What Lies in between Telomere and Organismal Ageing: Comparison between Replicative Senescence and Stress-Induced Premature Senescence

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Abstract. A mitotic cell that rests in permanent cell cycle arrest without the ability to divide is considered as a senescent cell. Cellular senescence is essential to limit the function of cells with heavy DNA damages. The lack of senescence is in favour of tumorigenesis, whereas the accumulation of senescent cells in tissues is likely to induce ageing and age-related pathologies on the organismal level. Understanding of cellular senescence is thus critical to both cancer and ageing studies.

Senescence, essentially permanent cell cycle arrest, is one of the results of DNA damage response, such as the ataxia telangiectasia mutated and the ataxia telangiectasia and Rad3-related signaling pathways. In other cases, mild DNA damages can usually be repaired after DNA damage response, while the cells with heavy damages on DNA end in apoptosis. The damage to the special structure of telomere, however, prone to result in permanent cell cycle arrest after activation of DNA damage response. In fact, a few previous pieces of research on ageing have largely focused on telomere and considered it a primary contributor to different types of senescence. For instance, its reduction in length after each replication turns on a timer for replicative senescence, and its tandem repeats specific to binding proteins makes it susceptible to DNA damage from oxidative stress, and thus stress-induced premature senescence. In most of the senescent cells, the accumulation of biomarkers is found around the telomere which has either its tail structure disassembled or damage foci exposed on the tandem repeats.

In this review, among several types of senescence, I will investigate two of the most common and widely discussed types in eukaryotic cells - replicative senescence and stress-induced premature senescence - in terms of their mechanism, relationship with telomere, and implication to organismal ageing.

1 Introduction

It was nearly sixty years ago when Hayflick and colleagues revealed the restricted ability in the proliferation of normal human cells (fibroblasts from adult or fetal tissue) in vitro (Hayflick & Moorhead, 1961). Despite the optimal conditions for cell growth, the accumulation of nondividing cells appears after a certain number of mitotic divisions. "Cellular senescence" was then termed to describe this permanent cell cycle arrest. It is a key feature to inhibit cell proliferation and thus prevent tumorigenesis (Acosta & Gil, 2012), while on the other side, the accumulation of non-functioning senescent cells are suspected to contribute to organismal ageing because biomarkers of senescent cells have been observed to increase proportionally with age (de Magalhaes & Passos, 2018).

Correspondingly, the properties of telomere made it a popular object of this topic. As telomere reduces in lengths after each replication due to the replication mechanisms, the replicative senescence (RS) proposed by Hayflick is attributed to the instability of critically short telomere (Harley, Futcher, & Greider, 1990). However, it is also a popular rumour that telomere length of cells in human tissue can determine the organismal longevity. Considerably low correlation between telomere length and age has been observed in a series of research (Kammori et al., 2002; Njajou et al., 2007; Nwosu et al., 2005; Serra & von Zglinicki, 2002), even though the replication capacity is directly proportional to the length (Allsopp et al., 1992). In addition, the telomere length of a eukaryotic cell at permanent cell cycle arrest does not have to obtain critically short telomere (Birch et al., 2015; Parrinello et al., 2003), which gives a clue to other types of senescence that do not involve telomere length, including stress-induced premature senescence (SIPS), the most common type. SIPS can be induced when stress including reactive oxidative species (ROS) causes DNA damage that can't be repaired (Hewitt et al., 2012). Despite RS and SIPS differ in terms of involvement of telomere length, it is common between them that DNA damage response (DDR) plays a significant role in bridging telomere and cellular senescence (Hewitt et al., 2012; H. Takai, Smogorzewska, & de Lange, 2003). Signaling pathways like ataxia telangiectasia mutated

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(ATM) and ataxia telangiectasia and Rad3-related (ATR) detect the presence of instability in DNA either caused by short telomere or oxidative damage and induce cell cycle arrest following a series of reactions (Marechal & Zou, 2013). A thorough understanding of the structure of telomere and the mechanism of DDR is an inevitable essential to learn senescence and how to manipulate it, for example, to induce senescence in cancer cells, or to reduce the senescent cells to slow down ageing.

