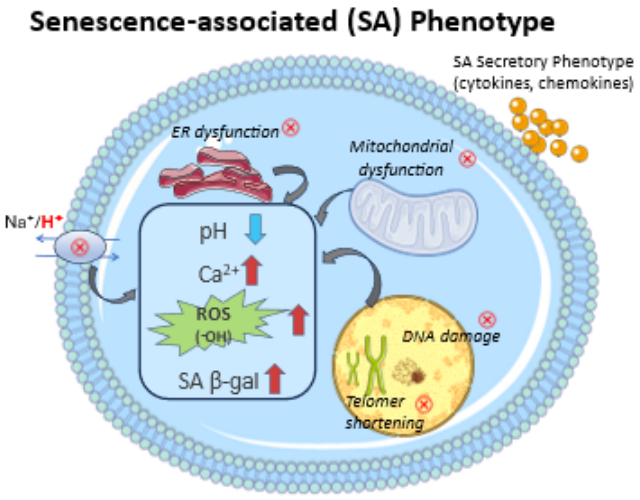
DECIPHERING THE INSIDE CELL´S STORY BY CYTOCHECK SPACHIP®!

Cell Senescence & the Links to Homeostasis of Calcium, pH, and Oxidative Stress

The complexity of senescence and multifaceted nature of cellular aging

Cell Senescence is a state of permanent cell cycle arrest that typically occurs in response to stress or damage, such as telomere shortening, DNA damage, or exposure to oxidative stress. Although these cells no longer proliferate, they remain metabolically active and secrete various factors collectively known as the senescence-associated secretory phenotype (SASP). While senescence can act as a tumour-suppressive mechanism by preventing the proliferation of damaged cells, it is also implicated in aging and age-related diseases due to the accumulation of senescent cells and the harmful effects of SASP.

Among the various physiological changes associated with senescence, alterations in **intracellular calcium ion concentration ([Ca²⁺]i), pH levels, and oxidative stress** play crucial roles in the initiation and maintenance of the senescent phenotype.



1. Intracellular Calcium Ion Concentration

Calcium ions (Ca^{2+}) are vital for various cellular processes, including signalling, metabolism, and apoptosis. Changes in $[Ca^{2+}]i$ are often associated with cellular aging and senescence. During senescence, dysregulation of calcium homeostasis is frequently observed, characterized by either an increase in basal $[Ca^{2+}]i$ or a disruption of the calcium signalling pathways.

Senescent cells exhibit altered calcium dynamics, often linked to a decline in the activity of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), leading to a decrease in calcium storage in the endoplasmic reticulum (ER). This imbalance in calcium signalling contributes to the dysfunction of mitochondria, which plays a critical role in the bioenergetic changes observed in senescence. Mitochondrial Ca^{2+} overload can lead to oxidative stress through the enhanced production of reactive oxygen species (ROS), which in turn can exacerbate mitochondrial dysfunction and contribute to the maintenance of the senescent state.

2. pH Changes

The intracellular pH (pHi) is tightly regulated under physiological conditions, but during senescence, cells often exhibit a shift in pH. Normally, the pHi is slightly alkaline (around 7.2), but senescent cells frequently display a more acidic cytoplasmic environment. This acidification is partly driven by the reduced activity of proton transporters, such as the Na^+/H^+ exchanger, and the accumulation of acidic metabolic byproducts as mitochondrial function declines.

The acidic pHi is thought to play a role in maintaining the senescent phenotype by altering enzyme activities, protein stability, and cellular metabolism. For instance, certain lysosomal enzymes, such as β-galactosidase, are activated at lower pH, and their upregulation has become a hallmark of senescence. Furthermore, the acidic environment can impair proper protein folding and clearance mechanisms, leading to the accumulation of damaged proteins that exacerbate cellular dysfunction.

3. Oxidative Stress and Hydroxyl Radical

Oxidative stress is a critical driver of cellular senescence, primarily through the excessive production of reactive oxygen species (ROS). Among the various ROS, the **hydroxyl radical (•OH)** is particularly reactive and damaging. It is primarily generated through the Fenton reaction, where hydrogen peroxide (H_2O_2) interacts with transition metals like Fe^{2+}, leading to the formation of •OH. This highly reactive species can cause significant damage to cellular components, including DNA, lipids, and proteins.

In senescent cells, there is often an increase in mitochondrial ROS production, leading to enhanced oxidative damage and further driving the senescence process. The generation of ROS, particularly hydroxyl radicals, can contribute to DNA damage and genomic instability, which activates cell cycle checkpoints

leading to permanent cell cycle arrest.
The role of ROS in general, and specifically hydroxyl radicals, in the induction of senescence is critical, as these molecules can modify key signalling pathways and regulatory proteins. For example, oxidative modifications of proteins involved in calcium handling and pH regulation can further dysregulate these systems, contributing to the progressive deterioration of cellular homeostasis.

