cellular senescence have an influence on TERT "travel"/localization and mitochondrial and cellular functions?
In the following sections we will describe the project progress for the different aims.

Aim 1:

One of the aims in the original joint proposal with our Russian partners was to follow the movement of microinjected recombinant TERT within cells. As an important prerequisite we first tested if the procedure itself distorts mitochondrial morphology and/or affects protein import into the mitochondria. Therefore, we microinjected an expression vector for mitochondrially-targeted emerald green fluorescent protein (emGFP) or a recombinant SNAP-tag protein with a mitochondrial targeting sequence. Despite these manipulations the mitochondrial structure was preserved and the function of the mitochondrial protein translocation machinery was not altered, demonstrating the feasibility of the microinjection approach (Bogorodskiy et al., 2021).
In other experimental settings, we aimed to express fusions between TERT and photoconvertible fluorescent proteins using lentiviral vectors. Our joint preliminary experiments with our Russian collaboration partners to setup the planned microscopy experiments had already shown that restricted local intracellular photoconversion of Dendra2 after expression from a lentiviral vector is feasible. For reasons of simplicity, these experiments had been performed in HEK293 cells with Dendra2 fused to a mitochondrial targeting sequence. Based on these encouraging results, we generated a lentiviral expression vector for a TERT-Dendra2 fusion protein, which would be suitable for delivery in any cell type. This vector was based on a design routinely used in our laboratories (Dyballa-Rukes et al., 2017; Goy et al., 2014) and included a puromycin resistance cassette as potential selection marker. However, the size of this expression vector, which would generate a lentiviral genomic RNA of roughly 8.400 nucleotides was in the upper range of the packaging limit of lentiviral vectors, which is between 8.000 and 10.000 nucleotides. First production experiments with multiple different conditions yielded poor infectious titers of $\leq 5 \times 10^5$ infectious particles per ml even after concentration making this vector design unsuitable for the planned experiments. Therefore, we recloned the TERT-Dendra2 expression cassette into a lentiviral backbone devoid of the selection marker, thereby reducing its size by approximately 1750 bp.
However, in the meantime the experiments of our Russian collaboration partners revealed that the SNAP-tag, which is much smaller than the photoconvertible fluorescent protein-moiety is superior to Dendra2 for the desired applications. Therefore, the transfer of TERT to mitochondria under conditions of oxidative stress was examined with the use of a SNAP-tagged TERT. These results were published in 2024 (Burkatovskii et al., 2024), however, at that point we refrained from being included as authors for political reasons after the breach of international law through the war of the Russian Federation against Ukraine and asked only to be mentioned in the acknowledgements.

Aim 2:

After the collaboration with our Russian partners was no longer possible, we resorted to a different method to characterize mitochondrial interaction partners of TERT as originally envisioned. Moreover, we used an approach that is not limited to predefined specific proteins, but allows for a comprehensive analysis of the complete mitochondrial TERT interactome. To identify interaction partners of TERT in the mitochondria, we performed BioID (Varnaitė and MacNeill, 2016) with our collaboration partner Dr. Alessandro Ori at the Leibniz Institute on Aging - Fritz-Lipman Institute (FLI) in Jena. This unbiased method has extensively been used to identify protein-protein interactions. It is based on the biotinylation of a proteins' interaction partners in close vicinity (≤10 nm distance) in living cells and thus, in the native context, even if the interaction is weak or only transient. Biotinylation is achieved by expression of a fusion between the protein of interest and BirA*, a mutant form of the *E. coli* biotin ligase, which promiscuously biotinylates vicinal proteins (Roux et al., 2012). After a biotin pulse, the biotinylated interaction partners are captured from cell lysates on the basis of their affinity for streptavidin and identified by mass spectrometry.

To this end, we first generated a plasmid vector for tetracycline-inducible expression of a fusion of TERT targeted to the mitochondria (mitoTERT) and BirA* and used it for recombinase-mediated single-copy insertion into a defined genetic locus of HEK293 cells using the Flp-In™ T-REx™ system (Ward et al., 2011) to generate a stable cell line for tetracycline-inducible expression of mitoTERT-BirA*. As a control, we established an isogenic cell line for tetracycline-inducible expression of a fusion protein between TERT targeted to the nucleus and BirA* (nucTERT-BirA*) with the rationale that TERT interaction partners identified in both cell lines could be regarded as unspecific. After validating tetracycline-dependent TERT-BirA* expression, we performed BioID in both cell lines. Principal component analysis demonstrated clustering of the data from the 4 biological replicates obtained with the mitoTERT-BirA* cell line and clear separation from the data of the nucTERT-BirA* cell line (Figure 1A). Moreover, BioID revealed multiple potential interaction partners of mitochondrial TERT (Figure 1B).