Therefore, to look at senescent phenotypes from molecular structures and activities, this review will examine the two most commonly discussed types of senescence, RS and SIPS, in terms of their relationship with telomere, associated DDR, ways to escape or resist senescence, and implication on ageing. Lots of pieces of research have already established a systematic understanding of this topic. A few unsolved problems including the epigenetics of the telomere and senescent cell dynamics in vivo are present.

2 Telomere

The telomere is the end portion of the mammalian linear DNA that protects the main chromatin sequence. The telomeric DNA is made up of double strand short tandem repeats, TTAGGG on 5' to 3' strand and AATCCC on 3' to 5' strand. The length of telomere varies between organisms. Typically, in newborn human, the telomere length range between 10-15 kilobases (kb) (1600~2500 repeats); the laboratory mice and rats can have telomere as long as 50 kb (8300 repeats) (de Lange et al., 1990). A fully functional telomere requires as little as 400 bp, about 67 repeats (Barnett et al., 1993; Farr, Fantes, Goodfellow, & Cooke, 1991). Due to the discrepancy of the double strands in cytosine and guanine proportion, the guanine-rich 5' to 3' strand is often referred to as the G-strand and 3' to 5' strand as the C-strand. The G-strand is usually longer than the C-strand, leading to a G-rich protrusion known as 3' overhang (Makarov, Hirose, & Langmore, 1997; McElligott & Wellinger, 1997), which has a length between 50-500 nucleotides (nt) in mammalians (Palm & de Lange, 2008). The shorter C-strand mostly ends up with ATC on 5' end, whereas the terminal sequence of the 3' overhang is variable (A. J. Sfeir, Chai, Shay, & Wright, 2005).

Instead of extended in a linear shape, looping structures are formed due to strand invasion. The base pair is formed between the 3' overhang (of the G-strand) and some site on the C-strand, this looping is named t-loop, first described in 1999 (Griffith et al., 1999) and visualized with electron microscopy in 2013 (Doksani, Wu, de Lange, & Zhuang, 2013). Different models of t-loops have been proposed, while either with solely 3' invasion or both 3' and 5' invasion (Tomaska, Nosek, Kar, Willcox, & Griffith, 2019), t-loop provides the terminus of telomere a more stable environment by sequestering the end of the chromatin, protecting the telomere itself from potential DNA damage (Griffith et al., 1999). The size of the t-loop varies, 30 kb in mice for example (Griffith et al., 1999), whereas it is independent of the length of 3' overhang (Tomaska et al., 2019). The t-loop structure is significant to the stability of telomere. Without it, the DDRs tend to recognize the end of linear DNA as a strand break, after which permanent cell cycle arrest will be provoked (de Lange, 2009). Multiple signaling pathways can detect these strand breaks and block cell cycles, which will be discussed later in the passage.

3 Shelterin and DNA Damage Response

It has been proved that shelterins, the telomere binding protein complexes that cover the telomeric repeats, play critical roles in t-loop formation and telomere strand protection. The six-unit shelterin complexes locate on the telomere including its t-loop. Two of the proteins, telomeric repeat binding factor 1 (TRF1) and TRF2, bind on the double-strand telomeric sequence as dimers. They connect to the rest four protein components including repressor/activator protein 1 (RAP1), protection of telomeres 1 (POT1), TRF1- and TRF2- interacting nuclear protein 2 (TIN2) and Adrenocortical dysplasia protein homolog (TPP1 or ACD). POT1 is the only one in the six units that bind on to single strand DNA. These proteins exist abundantly around the telomere throughout the cell cycle but have no function elsewhere in the nucleus (Palm & de Lange, 2008) (TPP1 and POT1 are of relatively lower abundancy)(K. K. Takai, Hooper, Blackwood, Gandhi, & de Lange, 2010). Among them, TRF2 and POT1 are especially critical because DDR can be immediately detected once either of TRF2 and POT1 is deleted.

