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Review

Telomeres and Cell Senescence - Size Matters Not

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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.

Contents

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

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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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pathway) mediating the senescence arrest following telomere dysfunction (Beausejour et al., 2003; Jacobs and de Lange, 2004).

There are several lines of evidence to suggest that telomere dysfunction may play an important role during ageing. Firstly, telomere dysfunction, assessed by the association between DDR proteins and telomeres, has been shown to increase with age *in vivo* in the skin of primates (Herbig et al., 2006), the liver, gut and lung of mice (Hewitt et al., 2012; Birch et al., 2015). Secondly, mouse models where telomere function has been compromised, suggest a role in the ageing process. Telomerase knock-out (mTERC$^{-/-}$) mice which carry a homozygous deletion of the RNA component of telomerase (Blasco et al., 1997) show a generation-dependent telomere shortening, which results in critically short telomeres and both senescence and apoptosis (Lee et al., 1998). In these mice, dysfunctional telomeres have been shown to impact on the function of stem cells, organ regeneration and lifespan (Wong et al., 2003). Moreover, it has been shown that reintroduction of telomerase activity in telomerase-deficient mice is able to revert the premature ageing phenotype observed in tissues such as the spleen, intestine and testes (Jaskelioff et al., 2011).

2. Stress Accelerates Telomere Shortening

Although cellular replication is a major contributor to telomere attrition, other factors can affect the rate at which telomeres shorten. For example, it has been extensively reported that telomere shortening is accelerated when cells are exposed to mild oxidative stress, leading to reduced replicative capacity and a phenotype that resembles replicative senescence (von Zglinicki et al., 1995; von Zglinicki, 2002; Saretzki et al., 2003). Indeed, reactive oxygen species (ROS) have been implicated as important players in telomere-dependent senescence, as overexpressing the antioxidant enzyme superoxide dismutase (SOD3) in human fibroblasts, or reducing the levels of intracellular peroxide using antioxidants, are effective in reducing the rate of telomere shortening and extending replicative lifespan of these cells (von Zglinicki et al., 2000; Serra et al., 2003). Moreover, studies have demonstrated that the antioxidant capacity of a cell correlates with its replication potential and rate of telomere shortening, suggesting a direct relationship between telomere attrition and the levels of intracellular oxidative stress (Richter and von Zglinicki, 2007). The role of ROS in telomere dysfunction has also been extended to *in vivo* studies, with one study suggesting that demyelination and axonal damage in multiple sclerosis (MS) patients are mediated by oxidative stress (Gilgun-Sherki et al., 2004). Indeed, a more recent report has shown that MS patients have increased markers of oxidative stress and shorter telomeres when compared to healthy controls (Guan et al., 2015). Although it is still unclear where ROS is originated, studies have suggested that mitochondrial-derived oxidative stress plays an important role in telomere shortening. For example, treating fibroblasts with Mito-Q, a mitochondria-targeted antioxidant, significantly reduces the rate of telomere shortening, as well as being sufficient to extend replicative lifespan under conditions of hyperoxia (Saretzki et al., 2003). Other studies have demonstrated that mild mitochondrial uncoupling, which reduces the accumulation of mitochondrial superoxide, is sufficient to extend the lifespan of human fibroblasts and decrease the rate of telomere shortening in these cells, reinforcing the importance of mitochondrial ROS in this process (Passos et al., 2007). On the other hand, FCCP treatment, which causes severe mitochondrial depolarization and thus mitochondrial dysfunction, increases ROS generation and leads to telomere attrition, telomere loss and chromosome fusions in mouse embryos (Liu et al., 2002). Moreover, patients with mitochondrial diseases such as MELAS and LHON, who display respiratory chain dysfunction, have shorter telomeres in white blood cells when compared to healthy subjects, further supporting a role of mitochondrial dysfunction in telomere shortening (Oexle and Zwirner, 1997). In addition, many age-related disorders such as Alzheimer's and cardiovascular disease have been associated with increased ROS production due to mitochondrial dysfunction (Dai et al., 2014), as well as with accelerated telomere shortening (Fyhrquist and Saijonmaa, 2012; Cai et al., 2013).