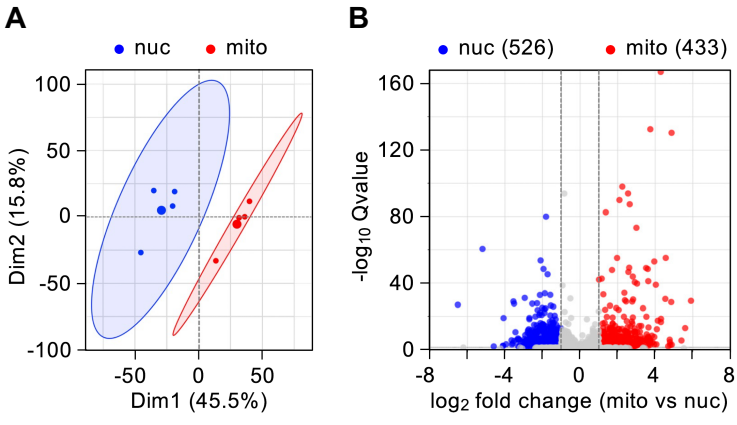

Figure 1: Identification of interaction partners of mitochondrial TERT by BioID. HEK293 cell lines expressing a mitoTERT or a nucTERT fusion with BirA* were used for 4 independent BioID experiments with each cell line. The data obtained using the mitoTERT-BirA* cell line are shown as red dots (mito), the data from the nucTERT-BirA* cell line as blue dots (nuc). (A) Principle component analysis. (B) Volcano plot. Grey dots represent proteins not significantly different between the two cell lines. The numbers on top indicate the interaction partners unique for nucTERT (nuc) and mitoTERT (mito).

The specificity of the approach was underscored by the fact that the GO terms mitochondrial matrix, mitochondrial membrane part, inner mitochondrial membrane, oxidoreductase complex,

and respiratory chain were among the top 6 enriched GO categories (normalized enrichment score ≥ 2.5, false detection ration <0.05) for interaction partners of mitochondrial TERT.
One of the interaction partners of mitochondrial TERT found in the BioID screen was the mitochondrial transcription factor A (TFAM), a protein originally envisioned to be analyzed with respect to interactions with TERT by two-color single molecule localization microscopy (SMLM) with photoswitchable fluorescent proteins. TFAM plays an essential role in transcription and replication of mitochondrial DNA and can also bind non-specifically to this circular molecule (Miranda et al., 2022). As we had previously shown that also TERT binds to mitochondrial DNA (Haendeler et al., 2009), it was intuitive that both proteins could come in close contact. Therefore, we used TFAM to exemplarily establish a validation procedure to detect protein-protein interactions of mitochondrial TERT in cells from the cardiovascular system. We used proximity ligation assays (PLA) (Hegazy et al., 2020), an antibody-based technique that is performed *in situ* in intact cells or tissue sections, thereby preserving the cellular structures. If two proteins are in close proximity allowing interactions, PLA creates a fluorescent signal up to a maximum distance of 30-40 nm between the target epitopes, thus, covering all interactions detected by BioID. We performed PLA in cardiac fibroblasts from our mitoTERT mice, in which TERT is localized exclusively in the mitochondria of all cells, and the corresponding nucTERT cells that contain TERT only in their nuclei (Ale-Agha et al., 2021) as a negative control (Figure 2).

