

21. Cellular Senescence

Dolly, a cloned sheep, was born in 1996 (Wilmut, I., et al., *Nature*, 1997). Dolly was generated by somatic cell nuclear transfer into an enucleated embryonic cell. One discussion was about the lifespan of Dolly. Telomeres, which are located at terminals of chromosomes and prevent the degradation of DNA, are known to be shortened by the replication of cell division. When telomeres are shorter than a critical length, which is about half of the initial length, cell division stops. Since the telomere length in the transplanted nuclei into embryonic cells should be shorter, the lifespan of Dolly was also thought to be shorter.

The reason why Dolly was so popular must be the expectation for a longer lifespan and self-conservation by humans. Although the concept of aging is often used in the context of whole organisms, it is also discussed in cell biology (cellular senescence).

Senescence: Mammalian somatic cells divide at the beginning of the cell culture, but their divisions stop after prolonged culture (Hayflick L, et al., *Exp. Cell Res.*, 1961). This is called Hayflick limit. In this state, cells are in cell cycle arrest, which is called cellular senescence. Cellular senescence is generally an irreversible event and is thought to prevent a cell from tumorigenesis. Senescent cells acquire a phenotype called SASP (senescence-associated secretory phenotype), secreting inflammatory cytokines, chemokines, growth factors, matrix metalloproteinases (MMPs), and exosomes. Since SASP is harmful to surrounding healthy cells, senescent cells are removed from the tissue by the natural immunity with the turnover time of several days and several weeks in younger and older mice, respectively (Karin, O., et al., *Nat. Comm.*, 2019). Senescent cells display other characteristics, including altered chromatin structure and increased activity in the acid β -galactosidase (*Cancer Meta.Rev.*, 29, 2010; Karin, O., et al., *Nat. Comm.*, 2019).

Molecular mechanisms for cellular senescence: By applying stimuli triggering cellular senescence to MEFs (mouse embryonic fibroblasts), MEFs skip mitosis before cell cycle arrest. This is mediated by the p53-dependent APC/CCdh1 protein activation, and pRb family protein-dependent inhibition of mitosis regulating protein, which are necessary and sufficient for inducing cellular senescence (Johmura, Y., et al., *Molec. Cell*, 2014).

Tumor suppressor proteins p16^{INK4a} and p19^{ARF} are involved in cellular senescence, which are especially important for the maintenance of cell cycle arrest (Johmura, Y., et al., *Molec. Cell*, 2014; Hashimoto, M., et al., *JCI Insight*, 2016). The stress signal that induces cellular senescence transcribes p16^{INK4a} and p19^{ARF} regulates pRb through a single step of CDK4/6 and 4 steps of MDM2, p53, p21, and CDK4/6 at the Cdkn2a gene loci (Sherr, C.J., 731, 2001; Karin, O., et al., *Nat. Comm.*, 2019). pRb transcription factor E2F that inhibits the expression of genes is required for the transition from G1 to S. Recently, the upstream and downstream signaling regulating apoptosis, necrosis, pyroptosis, and ferroptosis were found to be involved in the induction of cellular senescence (Shimizu, K., et al., *Seminars Cancer Biol.*, 2025).

Recently, an interesting report appeared, where exposure to restraint stress, a psychological non-social stress model, increased tumor-suppressor p21^{Cip1} exclusively in the brains of male, but not female mice, and in a p16^{Ink4a}-independent manner. Conversely, exposure to chronic subordination stress (males only were tested) increased key senescent cell markers (Lyons, C.E., et al., *Nat. Aging*, 2025).

Senescence and aging: This blog focuses on topics in molecular cell biology. However, we may want to know the possible link between cellular senescence and aging. Researchers in the field of senescence seem to hypothesize that there is some relation in cellular senescence to aging. The accumulation of senescent cells causes chronic inflammation (Karin, O., et al., Nat. Comm., 2019), and the elimination of p19^{ARF}-expressing cells enhances pulmonary function in mice (Hashimoto, JCI Insight, 2016). In addition, it was shown that AP20187 treatment extended median lifespan in both male and female mice (Baker, Nature, 2016). Clearance of p16^{Ink4a}-positive cells delayed tumorigenesis and attenuated age-related deterioration in the kidney and heart.

I have searched reports on cellular senescence, and learned that the Hayflick limit, which is characterized by a state of cell cycle arrest, is defined as the senescent state of a cell. However, there remains a basic question. Aging is a causal path to death in animals. Is this also true in cellular senescence? The division of yeast cells stops after about 20 cycles (replication lifespan). However, such yeast cells do not necessarily lead to cell death. Such cells might be closer to death than dividing cells. However, there remains a question in calling these cells senescent cells, because it might be tantamount to calling a 20-year-old person who has stopped growing an "old person."

Aging is a word used mainly to animals as a system, and it is not clearly defined. If we use such a word to cells, which we are going to explain by molecular mechanisms, I am afraid of any confusion. Is it valid using a word "senescence" to cells? This is a comment by a researcher who is not an expert in the field. If I have a chance to hear from an expert, the question might be solved.