EGYPTIAN ACADEMIC JOURNAL OF

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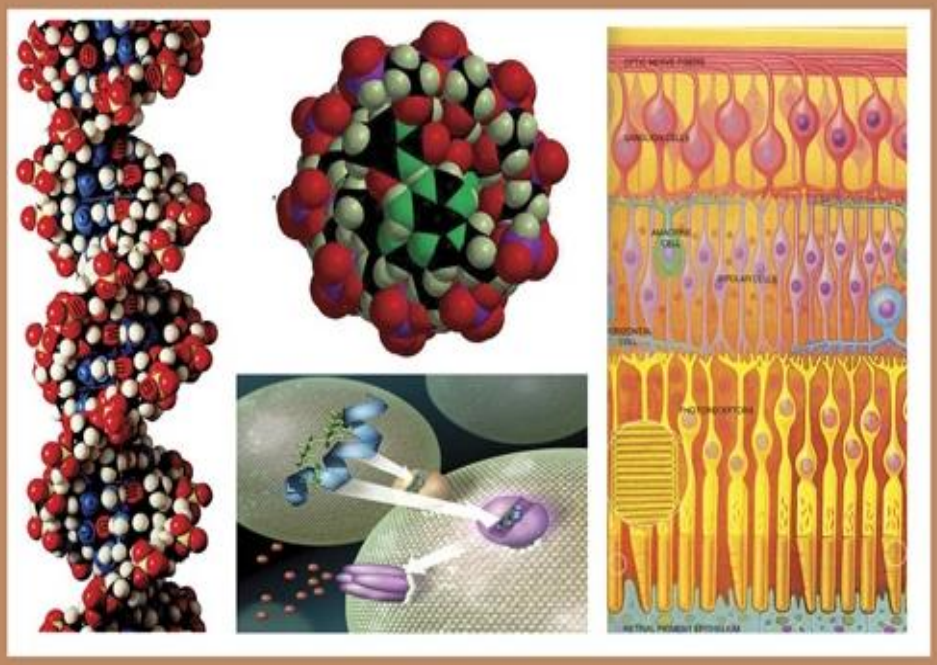


ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 13 No. 2 (2021)

Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 13(2) pp89-100 (2021)*

DOI: 10.21608/EAJBSC.2021.196932

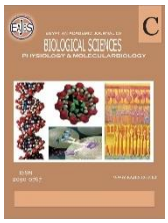
Egypt. Acad. J. Biolog. Sci., 13(2):89-100 (2021)

Egyptian Academic Journal of Biological Sciences

C. Physiology & Molecular Biology

ISSN 2090-0767

www.eajbsc.journals.ekb.eg



Role of Telomere Length in Cancer

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REVIEW INFO

Review History

Received:19/8/2021

Accepted:27/9/2021

Keywords:

Telomere, Telomere length, Telomerase, ALT, Cancer.

ABSTRACT

Telomeres are tandem repeats of DNA that are present at the end of a linear chromosome. They play a critical role in providing chromosome-end protection. There are certain binding sites of proteins, called shelterins, in the telomere structure that play a critical role in telomere protection. Telomeres are elongated by telomerase enzymes. In normal human somatic cells, telomerase is inactivated, and consequently, telomeres shorten with each cell cycle. Short telomeres can play opposing roles in carcinogenesis. First, short telomeres can play an essential role in stopping the occurrence of cancer. Second, short telomeres can lead to genome instability, which may result in the formation of some cancer cells. However, cancer cell telomeres can be lengthened by the reactivation of telomerase or alternative lengthening of telomeres (ALT) mechanisms. ALT is a homologous recombination-based mechanism that depends on the *RAD52* gene. This review summarises the role of short and long telomeres in the initiation and growth of cancer cells.

INTRODUCTION

Historical Background:

Telomeres were discovered by Muller (1938). He noticed that the ends of irradiated chromosomes in *Drosophila melanogaster* were protected against mutagenic X-rays. Chromosome ends have a specific protective structure, saving them from chromosome breakage, inversion, or deletion. Muller called this special chromosome end "telomere" (from the Greek words *telo*, meaning "end," and *mere*, meaning "part") (Muller, 1938).

In 1941, Barbara McClintock explained the fusion of chromosome ends that leads to dicentric chromosomes and revealed that the damage to chromosome ends can be repaired. Accordingly, telomeres play a critical role in chromosome protection and integrity (McClintock, 1941).

In 1961, Leonard Hayflick found that diploid normal human cells had limited time for duplications. Cells can divide only 40–60 times. This maximum time of cellular multiplication is called the "Hayflick limit". Consequently, cells reach a specific type of cellular arrest and undergo cell senescence upon reaching this limit (Hayflick & Moorhead, 1961).

In 1971, James Watson proposed the existence of the "end replication problem", based on the semi-conservative DNA replication mechanism.

