The ER - mitochondria junction as target for senolysis

Summary

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Supervisor:	Prof. Dr. Wolfgang Graier
Availability:	This position is available.
Offered by:	Medical University of Graz
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Description

Background:

During aging, a progressive loss in organ function occurs. This is due to an increasing number of senescent cells with a rather common phenotype that includes reduced cell functionality, halted proliferation, ER stress, and, increased cellular ROS levels (1). Notably, senescent cells also increase the expression of pro-survival networks that is consistent with their established resistance to apoptosis (2), thus, showing some similarities with cancer cells but without malignant features (3). Recently, a new class of drugs termed senolytics was identified. Senolytics selectively kill senescent cells and, thus, clears the organ/tissue from senescent cells (4, 5), and, consequently, facilitate tissue/organ repair by existing/recruited progenitor/stem cells (5). Our previous work using endothelial aging models provides evidence that senescent cells have elevated basal mitochondrial respiration due to an enforced ER-mitochondria tethering (MAMs) (6). Moreover, endothelial senescence is associated with enhanced protein arginine methyltransferase 1 (PRMT1) activity introducing the engagement of UCP2 for the activity of mitochondrial Ca^{2+} uptake (7). Remarkably, senescent cells strongly reduce the expression of UCP2, thus, limit the activity of mitochondrial Ca^{2+} sequestration to avoid devastating mitochondrial Ca^{2+} overload (6). Accordingly, overexpression of UCP2 reestablishes strong mitochondrial Ca^{2+} loading, resulting in ROS bursting and apoptotic cell death within 6-12 h (6). Notably, diminution of UCP2 expression by siRNA in senescent endothelial cells yielded cell arrest and slow cell death after 2-3 days, followed by a 7 times faster proliferation of the remaining progenitor/endothelial cells that were similar to that of young endothelial cells. Remarkably, all these effects were specific to aged but not young endothelial cells.

Hypothesis and objective:

We postulate that senescent (endothelial) cells have increased PRMT1 activity and manage their energy metabolism by an increased ER – mitochondria tethering, while UCP2 expression is strongly reduced to avoid mitochondrial Ca^{2+} overload and the induction of apoptosis. However, the small existing mitochondrial Ca^{2+} uptake that is achieved by the remaining UCP2 is essential for cell survival. We further hypothesize that inhibitors of the UCP2-dependent mitochondrial Ca^{2+} uptake protect senescent endothelial cells for sudden ROS burst and apoptotic cell death that harm neighboring healthy cells. Inhibitors of the UCP2-dependent mitochondrial Ca^{2+} uptake may yield slow, “quiescent” cell death of the senescent endothelial cells, thus, releasing existing (progenitor) cells (endothelial colony forming endothelial cells (9)) from the pressure of senescent cellsand, thus, facilitating vascular repair. Accordingly, inhibitors of the UCP2-dependent mitochondrial Ca^{2+} uptake might be powerful senolytic drugs for vascular repair. Based on our knowledge, we plan to design small peptides inhibitors of the UCP2-function on methylated MICU1 (7).

Methodology:

Small peptides that bind either to the intermembrane loop 2 of UCP2 (sequence KITIAREE) (8) or the UCP2 binding motif on MICU1 will be designed in collaboration with T. Madl. Binding of the peptide(s) on UCP2 and its intermembrane domain 2 will be verified using isothermal titration calorimetry (ITC) (7). Interorganelle tethering will be visualized utilizing the multiexcitation/multiemission high- and super-resolution (confocal/SIM) fluorescence microscopy (FRET; fluorescence recovery after photobleaching, FRAP; fluorescence loss in photobleaching, FLIP; analyses of organelle motility and dynamics; protein/organelle colocalization; organelle volume & surface rendering). Artificial tethering will be introduced by an optogenetic tool (10) to verify the impact of increased tethering to cell function and sensitivity to senolytics. Moreover, ion currents and membrane potential will be monitored by patch clamp technique simultaneously with the fluorescence measurement in collaboration with K. Groschner. In collaboration with G. Haemmerle, S. Sedej and D. Kratky, the student will perform organ bath experiments with isolated mice aortae and,

if promising peptides were found, animal experiments using the validated peptides in aged mice. The student will perform organelle/compartmental Ca^{2+} and H^{+} measurements, quantitative analysis of peptide-protein interaction, subcellular analyses of signaling pathways, protein expression, mutation and knock-down, real-time PCR, high-resolution respirometry, measurement of organelle/cellular ROS production, and visualization of spatial ATP concentrations. FACS analysis of progenitor cells (8) will be performed in the respective core facility at the MUG. Finally, cell proliferation and viability assays as well as tests for the initiation of apoptotic pathways will be performed (6).

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