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Review

Telomeres and Cell Senescence - Size Matters Not

Stella Victorelli *, João F. Passos

Institute for Cell and Molecular Biosciences, Newcastle University Institute for Ageing, Newcastle upon Tyne NE4 5PL, UK

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ABSTRACT

Telomeres are protective structures present at the ends of linear chromosomes that are important in preventing genome instability. Telomeres shorten as a result of cellular replication, leading to a permanent cell cycle arrest, also known as replicative senescence. Senescent cells have been shown to accumulate in mammalian tissue with age and in a number of age-related diseases, suggesting that they might contribute to the loss of tissue function observed with age. In this review, we will first describe evidence suggesting a key role for senescence in the ageing process and elaborate on some of the mechanisms by which telomeres can induce cellular senescence. Furthermore, we will present multiple lines of evidence suggesting that telomeres can act as sensors of both intrinsic and extrinsic stress as well as recent data indicating that telomere–induced senescence may occur irrespectively of the length of telomeres.

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1. Cellular Senescence and Telomeres

Cellular senescence was first described by Hayflick and Moorhead as the progressive and irreversible loss of proliferative potential of human somatic cells (Hayflick and Moorhead, 1961). This phenomenon is characterized not only by a loss in replicative capacity, but also by a series of dramatic changes in cell morphology, gene expression, metabolism, epigenetics and others (van Deursen, 2014). It is a stable phenotype, with senescent cells being able to be kept in culture for several years following the initial arrest.

So far, the best explanation for replicative senescence is the shortening of telomeres, regions composed of DNA repeats associated with proteins, found at the ends of chromosomes. In the 1990s, it was shown that telomere regions gradually shorten with cell division and that this correlates with the induction of cellular senescence (Harley et al., 1990). Importantly, it was demonstrated that ectopic expression of the enzyme telomerase, which is capable of elongating telomeres, counteracts telomere shortening driven by cell division and bypasses the senescence arrest (Bodnar et al., 1998). This experiment, demonstrated that telomere length was the limiting factor in the senescence arrest and therefore played a causal role in the process.

Since then, great advances have been made in the understanding of how telomeres are able to signal the senescence arrest. These

* Corresponding author.
E-mail address: s.g.victorelli@ncl.ac.uk (S. Victorelli).

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mechanisms are of particular importance in the field of ageing, since cellular senescence, driven by telomere dysfunction, has been shown to be a causal driver of ageing and age-related pathology (van Deursen, 2014).

1.1. Why Is Cellular Senescence Important?

In recent years, important conceptual advances have been made in terms of our understanding of the role of senescent cells *in vivo*. It is now clear, that the impact of senescence *in vivo* is not restricted to the loss of proliferative capacity. Apart from the cell-cycle arrest, senescent cells have been shown to experience dramatic changes in terms of gene expression, metabolism, epigenome and importantly, have been shown to have a distinct secretome profile, known as the Senescence-Associated Secretory Phenotype (SASP) (Coppé et al., 2008), which mediates the interactions between senescent and neighboring cells. The SASP includes pro-inflammatory cytokines as well as growth factors and extracellular matrix degrading proteins and is thought to have evolved as a way for senescent cells to communicate with the immune system (potentially to facilitate their own clearance), but also as an extracellular signal to promote the regeneration of tissues through the stimulation of nearby progenitor cells (van Deursen, 2014). Nonetheless, it has been shown that a "chronic" SASP is able to induce senescence in adjacent young cells, contributing to tissue dysfunction (Acosta et al., 2013) and paradoxically tumorigenesis (Demaria et al., 2017).

Recent data indicates that senescent cells play a variety of beneficial roles during processes such as embryonic development, tumor suppression, wound healing and tissue repair (Krizhanovsky et al., 2008; Muñoz-Espín et al., 2013; Demaria et al., 2014; Ritschka et al., 2017). On the other hand, senescent cells have been detected in multiple age-related diseases and in a variety of different tissues during ageing. However, only recently, senescent cells have been shown to contribute causally to the ageing process. Elimination of senescent cells by suicide gene-mediated ablation of $p16^{Ink4a}$-expressing senescent cells in INK-ATTAC mice has resulted in significant improvements in healthspan and lifespan suggesting that senescent cells are drivers of ageing (Baker et al., 2011; Baker et al., 2016). This has led the scientific community to identify new interventions to target senescence as a therapy against ageing and age-related diseases (Zhu et al., 2015; Chang et al., 2016). The positive and negative effects of senescence in different physiological contexts may be a reflection of the ability of the immune system to effectively clear senescent cells. It has been speculated that an "acute" type of senescence plays generally beneficial roles in processes such as embryonic development and wound-healing, while a "chronic" type of senescence may contribute to ageing and age-related disease (van Deursen, 2014). The role of telomeres in the induction of these two types of senescence is still unclear. However, senescent cells detected during development, which are present transiently in tissues (Muñoz-Espín et al., 2013) are not associated with the activation of a DNA damage response, which suggests that telomeres are unlikely involved in the process.