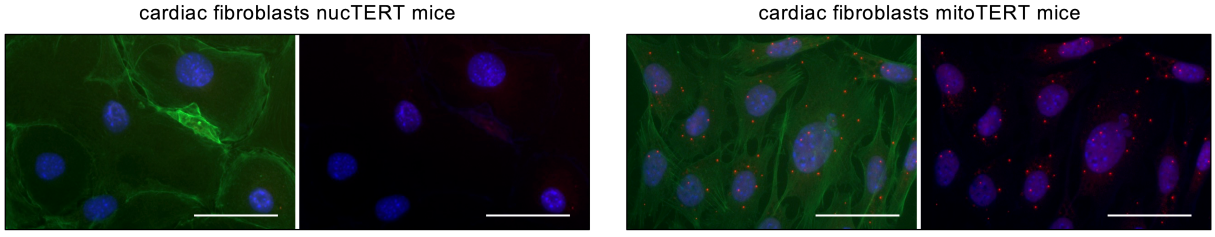

Figure 2: Interaction of mitochondrial TERT with TFAM in murine cardiac fibroblasts. Cardiac fibroblasts isolated from nucTERT and mitoTERT mice, respectively, were used for PLA with primary antibodies directed against endogenous TFAM and the myc-epitope tag at the C-terminus of nuclear and mitochondrial TERT. The PLA signals are visible as red dots, the cytoskeleton was counterstained with Phalloidin-Alexa 488 (green) and the nuclei with DAPI (blue); the scale bar is 50 µm. For better visualization of the PLA signals the microscopic pictures are also shown without the cytoskeletal staining.

The positive PLA signals in the mitoTERT fibroblasts show that TERT interacts with TFAM also in cells from the cardiovascular system. The lack of interaction in the nucTERT fibroblasts demonstrates that these interactions only occur with mitochondrially-localized TERT. To investigate if these interactions also take place in human cells and another cardiovascular cell type, we performed PLA in primary human endothelial cells. Therefore, the cells were transfected with an expression vector for mitochondrially-targeted TERT, which can be distinguished from endogenous TERT by a C-terminal myc-epitope tag, or an empty vector as control.
As in the murine cardiac fibroblasts, mitochondrial TERT interacts with TFAM also in primary human endothelial cells. The higher number of PLA signals in this cell type as compared to the murine fibroblasts is explained by the high-level overexpression of mitoTERT after transient transfection; the lack of signals in some cells is due to the transfection efficiency, as not all cells take up the mitoTERT expression vector (Figure 3).

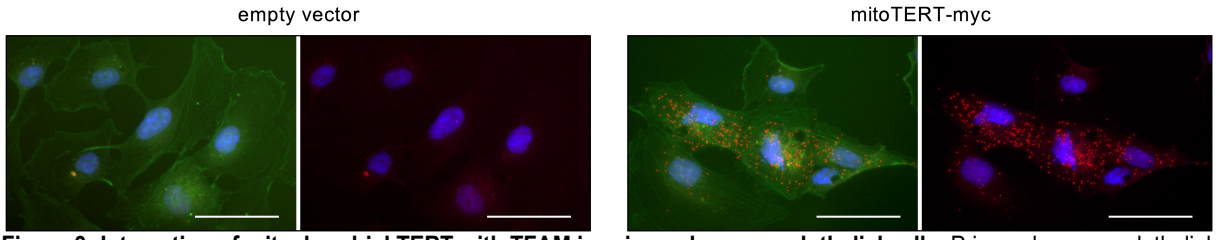

Figure 3: Interaction of mitochondrial TERT with TFAM in primary human endothelial cells. Primary human endothelial cells were transiently transfected with an expression vector for mitochondrially-targeted TERT with a C-terminal myc-epitope tag (mitoTERT-myc) or an empty vector as control. PLA was performed with primary antibodies directed against endogenous TFAM and the myc-epitope tag. The PLA signals are visible as red dots, the cytoskeleton was counterstained with Phalloidin-Alexa 488 (green) and the nuclei with DAPI (blue); the scale bar is 50 µm. For better visualization of the PLA signals the microscopic pictures are also shown without the cytoskeletal staining.

In summary, interactions of mitochondrial TERT with endogenous TFAM were observed in two different cell types of the cardiovascular system from different species, mice and humans, showing the feasibility of PLA to verify interactions detected by BioID. Moreover, the expression level of TERT in the mitoTERT mice is sufficient to detect interactions by this method, and, thus, could also be used in murine tissue sections.

Interestingly, among the extremely high-scoring interaction partners of mitochondrial TERT in our BioID experiments, we found two assembly factors for complex I (NDUFAF2/Mimitin: log2fold change/L2FC 2.58, Q-value 3.8x10-11; NDUFAF4: L2FC 4.03, Q-value 1.3x10-39) and three subunits of the mitochondrial contact site and cristae organizing system (MICOS) (IMMT/Mic60: L2FC 4.87, Q-value 3.4x10-131; CHCHD3/Mic19: L2FC 4.30, Q-value 2.9x10-17; CHCHD6/Mic25: L2FC 4.10, Q-value 6.1x10-13), a complex that is highly conserved from yeast to humans. The interactions with the complex I assembly factors, which are involved in different steps of supercomplex formation, give a hint as to why mitochondrial TERT improves complex I activity (see also next section) (Ale-Agha et al. 2021). On the other hand, the MICOS complex subunits are interesting because this complex is critical for formation and dynamics of cristae, invaginations of the inner mitochondrial membrane. Moreover, not only is the respiratory chain located in the cristae, but their shape also determines supercomplex assembly and, thereby, respiratory efficiency (Cogliati et al. 2013), directly linking cristae structure to respiratory chain function.