Telomeres are thought to be favored targets of oxidative attack compared to the rest of the genome due to their high content of guanine triplets, which are highly susceptible to oxidative modifications (Oikawa and Kawanishi, 1999). Indeed, single-stranded breaks have been shown to preferentially accumulate at telomeres in conditions of mild oxidative stress, and such lesions lead to replication fork stalling, resulting in incomplete replication of chromosome ends and thus accelerated telomere shortening (Petersen et al., 1998; von Zglinicki, 2000). Additionally, evidence suggests that oxidative damage at telomeres displaces shelterin proteins TRF1 and TRF2, which might be another mechanism by which oxidative stress leads to telomere dysfunction (Opresko et al., 2005). However, others have recently shown that TRF2 was often still present at damaged telomeres when cells were exposed to stress, suggesting that loss of shelterin components is not the sole cause of telomere dysfunction (Fumagalli et al., 2012). Another feature that makes telomeres unique is that lesions occurring at these sites are less efficiently repaired when compared non-telomeric damage (Kruk et al., 1995). This is mainly due to the presence of shelterin proteins, as TRF2 and RAP1 have been shown to inhibit NHEJ at telomeres by preventing the actions of DNA-PK, a double-stranded break repair complex protein, and also by inhibiting ligase-IV-mediated end joining (Bombarde et al., 2010). In support of this, recruitment of ligase IV as a response to DNA damage is impaired when double-stranded breaks (DSBs) are generated adjacent to telomeric repeats in budding yeast (Fumagalli et al., 2012). In addition, expressing TRF2 adjacent to a DSB in mammalian cells leads to persistent DNA damage, providing further evidence to the irreparability of telomeres (Fumagalli et al., 2012). As well as being involved in senescence induction *via* telomere dysfunction, ROS have also been proposed to act as an effector mechanism during senescence. We have shown that a DNA damage response can induce mitochondrial ROS and this is partially dependent on the activation of p53 and p21 pathways, but also signals from ATM towards mTORC1 (Passos et al., 2010; Correia-Melo et al., 2016). In accordance to this, late generation TERC$^{-/-}$ mice, which have dysfunctional telomeres, display higher levels of oxidative damage in tissues, which is ameliorated by deletion of p21 (Passos et al., 2010). Another report also shows that signaling downstream of short telomeres activates p53, which binds and represses PGC-1α and PGC-1β promoters leading to mitochondrial dysfunction (Sahin et al., 2011). It has been suggested that ROS generated by dysfunctional mitochondria is involved in a feedback loop generating further DNA damage such that the DDR is maintained, contributing to the stabilization of the senescent phenotype (Passos et al., 2010; Correia-Melo et al., 2016).

3. Telomere Dysfunction Can Occur Irrespectively of Length

So far, the majority of studies have given evidence to senescence as a result of telomere shortening. However, several reports now suggest that telomere dysfunction can also occur in a length-independent manner (Fig. 1). For example, persistent telomeric DDR signaling in response to genotoxic stress has been shown to occur irrespectively of length in human fibroblasts *in vitro* and in mouse neurons *in vivo* (Fumagalli et al., 2012). Longer telomeres signaling a DDR have also been implicated during the ageing process *in vivo*. Initially, it was believed that murine cell senescence was mainly mediated by oxidative damage, and occurred independently of telomeres (Parrinello et al., 2003). However, recent studies suggest otherwise; telomeric DNA damage has been shown to increase with age in the gut and liver of mice, which occurred irrespectively of length (Hewitt et al., 2012; Jurk et al., 2014). Length-independent telomere damage has also been observed in hippocampal neurons and liver of baboons with age, possibly indicating that this plays a role in age-related tissue dysfunction by inducing cellular senescence (Fumagalli et al., 2012). Moreover, analysis of individual telomeres in small airway epithelial cells in the lung of COPD patients,

