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Rejuvenation of senescent cells—The road to postponing human aging and age-related disease?

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ABSTRACT

Cellular senescence is the state of permanent inhibition of cell proliferation. Replicative senescence occurs due to the end replication problem and shortening telomeres with each cell division leading to DNA damage response (DDR). The number of short telomeres increases with age and age-related pathologies. Stress induced senescence, although not accompanied by attrition of telomeres, is also attributed to the DDR induced by irreparable DNA lesions in telomeric DNA. Senescent cells characterized by the presence of γH2AX, the common marker of double DNA strand breaks, and other senescence markers including activity of SA-β-gal, accumulate in tissues of aged animals and humans as well as at sites of pathology. It is believed that cellular senescence evolved as a cancer barrier since non-proliferating senescent cells cannot be transformed to neoplastic cells. On the other hand senescent cells favor cancer development, just like other age-related pathologies, by creating a low grade inflammatory state due to senescence associated secretory phenotype (SASP). Reversal/inhibition of cellular senescence could prolong healthy life span, thus many attempts have been undertaken to influence cellular senescence. The two main approaches are genetic and pharmacological/nutritional modifications of cell fate. The first one concerns cell reprogramming by induced pluripotent stem cells (iPSCs), which *in vitro* is effective even in cells undergoing senescence, or derived from very old or progeroid patients. The second approach concerns modification of senescence signaling pathways just like TOR-induced by pharmacological or with natural agents. However, knowing that aging is unavoidable we cannot expect its elimination, but prolonging healthy life span is a goal worth serious consideration.

1. Replicative and stress-induced senescence; the role of telomeres and DNA damage

Cellular senescence is defined as a permanent cell cycle arrest that is resistant to growth factors and other signals that induce cell proliferation (Stein et al., 1991). The senescence of human fibroblasts *in vitro*, described for the first time by Hayflick and Moorhead, was named replicative senescence and defined as a limit in divisions of cell population (Hayflick and Moorhead, 1961). Later on the replicative senescence of human fibroblasts was attributed to telomere shortening. Due to the nature of DNA replication (so called "end replication problem") and the lack of telomerase, telomeres become progressively shorter with every round of human cell division (Harley et al., 1990). Not only telomere shortening but also uncapping, that is losing telomere-binding proteins, leads to cellular senescence (von Zglinicki et al., 2003). Thus, in culture, human cells may continue to proliferate until the telomeres cease to function.

Senescent cells should be distinguished from quiescent or terminally differentiated cells, and generally it is possible to discriminate them by measuring the activity of so called senescence-associated β-galactosidase (SA-β-gal) and detecting other markers of cellular senescence (Sikora et al., 2011) which should mark only senescent cells.

Senescent cells should not be identified with dying cells as they might stay, at least in culture, alive and, although not able to divide, they are metabolically active. This is particularly well visible in the case of human fibroblasts which are even more resistant to apoptosis than young proliferating cells. Nonetheless, other cells such as human T cells, if not provided with an environment fully reflecting the organismal milieu (e.g. cytokines which are not needed by fibroblasts) will eventually die in culture (Brunner et al., 2012).

Although, the state of cellular senescence means the irreversibility of division arrest, it can be bypassed giving rise to immortalized cells. When the senescent human fibroblasts with short telomeres lose the activity of cancer suppressors such as p53, pRb or other proteins

Abbreviations: ATM, ataxia telangiectasia mutated; ATR, ATM and Rad3-related; BMP-2, bone morphogenetic protein-2; Cdk, cyclin-dependent kinase; CESP, cell-type exclusive senescence phenotype; CHK, check point kinase; DDR, DNA damage response; DSBs, double-strand DNA breaks; HGPS, Hutchinson–Gilford progeria syndrome; HUVEC, human umbilical vein endothelial cells; iPSCs, induced pluripotent stem cells; MRN complex, MRE11–RAD50–NBS1 protein complex; OIS, oncogene-induced senescence; RUNX-2, runt-related transcription factor 2; SA-β-gal, senescence associated-β-galactosidase; SASP, senescence associated secretory phenotype; SIPS, stress-induced premature senescence; VSMCs, vascular smooth muscle cells; TOR, target of rapamycin.