Therefore, we aim to validate the interactions of mitochondrial TERT with the aforementioned proteins as well as the functional consequences thereof in the future to get a more profound understanding of the molecular mechanisms underlying the functions of mitochondrial TERT.

Aim 3 and 4:

To understand the role of mitochondrially- and nuclear-localized TERT, we first investigated the amount of mitochondrial and nuclear TERT in our newly designed mitoTERT and nucTERT mice in comparison to wildtype mice. We found that in both cases TERT is increased approximately 8- to 10-fold. Moreover, we verified that in mitoTERT and nucTERT mice, TERT is only localized in the respective organelle (Ale-Agha et al., 2021).

Next, we measured the impact of TERT on respiratory chain activity. Therefore, mitochondria were isolated from the hearts of wildtype, mitoTERT, nucTERT, and TERT-deficient mice and

respiratory chain activity was measured. As published previously by us (Haendeler et al., 2009), we found that heart mitochondria of TERT-deficient mice had reduced complex I activity in comparison to wildtype littermates. Interestingly, mitoTERT mice showed increased complex I activity compared to wildtype mice, whereas nucTERT animals were similar to TERT-deficient animals, demonstrating that only mitochondrial TERT is able to maintain and increase complex I activity in the heart of those animals (Ale-Agha et al., 2021).

Two processes, which are described to depend on complex I activity in cardiovascular cells, are endothelial cell migration (Ale-Agha et al., 2018; Gonnissen et al., 2019) and myofibroblast differentiation (Ale-Agha et al., 2021; Negmadjanov et al., 2015). Therefore, we investigated the impact of mitochondrial and nuclear TERT on those processes. As expected from our respiratory chain measurements, we found that only mitochondrial TERT, but not nuclear TERT can improve migratory capacity of endothelial cells and differentiation of fibroblasts into myofibroblasts (Ale-Agha et al., 2021). To verify that indeed both processes completely depend on complex I activity, we treated those cells with rotenone, a specific complex I inhibitor, and demonstrated that the effects of mitochondrial TERT were abolished.

Increased complex I activity, improved endothelial cell migration, as well as enhanced myofibroblast differentiation are all processes, which have a positive impact in the early phases after myocardial infarction. Therefore, we also applied ischemia-reperfusion to the heart of those animals and found that mitoTERT mice in contrast to nucTERT mice have significantly smaller infarct size, as well as improved ejection fraction when compared to nucTERT mice (Ale-Agha et al., 2021).

To determine whether a substance can change the localization of TERT and has an impact on cellular functionality, we investigated the role of the Telomerase Activator 65 (TA-65), an FDA-approved extract from the plant *Astragalus membranaceus* used in traditional Chinese medicine. Over the last years, it has become clear that TA-65 does not protect cells from telomere shortening in the aging processes or under treatment with different stimuli, as measurements of average telomere length in humans and mice treated with TA-65 for up to one year did not change average telomere length (Bernardes de Jesus et al., 2011; Harley et al., 2011). In contrast, TA-65 improved glucose tolerance and reduced hepatic lipid deposition in mice when given for 6 months (Bernardes de Jesus et al., 2011). In healthy human subjects TA-65 had not shown adverse effects when given over a 1-year period and improved insulin sensitivity and lowered low-density lipoprotein levels (Harley et al., 2013). Recently, the Spyridopoulos group showed in a double blinded randomised controlled pilot trial evaluating the use of TA-65 in patients following myocardial infarction that TA-65 led to a significant increase in lymphocytes across all major subpopulations; TA-65 was well tolerated overall with a lower number of total adverse events (Bawamia et al., 2023).

Therefore, we determined the amount and localization of endogenous TERT in endothelial cells after treatment with TA-65. We found that short-term treatment only increased TERT in the mitochondria and not in the nucleus, which suggests that already existing TERT is predominantly imported in the mitochondria. Interestingly, treatment with TA-65 also improved myofibroblast differentiation and endothelial cell migration (Ale-Agha et al., 2021; Ale-Agha et

al., 2026). Moreover, we found that the TA-65 effects depend on TERT as TA-65 only increased differentiation of fibroblasts into myofibroblasts in TERT-proficient cells, but not in TERT-deficient fibroblasts (Ale-Agha et al., 2021).