TRF2 is essential for the t-loop formation by changing the topology of DNA by allowing telomere to wrap around the TRF homology (TRFH) domain (Benarroch-Popivker et al., 2016). Moreover, its cyclin-dependent kinase (CDK) phosphorylation site is like a switch coordinating t-loop's assembly and disassembly (Sarek et al., 2019). The disassembly of the t-loop can potentially lead to non-homologous end joining (NHEJ) and ATM signaling pathways. As TRF2 is deleted from cells, the chromosome ends of not necessarily homologous pair start to fuse with each other as a result of the misguided DDR, initiated by the binding of X-ray repair cross-complementing 5/6 (Ku70/80) to the double strand break (DSB). The phenotype of NHEJ is disastrous: there are two centrosomes in one chromosome, and that interferes with the anaphase of mitosis as two centromeres could go in the opposite direction (de Lange, 2009). The dysfunction of telomere justifies the importance of the presence of TRF2.

Furthermore, TRF2 also dedicates to block the ATM signaling pathway, in which MRN recruits ATM kinase on to DSB and amplifies the signal that inactivates CDK and impinges the cell cycle. As TRF2 deleted, the abundancy of Tumor suppressor p53-binding protein 1 (53BP1) at telomere, a DNA damage marker brought by ATM kinase, start to increase (Karlseder, Broccoli, Dai, Hardy, & de Lange, 1999). After removing the impact from DDR, super-resolution pictures taken also proves that TRF2 must be required in t-loop formation because the chromosome ends turn out to be linear when both TRF2 and DDR pathways are inhibited (Doksani et al., 2013).

ATR pathway, on the other hand, is impeded by POT1 on telomere, as the deletion of POT1 results in evidence of ATR signaling pathway activation, and the deletion of both POT1 and ATR kinase prevents DDR from happening (Denchi & de Lange, 2007). The exclusion of ATR by POT1 can be explained by the binding between POT1 and single strand DNA. It is also on the uncovered single strand break (SSB) where replication protein A (RPA) binds onto the single strand and recruits ATR kinase, which amplifies the signal and facilitates the repair, while phosphorylating Checkpoint kinase 1 (Chk1) and eventually leading to the inactivation of CDK and cell cycle arrest (H. Zhao & Piwnica-Worms, 2001). It was unclear why POT1 can excel in a competitive relationship with RPA in binding on the substrate, single strand DNA, while the RPA was much more abundant than POT1. One plausible hypothesis is that POT1 is tethered in the shelterin complex, having its concentration focus around telomere, whereas RPA can be all over the cell. The current experiment results have supported this hypothesis as the POT1 separated from the shelterin complex does not stop ATR signaling from happening (K. K. Takai, Kibe, Donigian, Frescas, & de Lange, 2011).

After all, the whole purpose of the telomere, including the t-loop, telomeric repeats, and the shelterin as a whole, or its individual components, is mainly aimed to resist disadvantageous DDR that blocks normal cell cycles. It can be viewed as a tradeoff mechanism to compensate for the linear structure of the eukaryotic chromosome. However, the replication problem with the 3' overhang constrains the telomere from a perfect solution to secure the chromosome.

4 Replicative senescence

While telomere's presence solved the end-protection problem for the chromosome, but there is an end-replication problem that impacts telomere's function. In other words, the telomere shrinks in length after each replication, and when it is critically short, it loses its ability of end-protection (Levy, Allsopp, Futcher, Greider, & Harley, 1992). It was back in 1961 when Leonard Hayflick and Paul Moorhead published their discovery that human diploid fibroblasts degenerate over 50 subcultivations in vitro, and the external factors are excluded (Hayflick, 1965; Hayflick & Moorhead, 1961). That reveals the senescence on the cellular level. In 1990, the cellular senescence was supported by the quantitative observation of telomeric DNA shortening (Harley et al., 1990). One of the most accepted explanations of telomere shortening is proposed earlier. It was suggested in 1973 that incomplete DNA synthesis on the edge of the sequence because the primer at the end of the leading-strand synthesis, or the whole Okazaki fragment may be missing due to limited space at DNA ends (Olovnikov, 1973). One problem with this explanation that got frequently ignored is that a blunt end double strand DNA will be formed on the leading-strand synthesis, which is not favourable for t-loop formation and leads to genomic instability (Wu, Takai, & de Lange, 2012). A telomere formed in this structure does not perform its end-protection duty.