1.2. What Are Telomeres and How Do They Signal Senescence?

Telomeres are repetitive sequences of DNA (tandem TTAGGG repeats), associated with a number of proteins which form a complex known collectively as the "Shelterin". It is believed that the "Shelterin" complex stabilizes a lariat-like structure, named the telomere-loop (or t-loop for short) with the purpose of shielding the exposed end of linear chromosomes (de Lange, 2005). Telomeres contain both a C-rich lagging strand and a G-rich leading strand, which contains a 3′ overhang comprising of single-stranded nucleotide repeats. The overhang is thought to bind to one of the double-stranded DNA regions and facilitate the formation of the t-loop structure (Griffith et al., 1999).

The "Shelterin" complex is composed of six proteins: telomeric repeat binding factor 1 (TRF1), telomeric repeat binding factor 2 (TRF2), TRF2 interacting protein (RAP1), TRF1-interacting nuclear factor 2 (TIN2), adrenocortical dysplasia protein homolog (TPP1) and protection of telomeres 1 (POT1). TRF1 and TRF2 bind to double-stranded telomeric sequences, whereas POT1 binds to the single-stranded 3′ overhang (Zhong et al., 1992; Bianchi et al., 1997; Bilaud et al., 1997; Baumann and Cech, 2001).

Why do telomeres shorten? Most somatic cells lack the activity of the enzyme telomerase and experience, with cell division, a phenomenon called the "end-replication problem". This occurs due to the intrinsic inability of DNA polymerases to completely replicate the telomere C-rich lagging-strand. During the process of lagging-strand synthesis, RNA primers come into play allowing DNA polymerases to initiate DNA replication. However, upon removal of the last primer at the 3′ end, the newly synthesized strand will inevitably be a few nucleotides shorter, resulting in loss of telomere repeats. This phenomenon was first hypothesized independently by Olovnikov and Watson in the early seventies (Olovnikov, 1971; Watson, 1972) and confirmed experimentally in the nineties (Harley et al., 1990).

How do telomeres signal senescence? It has been hypothesized that with the progressive loss of telomere repeats with cell division due to the "end-replication problem", shelterin components may be displaced from telomere regions and subsequently destabilize the abovementioned t-loop conformation (Griffith et al., 1999). This results in the exposure of the telomere end, which becomes recognized by the DNA repair machinery as a double-strand DNA break. Evidence supporting this hypothesis originated initially from studies which demonstrated that deletion of shelterin component TRF2 in human cells results in the activation and recruitment of proteins involved in the DNA damage response (DDR) such as 53BP1, the Mre11 complex and phosphorylated forms of ATM, H2A.X and Rad 17 (Takai et al., 2003). Similarly, conditional deletion of the 3′ overhang binding protein Pot1a in mice, results in the activation of a DDR specifically at telomere regions and induction of senescence (Wu et al., 2006). Consistent with the idea that progressive telomere shortening results in the exposure of telomere-ends and subsequent activation of a DDR, it was shown that replicatively senescent human fibroblasts accumulate proteins involved in the DDR at telomere regions, including γH2A.X, 53BP1, MDC1 and NBS1 (d'Adda di Fagagna et al., 2003). In this review, we chose not to embark on a detailed description of the DDR pathways, but will highlight some of the downstream effector pathways important for senescence induction. It is well established that a DDR can result in activation of transcription factor p53 which is involved in a variety of processes including DNA repair, cell-cycle arrest and apoptosis. P53 is a positive regulator of transcription of p21, a cyclin-dependent kinase inhibitor, which is involved in the cell-cycle arrest observed during senescence both *in vitro* and *in vivo*. Consistent with a key role for p21 in telomere-induced senescence, deletion of p21 improves the regenerative capacity of intestinal crypts and hematopoietic stem cells in late-generation telomerase-deficient mice, which contain critically short telomeres in a variety of tissues and accelerated ageing phenotype (Choudhury et al., 2007). Apart from the p53-p21 pathway, p16-Rb is also an important effector pathway of cellular senescence. However, the link between telomere-dysfunction and p16 activation is less understood. For instance, p16 has been shown to be activated independently from telomere dysfunction in human fibroblasts (Herbig et al., 2004). However, a more recent study reported that deletion of p16 in Wrn-deficient mice, which have dysfunctional telomeres, resulted in increased proliferative capacity of mouse embryonic fibroblasts (Zhang et al., 2012). Furthermore, deletion of TRF2 was shown to induce p16 activation (Jacobs and de Lange, 2004) However, p16 deficiency in these cells only partially restored the growth arrest imposed by telomere-dysfunction. Only when both p16 and p53 were simultaneously inhibited, a complete rescue in proliferation was observed. This and other studies suggest that p16 may act as a secondary mechanism (besides the p53-p21

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