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Telomeres and telomere dysfunctions

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Abstract The length of telomeres is an important determinant of cell viability and phenotype. In stem cells, telomere shortening affects their self-renewal potential and compromises the regeneration of a variety of tissues. Telomeropathies, also known as telomeres diseases or telomeres syndromes, are a suggestive example in this context. These diseases are caused by mutations in genes coding for proteins involved in telomere maintenance and repair. Telomere attrition is also a source of genomic instability. Cells that display frayed telomeres and dysfunctional tumor suppressor genes, often activate telomere maintenance mechanisms, telomerase or alternative lengthening of telomeres, and become immortal. Carcinogenesis is initiated more easily in stem cells, because they exhibit low levels of telomerase. Thus, in response to mutations induced by telomere shortening and environmental exposure, stem cells can enhance telomerase activity to stabilize their genome, gaining unlimited proliferation capacity at the same time. In this paper, we reviewed the literature on telomere biology in order to understand the molecular underpinnings of telomere maintenance and its involvement in human diseases, especially age-associated diseases and cancer.

Keywords: telomere shortening, telomeropathies, genomic instability, telomere maintenance mechanisms, telomerase, alternative lengthening of telomeres, stem cells, carcinogenesis

Introduction

The existence of particular structures at the ends of the chromosomes was postulated around the 1930s by the independent observations of Hermann Muller and Barbara McClintock (Gall, 1995). Yet, the telomeres structure was first described four decades later by Elizabeth Blackburn and Joseph Gall. They sequenced the ribosomal DNA macromolecules from *Tetrahymena thermophila* and observed that the chromosome extremities consisted of 20-70 tandem repeats of the 5'-TTGGGG-3 '/ 3'-AACCCC-5 hexameric sequence (Blackburn and Gall, 1978). The observations on ciliate telomeres remain valid nowadays as the telomeres of almost all species of eukaryotes, including humans, consist of a variable number of nucleotide repeats and the G-rich strand always forms the 3' end of the chromosome (de Lange, 2004; McKnight and Shippen, 2004; O'Connor, 2008).

Excepting cancer cells and embryonic cells, human cells telomeres shorten with aging. When the telomere length falls below a critical threshold, senescence and apoptosis occur (Shay and Wright, 2010). Telomeres shortening is accelerated if mutations in the proteins involved in monitoring and maintaining telomeres occur.

Dysfunctions in telomere maintenance machinery have serious consequences on the stem cells compartment (Martinez and Blasco, 2017). Dyskeratosis congenita (DC), Hoyeraal-Hreidarsson syndrome (HHS), Revesz syndrome, Coats Plus syndrome, idiopathic pulmonary fibrosis (IPF), aplastic anemia, and liver fibrosis also known as telomeropathies, telomeres diseases or telomeres syndromes, are suggestive examples in this context (Martinez and Blasco, 2017). In these diseases, cell proliferation is disturbed, which results in premature aging of a variety of tissues (bone marrow, epithelia, skin) (Holohan *et al*, 2014).

Telomeres are also involved in the control of genomic integrity and play a crucial role in initiating carcinogenesis (Gunes and Rudolph, 2013). Genomic instability can be considered a result of telomere uncapping (Sfeir and deLange, 2012; Nera *et al*, 2015), loss of DNA damage checkpoints (Wright and Shay, 1992), defective processing and repair of telomeric DNA (Rai *et al*, 2010; Gunes and Rudolph, 2013), replicative stress (Sarni and Kerem, 2017) and abnormal levels of telomeric proteins (Nera *et al*, 2015). Dysfunctional telomeres lead to end-to-end fusions, chromosome breakage or aneuploidy, all these events resulting in genomic instability (Shay and Wright, 2010).

In order to stabilize the genome, cells activate telomeres maintenance mechanisms (TMMs): i.e. telomerase or Alternative Lenghtening of Telomeres (ALT), and become capable of unlimited proliferation (De Vitis *et al*., 2018). Immortalization occurs more easily in stem cells, where telomerase is already active at a basal level (Gunes and Rudolph, 2013).
The possible mechanisms linking telomeres, telomeric proteins and human diseases will be described below in details.