There was a critical discovery that the lagging-strands often end with a specific position of the repeating sequence, while the leading strands end up at the random position (A. J. Sfeir et al., 2005). This opened a new vision to the mechanism of telomere shortening that there might be a specific lagging strand process going on at 5' end - the 3' overhang forms by active removal of 5' telomeric DNA instead of the inability of DNA synthesis. In other words, the telomere shortening is a tradeoff for genomic stability. Two nucleases, Exo1 and Apollo seem to play their role in 5' resection, with shelterin involved in the process (Wu et al., 2012). Further studies tried to explain the control of the resection as well, and a few enzymes are justified as inhibitors of 5' resection, including MAD2L2, 53BP1, RIF1, and shieldin complex (Boersma et al., 2015; Mirman et al., 2018; Xu et al., 2015; Zimmermann, Lottersberger, Buonomo, Sfeir, & de Lange, 2013).

Moreover, other than the gradual regression from replication, there are other means that a telomere can get shorter, due to telomere's fragility. The replication at the telomere region is difficult because too many factors are going on around the telomere simultaneously when the replication fork arrives. If a replication fork is interfered with while passing through the telomere, an unreplicated telomere may appear on the daughter DNA strands, and the telomere is much shorter than it should be after replication (von Zglinicki, 2002). The epigenetic factors including histone binding can also make the replication more difficult (Coluzzi, Leone, & Sgura, 2019). Therefore, interference with telomere replication is one example that stress can induce RS as well.

Telomeres shorten progressively, no matter through inability to perform complete synthesis or through lagging strand degradation by enzymes, at a rate of loss around 50~100 bp every cycle in mammalian cells, 37~85 bp in human cells in vitro and 75±9 bp in primary mouse fibroblast in vitro for example (Harley et al., 1990; Prowse & Greider, 1995; Y. Zhao et al., 2009). There is a tolerance for the loss of telomere, lasting for many cell divisions (as Hayflick limit describes). When the length reaches a critical value that the telomere is unable to protect its end (t-loop disassembles), DDR will be triggered (Griffith et al., 1999). This is similar to the removal of TRF2 from cells. When the 3' overhang is exposed, MRN complex and Ku70/80 can bind onto the end, and the ATM signaling pathway and NHEJ will be immediately activated (de Lange, 2009; Karlseder et al., 1999).

RS due to short telomere can be escaped when there are mechanisms that actively add nucleotides back to the telomere, such as the telomerase. The telomerase is a ribonucleoprotein enzyme leading the synthesis of telomeric DNA that normal DNA polymerase can't perform. The core of the telomerase is composed of an RNA subunit and a protein subunit; therefore, it is also referred to as RNP (RNA and protein) (Smith, Pendlebury, & Nandakumar, 2020). The RNA subunit is termed TR (Telomerase RNA) and the protein subunit is termed TERT (telomerase reverse transcriptase). These two components function jointly to achieve the purpose of

telomerase. Even though transcription of telomerase is turned off in most of the somatic cells, through safety assessed treatment, the cells are still able to attain a high level of telomerase (Hong & Yun, 2019; N. W. Kim et al., 1994). Similarly, alternative telomere lengthening is another mechanism that achieves the same purpose as telomerase. Although both of these two mechanisms will allow unlimited proliferation, it is believed that under active control, cells escaped senescence won't lead to tumour formation and cancer (Harley, 2002).

5 Stress-induced premature senescence

Stress-induced premature senescence (SIPS) is another type of senescence distinct from RS because it takes place independently of telomere length (Parrinello et al., 2003). Despite telomerase's expression in mouse embryonic fibroblast, which typically embraces a long telomere, senescence is still induced by long-term exposure to stress like oxidative species (Toussaint, Medrano, & von Zglinicki, 2000). This result is coherent to the experiment on human fibroblast cells with telomerase expression after genetic modification (Parrinello et al., 2003). The phenotype of the stress-induced senescent cell is comparable to the replicative senescent cell, with cell cycle arrested and biomarkers for DDR present, but a bit different on the protein expression (Dierick et al., 2002). However, instead of "telomere length-dependent", SIPS is often considered as "telomere-dependent" due to some special features including the repeating sequence, shelterin, and epigenetic factors of telomere that connive in stress and stress-induced DDR's persistent stay (de Magalhaes & Passos, 2018).