4. Induction of β-Galactosidase

One of the most used biomarkers for senescent cells is **senescence-associated β-galactosidase (SA-β-gal)**. This enzyme, which is normally present in lysosomes, becomes upregulated and more active at an acidic pH in senescent cells. Its increased activity is associated with the enlargement of lysosomal compartments and can be detected using histochemical assays.
SA-β-gal is not a causal factor in senescence but rather a consequence of lysosomal dysfunction and pH changes associated with the senescent state. Its use as a biomarker is particularly advantageous in identifying senescent cells in culture and tissues, as it provides a reliable indicator of lysosomal expansion and senescent cell accumulation.

5. Cell Senescence and SPAchip technology

SPAchip® is a groundbreaking technology which enables fluorescence-based intracellular analytics in single living cells and real-time (explanatory video).It is based on silicon chips functionalised for covalent tethering of either chemical or biological probes. Upon non-invasive cellular uptake, chips harbouring the probe remain accessible in the cytosol harmlessly without causing cytotoxicity. Signal from individual devices is quantified using fluorescence microscopy or flow cytometry. The technology is amenable to 2D and 3D biology.
A4cell have developed SPAchip® sensors for cellular messengers and markers such as **pH, Ca2+ and ROS, i.e. hydroxyl radical**. Furthermore, the technology is amenable to attaching oligonucleotides aptamers, proteins and fluorogenic enzyme substrates, hence it enables monitoring the induction or repression of enzyme biomarkers such as SA-β-gal.
If you do not find your detection kit of interest, please contact us and let´s work together in developing a tailored solution for you.

6. Conclusions

Cellular senescence is a complex process driven by multiple interconnected factors, including changes in intracellular calcium ion concentration, pH shifts, and oxidative stress. Among these, the generation of hydroxyl radicals plays a pivotal role in driving DNA damage and cellular dysfunction, thus reinforcing the senescent state. The induction of β-galactosidase serves as a hallmark of senescence, reflecting underlying lysosomal changes and acidification. The complexity of senescence means that multiple factors, including altered calcium signalling, pH dysregulation, and ROS generation, converge to maintain the senescent phenotype. Together, these changes reflect the multifaceted nature of cellular aging and its connection to oxidative damage, metabolic shifts, and homeostatic imbalances. Understanding these mechanisms not only provides insights into the biology of aging but also opens avenues for targeting senescent cells in age-related diseases and therapies aimed at mitigating the effects of cellular aging.

CytoCHECK SPAchip® assay kits by A4cell are novel fluorescence assays for the detection of intracellular Calcium, pH and ROS in live cells, which bring together the fields of nanotechnology and cell biology

Bibliography:

1.Childs, B. G., Durik, M., Baker, D. J., & van Deursen, J. M. (2015). Cellular senescence in aging and age-related disease: From mechanisms to therapy. Nature Medicine, 21(12), 1424-1435.
2.Mattson, M. P. (2007). Calcium and neurodegeneration. Aging Cell, 6(3), 337-350..
3.Sohal, R. S., & Orr, W. C. (2012). The redox stress hypothesis of aging. Free Radical Biology and Medicine, 52(3), 539-555..
4.Terman, A., & Brunk, U. T. (2006). Oxidative stress, accumulation of biological 'garbage', and aging. Antioxidants & Redox Signaling, 8(1-2), 197-204.
5.Bhatia-Dey, N., Kanherkar, R. R., Stair, S. E., Makarev, E. O., & Csoka, A. B. (2016). Cellular senescence as the causal nexus of aging. Frontiers in Genetics, 7, 13.
6.Campisi, J., & d'Adda di Fagagna, F. (2007). Cellular senescence: When bad things happen to good cells. Nature Reviews Molecular Cell Biology, 8(9), 729-740.
7.Kurz, D. J., Decary, S., Hong, Y., & Erusalimsky, J. D. (2000). Senescence associated β-galactosidase reflects an increase in lysosomal mass during replicative senescence of human endothelial cells. Journal of Cell Science, 113(20), 3613-3622.
8.Navarro, A., & Boveris, A. (2010). Brain mitochondrial dysfunction and oxidative damage in aging and Parkinson's disease. Journal of Bioenergetics and Biomembranes, 42(6), 291-297
9.Takahiro, S., Jun, I., Tomohiro, I., Hidetsugu, T., Michio, U., &, Shinichi, H. (2017). The novel antioxidant TA293 reveals the role of cytoplasmic hydroxyl radicals in oxidative stress-induced senescence and inflammation. Biochemical and Biophysical Research Communications, 482(4), 1183-1189.