Thus, it was tempting to speculate if TA-65 also improves complex I activity and ischemia-reperfusion injury. Indeed, we found that feeding of mice for 10 days with TA-65 pressed in food pellets, increased complex I activity comparable to the data obtained in heart mitochondria of mitoTERT mice (Figure 4).

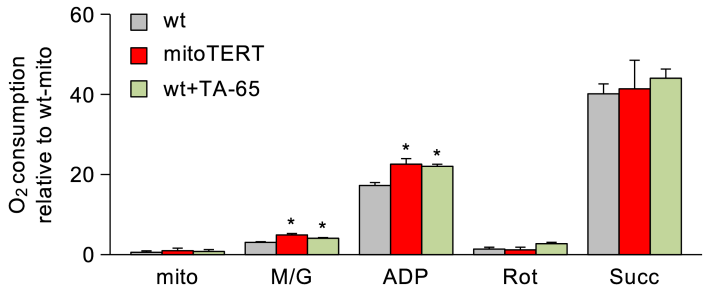

Figure 4: Mitochondrial TERT and TA-65 enhance mitochondrial respiration. O_2-consumption in heart mitochondria of adult wildtype mice (wt) and mitoTERT (mitoTERT) animals was determined in isolated cardiac mitochondria (mito), and after the sequential addition of malate/glutamate (M/G), ADP, rotenone (Rot) and succinate (Succ). One group of wt animals had received TA-65 with the food for 10 days (wt+TA-65). O_2 consumption of mean wt-mito was set to 1. Data are mean±SEM, n=5-11, *p<0.05 vs wt, one-way ANOVA with post-hoc Tukey test.

With respect to infarct size 2 days after ischemia-reperfusion injury, we found a significant reduction when mice were treated with TA-65 (Figure 5).

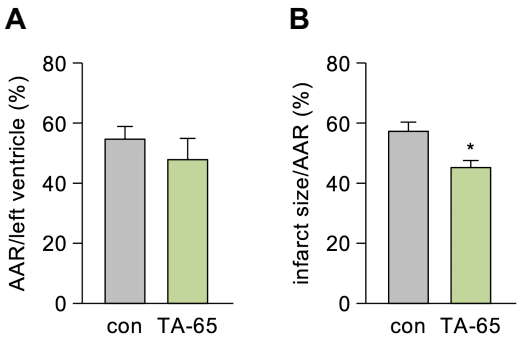

Figure 5: TA-65 reduces infarct size. Adult wildtype mice were fed regular chow (con) or received food pellets containing TA-65 for 10 days (TA-65). I/R injury was set and 2 days after infarction, area-at-risk (AAR) and infarct size were determined by Evans blue/TTC staining. (A) AAR. (B) Infarct size. Data are mean±SEM, n=4-5, *p<0.05 vs con, unpaired Student`s T-test.

It has been demonstrated that short-term treatment with H_2O_2 changes the localization of TERT towards the mitochondria (Spilsbury et al., 2015). As mentioned above, treatment with TA-65 also increased the TERT amount within the mitochondria. In contrast, long-term treatment with H_2O_2 leads to the induction of senescence in endothelial cells and TERT in general, as well as mitochondrial TERT, has been shown to be reduced in endothelial cell senescence (Büchner et al., 2013; Büchner et al., 2010; Haendeler et al., 2004; Rosen et al., 2020; Werner et al., 2009; Zurek et al., 2016). Therefore, increasing mitochondrial TERT could be a therapeutic approach to reduce endothelial cell senescence or to even revert it. However, so far, the impact of TA-65 on cellular senescence has not been investigated. Thus, we made use of our established human endothelial cell senescence model and determined the effects of TA-65. Therefore, human primary endothelial cells were treated with 50 µM H_2O_2 every second day for two weeks. In this model, apoptosis induction does not occur and typically the protein levels of endothelial NO Synthase (eNOS) are reduced and the nuclear localization of the cell cycle

inhibitor p21 is increased (Büchner et al., 2013; Goy et al., 2014; Merk et al., 2023). Our preliminary results demonstrate that preventive treatment, with TA-65 blocks the loss of eNOS protein as shown by immunoblot (Figure 6) and the upregulation of nuclear p21 as shown by immunostaining (Figure 7).