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involved in senescence signaling pathways, they can increase the number of divisions and continue telomere shortening until the state of crisis which is characterized by massive cell death. Some rare cells escape crisis and become immortalized through telomere length stabilization. Also ectopic expression of telomerase, which protects telomere erosion, leads to cell immortalization (reviewed in Fridman and Tainsky (2008)).

Cell types that are capable of entering replicative senescence include besides fibroblasts, epidermal keratinocytes, vascular smooth muscle cells, epithelial cells, glial cells, endothelial cells, melanocytes, T lymphocytes, adrenocortical cells and mesenchymal cells (Freedman, 2005; Sikora et al., 2011).

Although there are similarities in the senescent phenotype of various cell types, the process by which a cell senesces is likely to have features that are cell-type specific.

Epithelial cells undergo two stages of senescence. The first occurs after only a few cell divisions and is associated with an increase in p16 protein expression, that is not a consequence of telomere shortening. Following the inactivation of p16 and, in some cell types, the inactivation of p53, epithelial cells are able to emerge from this stage and continue to proliferate for another several dozen of population doublings before the second stage of growth arrest termed agonoescence (Stampfer and Yaswen, 2003).

Human lymphocytes (T cells) are resting cells and need to be activated by a mitogen to start the proliferation in culture. The T cell activation leads to cell divisions and to so called activation-induced cell death. Thus, many cells are dying in culture and those which survive and are considered as senescent CD8+ cells are lacking the CD28 co-receptor (CD8+CD28−). We showed that the subpopulation of CD8+ cells derived from umbilical cord blood contained only CD28-positive cells but the activation of cells in culture led quite rapidly to the selection of non-proliferating CD28-negative cells (Brzezinska et al., 2003). The CD8+CD28− cells have shorter telomeres than the CD8+CD28+ cells (Effros et al., 2003).

Recently, in the case of vascular smooth muscle cells (VSMCs) senescence the term "cell-type exclusive senescence phenotype"—CESP was coined. The senescent VSMCs have been shown to overexpress genes and proteins (including RUNX-2, alkaline phosphatase, type I collagen and BMP-2) associated with osteoblasts. This led to VSMCs calcification and to partial osteoblastic transdifferentiation (Burton et al., 2010).

The mesenchymal stem cell proliferation capacity and senescence are variables and depend on several factors, including tissue donor age, plating density and growth medium composition (Ksiazek, 2009). Also, the senescence of human umbilical vein endothelial cells (HUVEC) in culture depends very much on culture conditions which influence the rate of proliferation and apoptosis. It was found that the senescent HUVEC arrest in the G1 phase of the cell cycle but, unlike fibroblasts, accumulate with a 4 N DNA content suggesting polyploidization. Moreover, in contrast to human fibroblasts, senescent endothelial cells display a considerable increase in spontaneous apoptosis (Wagner et al., 2001).

The question is whether replicative cellular senescence is the phenomenon observed just in culture or it takes place also *in vivo*. As the main hallmark of replicative senescence is the limit of cell divisions caused by telomere attrition, the relatively easy way to detect senescent cells *in vivo* is to determine the length of telomeres. Indeed, telomere length has been shown to gradually decline with age in many human tissues. With a few exceptions, mainly brain and myocardium, reduction in telomere length was found in samples of over two dozen tissues taken from human subjects ranging in age from newborn to over 100 years (Takubo et al., 2010). Interestingly, even the cells that express telomerase undergo telomere shortening over time. Many studies have found a positive correlation between telomere shortening in human peripheral leukocytes and the risk of typical age-associated diseases. Moreover, premature aging syndromes are characterized by short telomeres (Aubert and Lansdorp, 2008; Blasco, 2005). Nonetheless, the length of the telomeres of the oldest old (centenarians) depends very much of the cell type (Mondello et al., 1999).