1. Telomeres and telomere maintenance

The extremities of linear eukaryotic chromosomes possess a particular segment of repetitive sequences called telomeres, which form a "cap" at each end of the chromosomes. Human cell telomeres consist of tandem repeats of the nucleotide sequence 5'TTAGGG3 '/ 3'AATCCC5'; these double-stranded structures have a length between 9 and 15 kb and end with a 3 'single-stranded-DNA overhang (O'Sullivan and Karlseder, 2010). This overhang invades the telomeric duplex and through complexation with specific proteins forms a lariat structure called T-loop, which contributes to the maintenance of the telomeres' protective cap (Fig. 1) (Sfeir, 2012).

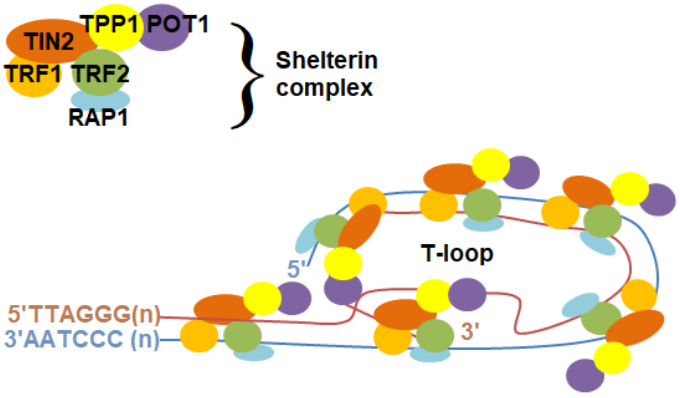

Fig. 1. Schematic representation of telomere structure.

Telomere length homeostasis is carefully controlled by numerous proteins, acting either independently or organized into complexes. Some of these proteins are found exclusively at telomeres, while others have subnuclear or subcellular localization (Campisi *et al*, 2001). By far, the most important proteins involved in telomere lenght maintenance are the six molecules organized in the shelterin complex (Figure 1) (Palm and de Lange, 2008). Telomeric Repeat-binding Factor 1 (TRF1) and Telomeric Repeat-binding Factor 2 (TRF2) bind duplex telomeric DNA (Broccoli *et al*, 1997; Palm and de Lange, 2008), whereas Protection Of Telomeres 1 (POT1) binds to single stranded DNA (Baumann *et al*, 2002; O'Sullivan and Karlseder, 2010). TRF1 assures correct telomere replication (Martinez, 2009), while TRF2 regulates telomeric DNA topology (Amiard, 2007) and assist the T-loop assembly, a lariat structure that protects telomeres against DNA Damage Repair (DDR) complex (Denchi and de Lange, 2007). The other proteins do not interact with telomeric DNA. TRF-Interacting Nuclear protein 2 (TIN2) stabilizes the interaction between TRF1 and TRF2 (Ye and Donigian, 2004) and recruits Tripeptidyl-peptidase 1 (TPP), which interacts with POT1 (Takai *et al*, 2011). TPP1 and POT1 compete with telomerase for the 3 'overhang end. TPP1 interaction with telomerase enhances enzyme processivity, while increased loading of POT1 along the overhang blocks the interaction of telomerase with its substrate (O'Sullivan and Karlseder, 2010). In addition, Repressor and Activator Protein 1 (RAP1) does not bind directly to the repetitive sequences in the telomere structure and its presence in the Shelterin complex depends on interaction with TRF2 (Figure 1) (Li and Oestreich, 2000; Yang *et al*, 2005).
Members of the shelterin complex interact with a series of cofactors to induce the T-loop conformation assembly and to protect the terminal ends of the chromosomes. Proteins recruited at telomeres by shelterins include helicases, nucleases and proteins responsible for telomeric DNA replication and repair (Raynaud *et al*, 2008). Mutations in these accessory molecules have serious implications for aging and health, causing telomere diseases (Martinez and Blasco, 2017).
Replication of telomere occurs after replication of the entire chromosome and is facilitated by telomeric proteins (Raghuraman *et al*., 2001). Conventional polymerases do not have the ability to replicate the entire parental DNA on the lagging strand, therefore, human telomeres progressively shorten along successive cell divisions, and each round of division results in the loss of 50-200 base pairs (Huffman *et al*., 2000). Once telomeres reached a critical length, they are recognized by the machinery involved in DDR, which will impose cell cycle arrest or apoptosis. The entry of cells into the replicative senescent state is a mechanism designed to ensure genomic stability and prevent malignant transformation of normal cells (Shay and Wright, 2010).
Over the time, the study of epigenetic changes of telomeric chromatin revealed that epigenetic regulation is another key factor that controls telomeric length and function. The telomeres are highly compacted structures, normally endowed with the epigenetic markers of constitutive heterocromatin: trimethylation of histone H3 at lysine 9 (H3K9me3) and histone H4 at lysine 20 (H4K20me3), hypoacetylation of H3 and H4 and subtelomeric DNA hypermethylation (Blasco, 2007).
Changes in the epigenetic pattern of telomeric chromain trigger abnormal events in living organisms, for instance, diminished histone methylation leads to aberrant elongation of telomeres (Garcia-Cao *et al*, 2004; Benetti *et al*, 2007b), while decreased telomeric and subtelomeric DNA methylation leads to telomeric fusions (Gonzalo *et al*, 2006). These observations are of particular importance in the context of neoplasms, where low methylation