Compared to the main DNA genomes, the efficiency of DNA damage repair at the telomeric region is significantly slower, left as a persistent DDR that is unresolved and consistent (Fumagalli et al., 2012; Hewitt et al., 2012). Therefore, a special feature of telomere that does not associate with the length of it must be present to explain the difficulty in repairing. Analysis of oxidative stress-induced premature senescence, the most common SIPS type, shows its interaction with reactive oxygen species (ROS).

ROS can accumulate through multiple endogenous and exogenous sources (Marnett, 2000), or be produced during pathogenesis (Kohen & Nyska, 2002). Macromolecules, including DNA, are vulnerable to these highly reactive species. 8-oxoguanine (8-oxoG) is the marker of oxidative damage. It is highly mutagenic and is often induced when DNA polymerase bypasses an unrepaired oxidized base, or in the dNTP pool so that the A:T-C:G transversions in the replication or subsequent replication prone to occur (Grollman & Moriya, 1993). Besides nucleotide modification, SSB, stall of replication, and a series of subsequent events always indicate the presence of oxidative stress (Wallace, 2002). After all, the high concentration of guanine in the telomeric tandem repeat (TTAGGG) allows the most frequent DNA lesion, represented by 8-oxoG (Oikawa, Tada-Oikawa, & Kawanishi, 2001). Furthermore, researchers believe that the association between shelterin and telomeric DNA is also reduced by 8-oxoG. As discussed earlier in the shelterin's structure, the enzyme TRF1 and TRF2 bind specifically to the tandem repeats. Since the tandem repeat's nucleotides are modified by ROS, the binding capability of TRF1 and TRF2 are altered (Opresko, Fan, Danzy, Wilson, & Bohr, 2005). In fact, a significant reduction in these two enzymes at telomeres was observed 48hrs after H2O2 treatment (Opresko et al., 2005). The absence of TRF1 and TRF2 's protection of DNA from SSB and DSB could explain the subsequent instability of telomere (de Lange, 2009). The mid-step that lies between the senescence and shelterin binding reduction, is likely to be associated with γH2AX, a marker of DNA damage that experiences phosphorylation during both DSB and replication fork arrest and increases significantly after 48 hrs, persisting for 72 hrs after hydroxide treatment. Since another biomarker associated only with DSB does not show a significant elevation in concentration after the same treatment, it is suggested that 8-oxoG modification, as the principal telomere lesion, leads to shelterin reduction, replication fork arrest, telomere dysfunction and senescence (Coluzzi et al., 2019). It is worth mentioning that due to the replication fork block in SIPS could potentially contribute to RS because the telomere length is considerably reduced (Sirbu et al., 2011). Other factors including fragile telomere or epigenetic factors resulted from SIPS also surge RS as well (Coluzzi et al., 2019; A. Sfeir et al., 2009).

Another important phenotype that a senescent cell, no matter from RS or SIPS, is the Senescence-Associated Secretory Phenotype (SAPS). Its unique secretome profile including pro-inflammatory cytokines, chemokines, and growth factors (Coppe et al., 2008). SAPS is a way of communication between senescent cells and the immune system, a signal that promotes the clearance of itself and provokes regeneration of tissue (Xue et al., 2007). However, a chronic SASP that is not cleaned out by the immune system also induces the senescence in other nearby healthy cells. The increase of ROS in senescent cells and pro-inflammatory factors become an active source of stress which increases the occurrence of SIPS (Coppe et al., 2008; Jurk et al., 2014; Nelson et al., 2012; Passos et al., 2007). This positive feedback allows the interference with tissue homeostasis and function even if there were only a few senescent cells (Coluzzi et al., 2019). To reduce the effect of SASP, one feasible method is to remove senescent cells by senolytics, facilitating the function of the immune system. For instance, one senolytics, dasatinib and quercetin, has successfully improved vasomotor in mouse (Roos et al., 2016). There are reviews that summarized the result of senolytics treatment in tables, in terms of types of senolytics, or the targeted diseases (Chapman, Fielder, & Passos, 2019; E. C. Kim & Kim, 2019). Recently, it has been proved to function on human as well (Justice et al., 2019).