Replicative senescence characterized by telomere erosion occurs also in tissue stem cells particularly those present in continuously renewing tissues. Stem cells undergo many rounds of division to maintain normal tissue homeostasis. Experimental manipulations, such as serial transplantation, clearly reveal that adult stem cells have a finite replicative life span that can be exhausted. Senescing of stem cells may be responsible for a decline with age in the homeostatic and regenerative capacities of all tissues and organs. Although in some cases, such as neural stem cells and melanocyte stem cells, senescence may lead to a depletion of the stem cell pool, in most stem cell compartments, the number of stem cells does not decline significantly with age; rather, they lose their functionality due to the low turnover rate, which allows for the accumulation of damages during the state of quiescence, just like in postmitotic cells (reviewed in Liu and Rando (2012)).

Assuming that telomere erosion due to the "end replication problem" is a "replicometer" counting cell divisions the question is how the signal from short telomeres is transduced to the cell cycle machinery? It has been known for many years that the onset of cellular senescence is regulated, together or sometimes independently, by two suppressor proteins, p53 and Rb, whose genes are frequently lost or mutated in cancer cells, thus promoting indefinite proliferation. Phosphorylated and stabilized p53 activates its target genes, including the cyclin-dependent kinase inhibitor p21, whose protein product activates Rb through inhibition of a cyclin-dependent kinase complex (E/Cdk2). The hypo-phosphorylated Rb inhibits the transcription of E2F target genes arresting cells in the G_1 phase of the cell cycle. Rb is also activated by another Cdk inhibitor, p16, acting through the cyclin D/Cdk4,6 complex (Campisi, 2001). The p53 protein is the main mediator of double-strand DNA breaks (DSBs) which induce the so called DNA damage response (DDR). When telomeres reach a critical minimal length, they are recognized as DNA double-strand ends, resulting in the DDR activation. Senescence is determined not by the average telomere length within a cell, but by the presence of a few telomeres that are sufficiently short to trigger DDR. Moreover, it was shown that not only short telomeres, but also telomeres that are dysfunctional due to the loss of telomere-bound proteins can induce DDR (d'Adda di Fagagna, 2008).

DDR is mediated by DNA damage protein sensors, such as the MRN complex, which trigger the activation of a signal transduction system including the protein kinases: ATM (ataxia telangiectasia mutated), ATR (ATM and Rad3-related), CHK1 and CHK2 (checkpoint kinases 1 and 2). Ultimately, DDR activates p53 (reviewed in d'Adda di Fagagna (2008); Prieur and Peeper (2008)). Consistent with the concept that DDR is activated in senescence, the number of γ-H2AX foci, which consist of accumulated H2AX phosphorylated by ATM kinase and are a common and reliable DSB marker, increases in both mouse and human senescent primary cells in tissue culture (Nakamura et al., 2008) as well as in the skin of old primates (Herbig et al., 2006). SA-β-gal and γ-H2AX-positive cells co-localize in old mice (Wang et al., 2009) and the number of γ-H2AX foci in lymphocytes in humans increases with age (Sedelnikova et al., 2004). Fibroblasts from people suffering from Hutchinson–Gilford syndrome display persistent markers of an increased basal DDR, such as γ-H2AX, ATM and ATR foci, as well as an increased level of phosphorylated CHK1 and CHK2 (Burtner and Kennedy, 2012).

DDR plays an essential part in both senescence initiation and maintenance. Functional inactivation of CHK2, or deletion of genes encoding p53 and p21, extends the proliferation of human fibroblasts in culture beyond their senescence limit. Also transient inactivation of ATM, alone or together with ATR, and combined CHK1 and CHK2 inactivation lead to an escape from senescence and re-entry into the S phase of the cell cycle (d'Adda di Fagagna, 2008).