levels of chromatine have been reported, and where TMMs-mediated telomere elongation confers infinite replicative potential to cancer cells (Blasco, 2007). Regarding aging-associated conditions, it is supposed that the existence of extremely short telomeres leads to epigenetic changes that weaken telomeric silencing. Besides hypomethylation, extremely short telomere are characterized by the hyperacetylation of telomeric and subtelomeric chromatin, which is a feature of the transcriptionally active chromatin (Benetti *et al*, 2007a). These observations are in line with the telomeres position effect (TPE), where reversible silencing of the genes located near telomeres relies on telomeric elongation (Blasco, 2007).

Moreover, the lifestyle choices can have a significant influence on the rate of telomere shortening. Healthy diets including vegetables, vegetable oils, nuts and fish are rich in biological compounds with antioxidant and anti-inflammatory action and promote the stability of telomeres (Marin *et al*, 2012; Crous-Bou *et al*, 2014). In contrast, diets based on red meat and sweetened carbonated beverages (Lee *et al*, 2015; Leung *et al*, 2008), along with stress exposure (Epel *et al*, 2004), smoking and a sedentary behaviour (Valdes *et al*, 2005) have been shown to accelerate telomere shortening, leading to modification in telomeric and subtelomeric chromatine that promote the development of age-related conditions (Blasco, 2007).

2. Telomerase

Telomerase is a specialized polymerase capable of synthesizing TTAGGG repeat sequences at the 3 'end of telomeric DNA (Greider and Blackburn, 1985). The discovery of this mechanism is one of the most important advances in the history of modern molecular biology, for which Greider and Blackburn were awarded Nobel Prize in 2009.

Human telomerase has two essential components: a catalytic subunit, hTERT, with reverse transcriptase activity and a RNA molecule (hTR or hTERC). Telomerase uses its own RNA, which has a 11 bp sequence complementary to repetitive motifs, as a template for telomeric DNA synthesis (TTAGGG)n (Brown *et al*, 2014). Dyskerin (DKC), NHP2, NOP10 and GAR1 are also part of the telomerase ribonucleoproteic complex (Egan and Collins, 2010) (Fig. 2). Mutations in telomerase core components cause diseases known as telomeres dysorders (Holohan *et al*, 2014; Martinez and Blasco, 2017).

The inactive telomerase complex is assembled in nucleus, in the Cajal bodies, and these corpuscles are then transported near the telomere by the Telomerase Cajal Body Protein 1 (TCAB1) (Venteicher *et al*, 2009). The ATP-ases pontin and reptine interact with the inactive complex, in order to activate and initiate the synthesis of repeating units at the 3 'end of the telomeric DNA (Venteicher *et al*, 2008).

In humans, telomerase is well expressed in the early stages of embryogenesis, but the gene encoding the hTERT subunit is repressed, thus, the enzymatic activity of telomerase is diminished considerably. Telomerase remains activated in male germ cells and in several somatic cell types, such as activated lymphocytes and stem cells from tissues with increased rates of regeneration (Wright *et al*, 1996).

