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Thesis title: The Role of Calcium Ion Dynamics and Inter-Organelle Communication During Plasma Membrane Damage- Dependent Cellular Senescence

Research aim:

Cellular senescence is a state of stable cell cycle arrest that contributes to various physiological and pathological processes, including organismal aging, tissue repair, and tumorigenesis. While various stresses have been shown to induce cellular senescence in vitro, the triggers of cellular senescence in vivo remain unclear. This thesis aimed to investigate the role of calcium ion (Ca^{2+}) dynamics and inter-organelle communication during plasma membrane damage-dependent cellular senescence. The study hypothesized that external stress such as plasma membrane damage induces cellular senescence.

Material and method:

Human normal fibroblast, cancer cell, yeast cell were used. Various methods were utilized, including Cell culture, vector construction, transfection, yeast lifespan assays, plasma membrane damage and cellular senescence induction with SDS, SLO, silica and laser, penetration assays, live cell imaging with genetically encoded calcium indicators, immunocytochemistry, qRT-PCR, Western blotting, analysis of mitochondrial homeostasis, proximity ligation assay, and split-TurboID with mass spectrometry analysis.

Result:

Transient plasma membrane damage induced by SDS, SLO, silica and laser triggered premature senescence in normal human fibroblasts, termed plasma membrane damage-dependent cellular senescence (PMD-Sen). PMD-Sen was suppressed by overexpression of the ESCRT-III protein CHMP4B. Plasma membrane damage also limited the replicative lifespan of budding yeast. PMD-Sen required p53 but not the classical DNA damage response pathway. Ca^{2+} influx was necessary and sufficient to induce PMD-Sen, leading to sustained calcium elevation in the mitochondria and increased mitochondrial ROS. Ca^{2+} influx increased ER-mitochondria contact sites, and Ca^{2+} transport from the ER to mitochondria via these contacts was necessary for cytosolic Ca^{2+} buffering and cell survival after Ca^{2+} influx. Proteomics identified several novel ER-mitochondria contact site proteins required for this Ca^{2+} transport and cell survival during Ca^{2+} influx into cytosol.

Conclusion:

This study identified a novel subtype of cellular senescence, PMD-Sen, triggered by plasma membrane damage and mediated by Ca^{2+} influx, mitochondrial ROS, and p53. Ca^{2+} influx into cytosol induces ER-mitochondria contacts that enable ER-mitochondria Ca^{2+} transport. These findings provide new insights into the triggers and mechanisms of cellular senescence.