6 Implication of senescence to ageing

It has long been pondered whether the cellular level senescence is a direct contributor to tissue and organismal ageing. Many biomarkers such as β-galactosidase for the

senescent cells are shown to correlate with the increase in ageing (Dimri et al., 1995). As the field of study develops, the question now is what differences there are between RS and SIPS, and which contributes more to the ageing. Even though the exact dynamics of senescence is yet unknown, the characteristics of RS and SIPS has already implied some answers to how senescence leads to ageing. The main property that distinguishes RS from SIPS is the critical short telomere. However, in research conducted with over 4500 samples at a duration of 10 years found no significant relationship between telomere length and mortality of the population (Weischer, Bojesen, & Nordestgaard, 2014). A Mendelian randomization study on over 260,000 British seniors over 7.5 years also found no significant correlation between telomere length and age-related phenotypes, but instead strong suggestions for cancer (Kuo, Pilling, Kuchel, Ferrucci, & Melzer, 2019). In fact, the distribution of telomere length in vivo is also very random, nonspecific to tissues and characteristic to individuals (Takubo et al., 2002). And since it is only telomeres that are critically short will induce RS, the average length of telomere might not be a direct and efficient indication (Hemann, Strong, Hao, & Greider, 2001). One research that supports the role of telomere length in ageing is conducted by removing telomerase from mouse cells and then activate it again (Jaskelioff et al., 2011), which is not a surprise, however, as the telomerase is commonly expressed in mouse cells. Returning telomerase to cure the pathologies caused by the removal of telomerase does not really justify the molecule as a feasible treatment to improve the condition of normal cells. Therefore, these observations seem to suggest that a weak correlation between RS and tissue and organismal ageing. SIPS, however, is of a closer relationship with ageing based on the current researches. The positive feedback loop between SIPS and SASP has made the spread of senescence on a large scale, which is more likely to lead impacts on the whole tissue (Coluzzi et al., 2019). Compared to RS, SIPS is a lot less confined as it does not require the t-loop disassembly. Even though there is currently more potential in SIPS to cause ageing, a specific experiment should be done to distinguish RS and SIPS. For example, observing the concentration of senescent cells in samples while examining the telomere length or distinguishing proteins to determine the type. It has been a challenge to discover the cell turnover rate, especially for senescent cells, but this has to be overcome to further the study between cellular senescence and ageing

7 Conclusion

To achieve the purpose of end-protection through the t-loop, telomere has to decrease in length for every replication. When the telomere is too short to form a t-loop or load enough shelterins, DDR is triggered, and the cell becomes senescent. Thus, RS is telomere-length-dependent and only induced when the telomere is critically short. The widest studies treatment to slow down or reverse RS is through the activation of telomerase expression. RS was found early and studied well-roundedly. Nevertheless, many pieces of research have cast doubts on the essential relationship between RS and ageing because the length of telomere has not much statistical significance with organismal ageing. To those who have believed that the length of the telomere is an important indicator of physical age, it has been suggested that it is much less accurate than the real age (Coluzzi et al., 2019). On the other side, before telomere eventually reaches the critical length, oxidative stress may have already damaged the telomere. The telomere is a preferred target of ROS as the concentrated guanine in telomeric repeats is high mutagenic under ROS, following which the shelterin binding and replication are all interfered with. Due to the inefficient repairing of DNA lesion on telomere, SIPS is evoked independently to telomere length. The presence of SIPS also forms a positive feedback relation with SASP, implying a strong connection to macro-scale tissue ageing. Senolytics treatment has already been practicing the efficiency of senescent cell removal as an intervention of SIPS's ROS accumulation. Upon the researches so far, the new researches following up can focus on the cell turnover rate to understand the difference or similarity between RS and SIPS, and their contribution to ageing separately.

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