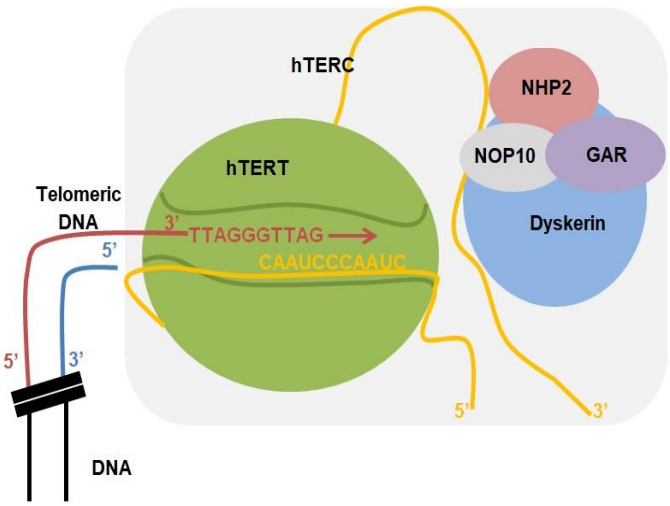

Fig. 2. Telomerase core components and the mechanism of telomere maintenance.

Due to its activity, telomerase counteracts telomere shortening that occurs during DNA replication. In normal somatic cells, telomerase is in an almost inactive state, which allows the telomeres to be shortened with each replication (Wright *et al*, 1996). Instead, telomerase is active in about 90% of the tumor cells studied, where its activity cancels out telomere shortening effects and gives immortality to cell clones (Stewart *et al*, 2002; De Vitis *et al*, 2018). Maintaining telomere is an essential feature of tumor cells capable of continuous proliferation, so tumor cells in which telomerase is not active use another TMM to ensure immortality: ALT (Cesare and Reddel, 2010).

Studies of Artandi and De Pihno (2000) have shown that telomerase plays a dual role in somatic cells. Thus, if telomerase is activated before the telomere shortening reached the critical threshold (Hayflick limit), there is no chromosome fusion; in this case, telomerase acts against the accumulation of chromosomal aberrations and against genomic instability. Consequently, cells do not undergo malignant transformation processes. In contrast, if telomerase is activated when chromosomal fusion occurred and the tumor suppressor signals have been lost, then, telomerase supports cellular immortalization and carcinogenesis (Artandi and De Pihno, 2000).

3. Telomeropathies and aging-associated diseases

The length of telomere is correlated with cell longevity. The importance of telomere homeostasis is emphasized by the emergence of a broad spectrum of related diseases triggered by the existence of shortened telomeres, often referred to as telomeropathies, telomere disorders or telomere syndroms (Holohan *et al*, 2014). In telomeropathies, telomere attrition occurs as a consequence of germline mutations present in genes coding for components of telomerase and shelterin complexes or other proteins involved in telomere replication and maintenance. All these diseases share similar mutations and clinical symptoms, and are considered a single spectrum disorder (Fig. 3) (Martinez and Blasco, 2017). However, the age of onset and clinical manifestations are highly variable among affected individuals. DC, HHS, Revesz syndrome and Coats Plus syndrome are telomere disorders that manifest in childhood and are rarely found in the population. In contrast, IPF, aplastic anemia, liver cirrhosis and acute myeloid leukemia (AML) are less complicated telomere diseases that manifest in adults and are more frequent in the population (Holohan *et al*, 2014).

Dysfunctions in the telomere maintenance machinery have serious consequences on the stem cells compartment. In this context, telomeres of stem cells are gradually shortening at each cell division and get frayed faster than in healthy individuals, compromising the self-renewal of tissues (Martinez and Blasco, 2017).