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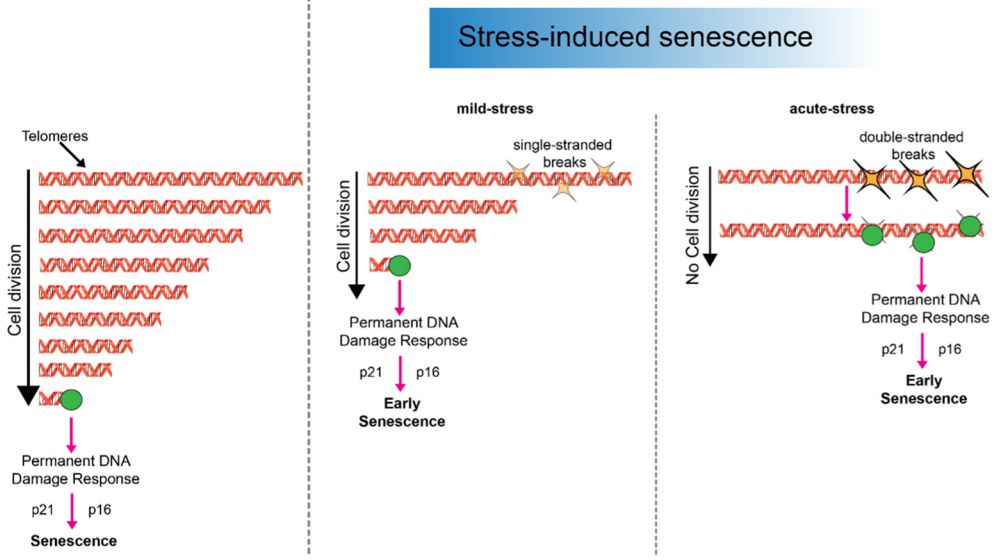

Fig. 1. Length-independent telomere damage. Telomere shortening that occurs naturally with each round of cell division ultimately leads to chromosome ends becoming exposed and activating a DNA damage response, which results in a permanent arrest known as replicative senescence. However, recent evidence suggests that telomeres may serve as highly sensitive sensors of stress. It is known that mild oxidative stress causes single-stranded breaks to accumulate at telomeres, leading to accelerate shortening and premature cell cycle arrest. However, it is possible that acute stresses induce telomeric double-stranded breaks which are not efficiently repaired. This results in a persistent DDR signaling, preventing cells from undergoing further rounds of replication irrespective of their telomere length, a state which can also be called "telomere length-independent senescence".

which show senescence markers such as increased p16 expression, has also revealed that damaged telomeres are not significantly shorter than those not associated with DDR proteins (Birch et al., 2015). This phenomenon also extends to oncogene-induced senescence (OIS), as a study has demonstrated that dysfunctional telomeres in melanocytic nevi were not shorter than functional ones (Suram et al., 2012). In fact, TRF2 was shown to still be present at a fraction of dysfunctional telomeres, suggesting that shortening and uncapping (*i.e.* loss of shelterin proteins) are not the only causes of DDR activation (Suram et al., 2012). In accordance to this, length-independent telomere dysfunction has also been shown to occur in replicative senescent cells, where TRF2 and RAP1 were still present in some telomeres positive for DDR proteins, suggesting that DDR activation at telomeres is not always a consequence of loss of shelterin components (Kaul et al., 2012).

It has been suggested that DNA damage is more likely to occur at long telomeres as they represent a more abundant target for lesion formation, possibly explaining the occurrence of length-independent DDR activation (Fumagalli et al., 2012). Indeed, in a study where telomeres were elongated in human cancer cells, it was shown that cells with very long telomeres were more sensitive to ionizing irradiation, suggesting that telomeres above a critical length are more likely to accumulate DSBs (Fairlie and Harrington, 2015). Moreover, a recent Mendelian randomization study has shown strong associations between long telomeres due to germline genetic variation and increased risk for certain types of cancer (Haycock et al., 2017). However, thus far evidence does not clearly point out that longer telomeres are preferential targets of damage over shorter ones. Studies where individual telomere length was analyzed both in melanocytic nevi and in mice were unable to identify a significant difference in length between damaged and undamaged telomeres (Hewitt et al., 2012; Suram et al., 2012). One possibility is that the sensitivity of current techniques available to measure individual telomere length in tissues is not great enough to detect very short telomeres, masking any possible significant differences in length between dysfunctional and functional telomeres.