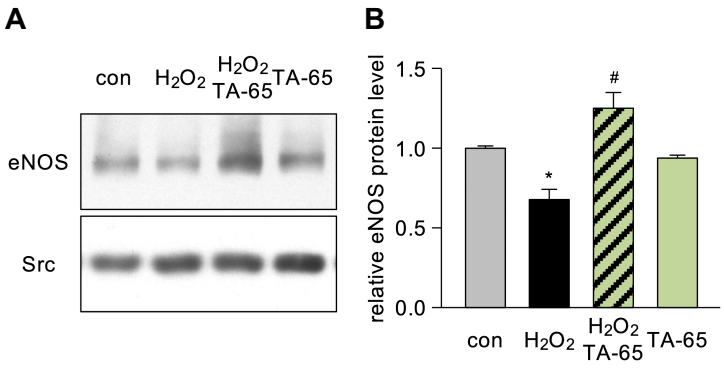

Figure 6: Co-incubation with TA-65 prevents loss of eNOS protein in H_2O_2-induced endothelial cell senescence. Primary human endothelial cells were treated with 50 µM H_2O_2 and 1 µM TA-65 (H_2O_2 TA-65) every second day for two weeks or with 50 µM H_2O_2 and DMSO as solvent control for TA-65 (H_2O_2). As control, cells were either incubated with DMSO (con) or 1 µM TA-65 (TA-65) for two weeks. Immunoblots were performed with an antibody against eNOS. Src served as loading control. (A) Representative immunoblots (upper panel eNOS, lower panel Src). (B) Semiquantitative analysis was performed using ImageJ. eNOS protein levels were normalized to Src protein levels. Data are mean ± SEM, n=3, *p<0.05 vs con, #p<0.05 vs H_2O_2, one-way ANOVA with post-hoc Tukey test.

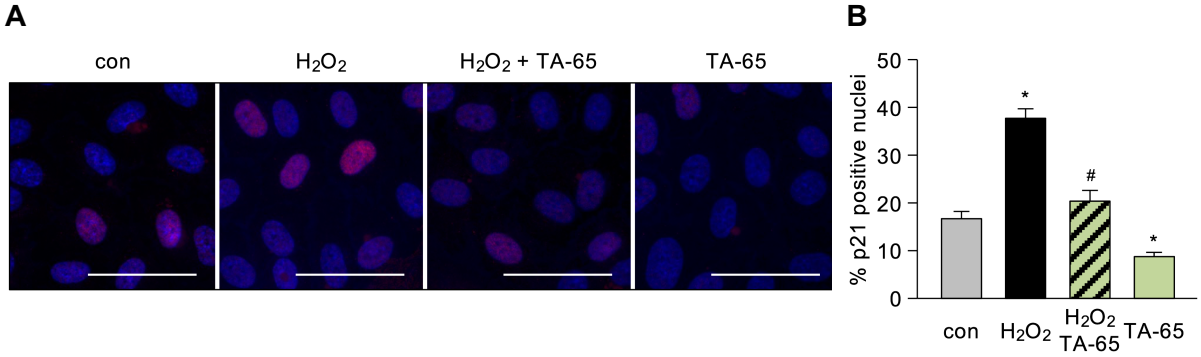

Figure 7: Co-incubation with TA-65 prevents increase of nuclear p21 in H_2O_2-induced endothelial cell senescence. Primary human endothelial cells were treated with 50 µM H_2O_2 and 1 µM TA-65 (H_2O_2 TA-65) every second day for two weeks or with 50 µM H_2O_2 and DMSO as solvent control for TA-65 (con). As control, cells were either incubated with DMSO (con) or 1 µM TA-65 (TA-65) for two weeks. Immunostaining was performed with an anti p21 antibody and nuclei were stained with DAPI. (A) Representative immunostainings; p21 is shown in red and nuclei in blue, the scale bar is 50 µm. (B) The percentage of p21 positive nuclei was determined using ImageJ. Data are mean ± SEM, n=3, *p<0.05 vs con, #p<0.05 vs H_2O_2, one-way ANOVA with post-hoc Tukey test.

So far, it is not known how TA-65 induces the translocation of TERT into mitochondria. Therefore, we aim to elucidate the underlying molecular mechanisms. The detailed mechanistic knowledge might help to design small molecules, that could replace the natural substance as a defined chemical could be manufactured more consistently than a plant extract.

A long-term goal would be to cooperate with clinicians in geriatric medicine for a study addressing the question if TA-65 can improve cardiovascular health in the elderly.

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4 Published Project Results

4.1 Category A – Articles in peer-reviewed journals, contributions to peer-reviewed conferences or to anthology volumes, and book publications

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