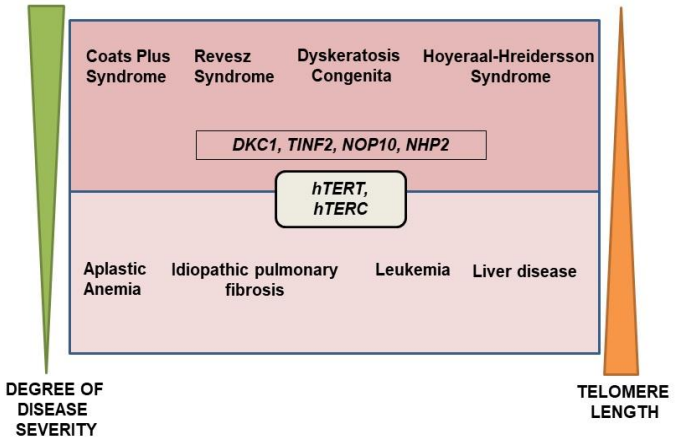

Fig. 3. Telomeropathies represent a broad spectrum of related diseases caused by mutation in telomere maintenance genes. Dyskeratosis congenita and its associated conditions are severe diseases. In telomeropathies, the presence of very short telomeres is a specific event, but DC patients display extremely short telomeres.

DC is the first disease associated with failure of the telomere maintenance machinery and causes in 80% of cases bone marrow failure and defects in hematopoiesis (Dokal, 2011). The disease begins in childhood and is characterized by extremely short telomeres. In DC, were registered mutations in 6 genes whose products are essentials to the optimal functionality of telomeres (DKC1, TERC, TERT, NOP10, NHP2 belong to the telomerase complex and TIN2 to shelterin complex) (Vulliamy and Dokal, 2008; Dokal, 2011). In addition, three molecular subtypes of DC were defined: X-linked recessive, autosomal dominant and autosomal recessive (Kirwan and Dokal, 2008). In accordance with this genetic heterogeneity, beyond the classical triad of associated symptoms (oral leukoplakia, nail dystrophy and skin hyperpigmentation), a plethora of new DC associated symptoms such as epiphora, cirrhosis, pulmonary disease, mental retardation, deafness, tooth loss and osteoporosis were frequently reported (Dokal, 2011; Kirwan and Dokal, 2009). However, many studies have shown that cases of DC in which the three key symptoms do not manifest could registered (Holohan *et al*, 2014).

HHS, a severe form of DC, is a multisystem genetic disorder characterized by bone marrow failure, severe growth retardation, immunodeficiency, microcephaly and cerebellar hypoplasia (Berthet *et al*.,1994; Aalfs *et al*, 1995). Previous studies have revealed that HHS can be attributed to *DKC* (Knight *et al*, 1999), *TINF2* (Walne *et al*, 2008) gene mutations or might be caused by homozygous mutations in *hTERT* (Marrone *et al*, 2007). Moreover, a mutant form of Apollo nuclease, one of the proteins recruited to the telomeres by TRF2 and involved in the repair of DNA interstrand cross-links (ICLs), has been identified in patients with HHS (Touzot *et al*., 2010). Mutations in the helicase RTEL1, an enzyme that performs the T-loop release, enabling telomeric DNA replication, enrich the list of mutations commonly found in HHS (Ballew *et al*., 2013, Le Guen *et al*., 2013).

Revesz syndrome and Coats Plus syndrome are two other severe forms of DC, extremely rarely found in human population (Holohan *et al*, 2014). The main difference between these two disorders are that exsudative retinopathy (Kajtar and Mehes, 1994) is usually associated with Revesz syndrome, while in the Coats Plus syndrome *CTC1* mutations were recurrent (Anderson *et al*., 2012; Polvi *et al*., 2012). CTC1 is a member of the CST (CTC1-STN1-TEN1) complex that is essential for telomere capping and replication (Rice and Skordalakes, 2016).

IPF is the most common condition that arise from dysfunctional telomeres and usually begins in adulthood (Armanios and Blackburn, 2012). This disease affects lung parenchyma and in most cases, the cessation of respiratory function occurs within 5 years (Lawson and Loyd, 2006). IPF is mainly determined by gene mutations that encode hTERT and hTR components of telomerase, these mutations being found in 8-15% of family cases and in a small proportion in sporadic cases (Tsakiri *et al*, 2007). The sporadic form of IPF can manifest even though there are no defects in telomerase core components, and extremely short telomere are a common event in these situations (Liu *et al*, 2013). Because

telomere length is genetically determined, this observation draws attention to the fact that individuals with short telomere may have a higher predisposition to develop IPF.