Roger Reddel's group have suggested that telomeres may exist in three different states, providing an explanation to DNA damage signaling at telomeres that still contain shelterin proteins. The first state is the so-called fully capped or closed state, where telomeric repeats are sufficiently long such that the T-loop conformation is not compromised, and shelterin proteins inhibit NHEJ, preventing DDR activation. However, if T-loop conformation is lost, telomeres may adopt an intermediate state, activating a DDR without leading to end-to-end fusions due to the presence of sufficient shelterin proteins. This may occur in a length-dependent and –independent manner, possibly explaining the presence of DDR proteins at longer telomeres that still contain components of the shelterin complex. Lastly, extreme telomere shortening can lead to disruption of the T-loop, causing DDR activation, a state also known as fully uncapped. In the latter, telomeric repeats have reached critical lengths such that there is a loss of shelterin proteins and NHEJ is no longer inhibited at telomeres, causing end-to-end fusions (Cesare et al., 2009). Moreover, recent data from our lab and others indicate that a fourth state may also exist, whereby persistent DDR signaling within telomeric repeats can occur even in the presence of an undisrupted T-loop and shelterin components.

A major question which arises from the aforementioned work is if activation of a DDR within telomere repeats indicates physical DNA double stranded breaks or is merely changes in chromatin without an associated lesion. Recently, a new method has been developed which allows the accurate detection of physical DNA damage breaks by DNA damage *in situ* ligation, followed by proximity ligation assay. Using this method, the authors observed that following acute genotoxic stress, persistent DNA double stranded breaks occur, *in vitro* and *in vivo*, which co-localise with DDR proteins (Galbiati et al., 2017).

4. Telomere-persistent DNA Damage Is Important For Stabilizing senescence

Normally, cells can efficiently and rapidly repair DNA damage that occurs as a result of daily insults; however, studies have shown that telomeric lesions remain unrepaired for several months both *in vitro* and *in vivo* (Fumagalli et al., 2012; Hewitt et al., 2012). In fact, our group has demonstrated through live-cell imaging that most of the long-lived foci co-localized with telomeres in stress-induced senescence (Hewitt et al., 2012). This was also shown to occur *in vivo*,

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where telomeres signaling a persistent DDR were observed in hippocampal neurons of mice even three months after they were exposed to genotoxic stress (Fumagalli et al., 2012). Research also indicates that damaged telomeres play a role in oncogene-induced senescence, preventing human cancer progression. One study showed that persistent telomeric DDR foci occur as a result of oncogene activation in human cells, as well as replication fork stalling at telomeres and DNA replication stress (Suram et al., 2012). Accumulation of damage at telomeres is also a feature of cancer precursor lesions, such as human melanocytic nevi, ductal breast hyperplasia, and colonic adenomas, implicating long-lived telomere damage as an important contributor to tumor-suppressor mechanisms *in vivo*. It is believed that cells undergo replication stress as a response to oncogene activation, which causes telomere shortening and dysfunction in cells that do not express telomerase. Ultimately, this induces senescence, and prevents malignant transformation of somatic cells (Suram et al., 2012). However, recent studies have demonstrated that telomerase expression in cells arrested due to OIS is capable of resolving existing telomere damage, and prevents further telomere dysfunction in these cells. Therefore, telomerase alleviates telomere damage as a result of replication stress in pre-malignant cells, allowing these cells to escape senescence, and contribute to cancer progression (Meena et al., 2015; Patel et al., 2016).