Aplastic anemia, liver cirrhosis and AML, other telomere diseases that occur in adults are all caused by somatic or germline mutations in the genes encoding the hTERT and hTERC components of telomerase (Holohan *et al*, 2014). Certain patients with h*TERT* or *hTERC* mutations and bone marrow failure have experienced severe liver conditions (Calado *et al*, 2009), in other cases, hepatic cirrhosis may be associated with IPF and aplastic anemia (Parry *et al*, 2011). According to these studies, telomerase mutations can affect different organs at the same time, having important clinical implications for both patients and clinicians (Holohan *et al*, 2014).

Telomeropathies support the onset of other age-related diseases, such as hypertension, Alzheimer's disease, type 2 diabetes and, last but not least, cancer (Martinez and Blasco, 2017). This event might be in part explained by the fact that cells that have lost their viability accumulate in the body and release substances that disrupt tissue homeostasis (Tchkonia *et al*, 2013).

Another mechanism by which telomeres are involved in human diseases is TPE (Martinez and Blasco, 2017). Progressive telomere shortening enhance the expression of nearby genes that were initially repressed, and this leads to physiological dysfunctions with serious implications for the body (Doheny *et al*, 2008). Stadler *et al*. reported that gradual telomeres shortening triggered overexpression of the *DUX4* gene, located near 4q telomere. *DUX4* is a gene encoding a protein involved in the pathogenesis of facioscapulohumeral muscular dystrophy (FSHD), a disorder that causes the weakness and atrophy of the scapular, facial and upper limbs muscles (Stadler *et al*, 2013). This study was the first to reveal a new contribution of telomeres shortening in triggering aging-related diseases.

4. Telomeres and their involvement in carcinogenesis

Besides the function of "molecular clock" that indicates the degree of viability and proliferation of the cell, telomeres are involved in the control of genomic integrity and play a pivotal role in initiating carcinogenesis. Although studies have focused particularly on the role of telomere shortening and activation of telomere elongation mechanisms in this process, telomere fragility, aberrant repair responses and abnormal levels of shelterins are also additional sources of genomic instability (Fig. 4). All of these issues will be discussed below.

4.1. Somatic cells and cancer

In most human somatic cells, the DNA replication machinery is unable to completely replicate the end of the lagging strand of telomeric DNA, and consequently, telomeres progressively shorten until reach a critical length. Without delay, a DNA damage response is launched and cells enter replicative senescence (M1). Also, this process occurs in stem cells. Cells in which DNA damage checkpoints (p53, pRB) are inactivated continue to divide beyond the senescence phase, and although their lifespan is extended, their telomeres continue to fray. These cells will enter the second state of cell cycle arrest, called crisis (M2), where the eroded ends of the chromosomes tend to fuse with each other, leading to breakage-fusion-bridge (BFB) cycles that are an important source of chromosome aberration. To prevent the generation of chromosomal instability, cells are programmed to suffer apoptosis (Wright and Shay, 1992). However, 1/10 millions cells activates TMMs to counteract the genetic chaos induced by telomere dysfunction and gain immortal proliferation capacity (Shay and Wright, 2010). In mammalian cells, two main mechanisms of TMMs have been identified. Most tumors use telomerase to expand their telomeres (Stewart *et al*, 2002; De Vitis *et al*, 2018). In contrast, in 10-20% of tumors has been described ALT, which consists of exchange of telomeric sequences between sister chromatids (Bryan *et al*, 1995; Cesare and Reddel, 2010). Therefore, M1 and M2 mechanisms are two mechanisms designed to prevent genomic instability and carcinogenesis (Shay and Wright, 2010).

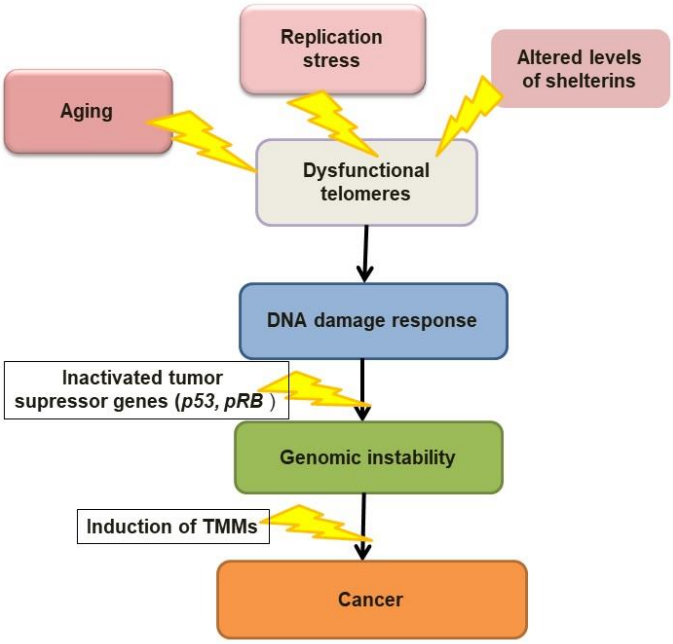

Fig. 4. Molecular mechanisms that contribute to genomic instability and carcinogenesis in somatic cells with dysfunctional telomeres.

The telomeric sequences are fragile sites, very susceptible to mutations (Sfeir *et al*, 2009). Due to chronic replication stress at telomeres, these fragile regions become prone to chromosomal breakage and recombination events. At the moment, oncogene activation is considered one of the most important sources of replication stress. Furthermore, replication

stress has very important implications at the level of G-quadruplex (G4) secondary structures. G4 are stable DNA structures that are known to hinder replication fork progression, leading to DNA damage, chromosomal fusions and ultimately, to genomic instability, which is the driving force of tumorigenesis (Gunes and Rudolph, 2013; Sarni and Kerem, 2017). The presence of the shelterin complex is essential for the protection of chromosomal extremities. When telomere uncapping occurs due to the loss of shelterin proteins, eroded telomeres are recognized by cells as aberrant DNA (Sfeir and deLange, 2012; Nera *et al*, 2015). Consequently, DDR mechanisms are activated and perform a faulty repair of telomeric DNA. For example, it has been found that Non-homologous end joining (NHEJ) activation leads to chromosomal fusions, whereas Homology directed repair (HDR) facilitates telomeres lenghtening by ALT. Moreover, it seems that DDR pathways activated in response to loss of shelterins differs from the DDR pathways that are activated when telomeric dysfunctions are caused by other factors, but further research around this subject is needed to completely understand this selectivity (Gunes and Rudolph, 2013). Using a murine experimental model, Rai *et al*., concluded that the removal of the Trf2 with retrovirus-mediated shTrf2 led to chromosomal fusions induced by the classical (c-)NHEJ pathway. In the same study, where different variants of mutant mice were used, it was found that potential loss of Pot1 / Tpp1 complex may lead to alternative (alt-) NHEJ activation. These studies, consistent with others performed on murine experimental models, suggest that NHEJ is the main mechanism to process dysfunctional mice telomeres that lost shelterins during aging (Rai *et al*, 2010); therefore, complete understanding of this pathway and associated mechanisms in humans may provide new insights about the link between aging, genomic instability and cancer (Gunes and Rudolph, 2013).

Activation of DDR pathways also occurs as a response to telomere DNA processing and it may significantly influence the acquisition of the malignant phenotype. The 5'-3 'end resection, an event required to yield 3' single-stranded DNA (ssDNA), inhibits c-NHEJ and activates HDR, which in turn activates ALT. Resection of the 5 'DNA strand can also promote alt-NHEJ, leading to end-to-end fusions (Gunes and Rudolph, 2013). Thus, additional research into DDR pathways involved in telomere processing may reveal new mechanisms that promote malignant transformation.