The irreparability of telomeric DNA damage has been recently challenged by studies which have provided evidence that DSB repair can occur at telomeres (Doksani and de Lange, 2016; Mao et al., 2016). However, repair at telomeres was only observed in proliferating cells, such as BJ fibroblasts and HeLa cells. Interestingly, HeLa cells, which are faster dividing cells, display a faster repair kinetics compared to slower dividing fibroblasts, suggesting that proliferation rate is an important determinant of telomeric DSB repair (Mao et al., 2016). On the other hand, when senescence was induced in fibroblasts either by genotoxic stress or replicative exhaustion, both of which were associated with increased telomeric DDR signaling, no significant repair at chromosome ends was detected (Mao et al., 2016). By using the CRISPR-Cas9 system to specifically target telomeric repeats, the authors were able to induce double-stranded breaks specifically at telomeres in 293T cells, and demonstrated that homologous recombination (HR) plays an important role in telomere damage repair (Mao et al., 2016). Evidence for break repair at telomeres has also been provided by de Lange's group, who expressed an endonuclease coupled to TRF1 (TRF1-*Fok*I) in order to induce telomeric DSBs in mouse embryonic fibroblasts (MEFs) (Doksani and de Lange, 2016). Indeed, TRF1-*Fok*I expression led to increased telomere-associated DNA damage foci and activation of ATM kinase-dependent signaling in S-phase; however, these breaks were shown to be efficiently repaired (Doksani and de Lange, 2016). As well as showing the involvement of homologous recombination in telomeric DSB processing, it was also proposed that alternative non-homologous end-joining (alt-NHEJ) plays an important role in repairing *Fok*I-induced DSBs at telomeres (Doksani and de Lange, 2016). The aforementioned studies suggest that in dividing cells, particularly in S phase, telomeric breaks are subjected to repair *via* HR; however, this does not occur in senescent cells. It has been suggested that this could be a consequence of heterochromatin changes that occur during senescence (Narita et al., 2003), making telomeric DNA less accessible for HR to occur, thus contributing to the persistent DDR signaling observed (Mao et al., 2016). However, further studies are required to confirm this hypothesis. In addition, even though it was reported that generating telomeric DSBs did not lead to senescence in both cases, it is important to note that the cells used in these studies are immortalized (Doksani and de Lange, 2016; Mao et al., 2016). Therefore, it is important to determine the response of non-immortalized cells to telomere-specific damage as they represent a more physiologically relevant model.

Since persistent telomeric DNA damage is a feature of replicative, stress- and oncogene-induced senescence, it has been suggested that unrepaired telomeres provide a source of persistent DDR signaling which is important to the establishment of senescence (Fumagalli et al., 2012). However, damage at non-telomeric sites also contributes to the senescent phenotype. Short-lived, non-telomeric lesions are constantly renewed during replicative and stress-induced senescence. In fact, these make up about half of the DNA damage foci, and their constant turnover has been suggested to be a result of increased ROS production during senescence, as inhibiting ROS generation results in a rescue of the proliferation arrest in a number of cells (Passos et al., 2010; Hewitt et al., 2012). Therefore, DNA damage signaling emanating from both telomeric and non-telomeric regions are important for the senescent phenotype, although distinguishing the extent of their individual contribution to senescence may prove technically challenging.

In summary, substantial evidence is emerging suggesting that telomeres do not only act as replicometers, simply determining how many rounds of division a cell is capable of undergoing. Instead, they act as molecular sensors of intrinsic and extrinsic stresses, and maintain genomic stability by limiting replication of cells that have accumulated significant genomic damage.

5. Outstanding Questions

While data strongly suggests that telomere-associated foci, which can occur irrespectively of telomere length, increase with age and in several age-related diseases, it remains to be determined whether it is a better biomarker than telomere length alone particularly in humans. Further larger studies need to be conducted, however tissue availability may be a limitation.

Search Strategy and Selection Criteria

Data included in this review were obtained by searches of Pubmed, and references from relevant articles using the search terms “senescence”, “telomeres”, “telomere damage”, and “ageing”. Only articles published in English between 1970 and 2017 were included.

Author Contributions

Stella Victorelli and João F. Passos contributed equally to the writing of the review. João F. Passos contributed mostly to chapter 1 and Stella Victorelli to chapters 2–4.

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