Altered levels of shelterins may be another source of genomic instability. As we mentioned above, the loss of shelterins causes the merger of chromosome ends, leading to BFB cycles that promote chromosomal instability. However, significant increased levels of shelterins are not desirable, because events can follow the same path (Nera *et al*, 2015). A robust body of clinical evidence revealed the presence of significant amounts of shelterins, especially TRF1, TRF2 and TIN2 in different forms of cancers. Gastric carcinomas with short telomeres expressed proeminent levels of telomerase in addition to TRF1, TRF2 and TIN2 overexpression (Matsutani *et al*, 2001). *TRF1* and *TRF2* genes, and often *TIN2* gene were also found to be upregulated in adult T-cell leukemia (Bellon *et al*, 2006), lung cancer (Nakanishi *et al*, 2003), renal cell carcinoma (RCC) (Pal *et al*, 2015) and hepatocarcinoma (Yokota *et al*, 2003). However, at the moment, the carcinogenic mechanisms triggered by telomeric binding proteins (especially TRF1 and TRF2) overexpression remain elusive. Several studies have shown that *TRF1*(van Steensel and de Lange T, 1997) and *TRF2* overexpression result in gradual telomere shortening (Smogorzewska *et al*, 2000). Experimentally induced overexpression of *TRF2* results in replication fork stalling, subsequent formation of telomeric ultrafine anaphase bridges (UFBs), finally leading to chromosome fusions and extensive deletion of telomeric sequences. Chromosomal fusions and ruptures generated by *TRF2* overexpression are similar to those found in human cancers (Nera *et al*, 2015). Interestingly, *TRF1* and *TRF2* genes silencing triggered apoptosis and cell cycle arest in a RCC cell line. Thus, *TRF1* and *TRF2* inhibition may hold substantial promise for the attenuation of genomic instability and treatment of cancers (Pal *et al*, 2015).

4.2. Stem cells and cancer

Stem cells have the remarkable potential to diferentiate into various types of cells during early life and growth (Montagnani *et al*, 2016). Stem cells reside in tissues in a dynamic and specialized local microenvironment termed ”niche” and have a low growth rate and longer telomeres than those of differentiated somatic cells (Ferraro *et al*, 2010; Shay and Wright, 2010).

Stem cells are presumed to be the origins of cancers (Gunes and Rudolph, 2013). Unlike normal somatic cells, they express low levels of telomerase, but this is not enough to prevent telomere shortening during aging (Shay and Wright, 2010). Moreover, although they have developped more efficient mechanisms of genomic integrity protection compared to normal somatic cells, stem cells are also prone to mutations. Thus, in order to alleviate the genetic chaos induced by telomere shortening and acquired mutations, cells enhance telomerase activity and become immortal (Gunes and Rudolph, 2013).

The most important clinical evidence in support of this theory comes from the study of hematopoietic cancers. AML is an agressive cancer of the bone marrow characterized by uncontrolled proliferation of hematopoietic stem cells (HSCs). Preleukemic HSCs harbor a pattern of mutations that is found recurrently in AML patients, exhibit short telomeres and elevated telomerase levels (Corces-Zimmerman *et al*, 2015). Thus, telomere attrition in HSCs is considered an event that contributes to the development of chromosomal instability and neoplastic transformation (Hiyama and

Hiyama, 2007). Furthermore, telomere shortening in DC is associated with an increased risk of hematologic malignancy (Mason *et al*, 2005). In a similar manner to hematopoietic cancers, a plethora of human solid tumors originate from cancer stem cells (CSCs): breast, prostate, brain and melanoma (La Porta, 2012).

Conclusions

The study of telomere biology has improved significantly our knowledge regarding the functions that telomere and telomere- associated proteins exert at cellular level. Now it is well known that telomeric dysfunction is responsible for premature degeneration of tissues, induction of chromosomal aberrations and acquisition of malignant phenotype. It is desirable for this information to be further translated into clinic, but the process is difficult because it requires extensive testing and sophisticated equipment.

Telomerase gene therapy and shelterin chemical inhibitors have proved to be effective therapeutic strategies for the treatment of telomeres syndromes or aging-associated diseases, but additional studies are needed to estimate the risks and effects of long term use (Martinez and Blasco, 2017).

In cancers, therapeutic strategies are focused on targeting TMMs, considered the molecular foundations of unlimited proliferation. An arsenal of telomerase inhibitors are available, but their effectiveness is still doubtful.

In consequence, current research efforts aim to identify novel telomerase blocking agents, showing high specificity for the enzyme, low toxicity to normal cells and fewer side effects (Arndt and MacKenzie, 2016). Further complicating this scenario, tumors have the ability to switch TMMs under selective pressure of drug administration or to present both TMMs at the same time, which hampers therapeutic approaches in cancer (De Vitis *et al*, 2018). Therefore, additional research is needed around this topic to fully understand the molecular underpinnings of carcinogenesis and to design more effective therapies against cancer.

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