According to the mechanism of DNA replication, Watson anticipated that after several cell divisions chromosomes would lose their ends. Eventually, chromosome end shortening with each cell division would lead to cell senescence or death. Additionally, he assumed that a protective mechanism would exist to prevent the chromosome end reduction (Watson, 1972). Moreover, as proposed by Alexsey Olovnikov in 1971, cellular aging may occur as a result of telomere shortening. Telomere erosion may reach nearby essential genes, and thus, influence human aging (Olovnikov, 1973).

In 1978, Blackburn and Gall found that extrachromosomal telomeres in *Tetrahymena thermophila* contained 20–70 tandem repeats. This repeat was a hexanucleotide of 5′-CCCCAA-3′ sequence on one strand and 5′-TTGGGG-3′ on the complementary strand (Greider & Blackburn, 1985).

The enzyme that elongates telomeres was discovered by Blackburn and Carol Greider in 1988. It was initially named terminal telomere transferase but later changed to telomerase (Greider & Blackburn, 1985).

Telomerase activity in humans was first reported by Morin in 1989. Moreover, he revealed that human telomeres consist of repeats of the TTAGGG sequence (Morin, 1989). In 1994, Shay *et al.* demonstrated telomerase activity in approximately 90% of human cancers (Kim *et al.*, 1994). Moreover, they found that telomerase immortalised normal cells (Bodnar *et al.*, 1998).

Telomere Structure and Function:

Telomeres are nucleoprotein structures that exist at the ends of linear chromosomes. Telomeres consist of TG tandem repeats that vary among different organisms. For instance, telomeres in protozoans contain approximately 20-70 TTGGGG tandem repeats (Blackburn, Greider, & Szostak, 2006). In yeast, the GGTTACA repeat extends up to approximately 300 bp (Pfeiffer & Lingner, 2013). Telomeres in plants comprise TTTAGGG repeats in the range of 2–100 kb (Fajkus *et al.*, 2019). Telomeres in vertebrates consist of TTAGGG tandem repeats (Shay & Wright, 2019). Human telomeres are normally between 10 and 15 kb in length (Heidenreich & Kumar, 2017; Pfeiffer & Lingner, 2013; Webb, Wu, & Zakian, 2013). Telomeres consist of two parts: subtelomeres and telomeric repeats. The telomere repeat contains double-strand and single-strand regions (Fig. 1) (Baird, 2018; de Lange, 2018). Single strands in mammalian telomeres are mostly 50–400 bp long. Six proteins, called shelterin complex proteins, bind telomeres to provide telomere stability (Heidenreich & Kumar, 2017; Sfeir & de Lange, 2012). The tandem repeat single strand at the end of telomeres, called the 3 overhang, is created by nucleolytic degradation (Wu, Takai, & de Lange, 2012). The 3 overhang folds back and invades the telomeric double strand to form a T-loop structure (Fig. 2) (Doksani, Wu, de Lange, & Zhuang, 2013; O'Sullivan & Karlseder, 2010).

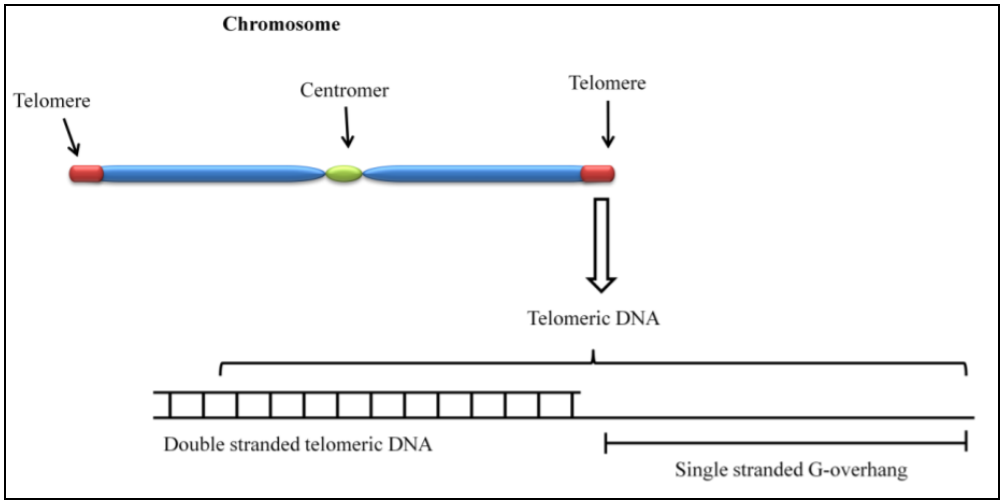

Fig. 1. Telomere structure includes tandem repeats of DNA that exist at the end of the linear chromosome, and consist of double-stranded DNA and 50–300 nucleotide single-stranded G-overhangs.

Many proteins are associated with telomeres. These proteins can be broadly grouped into three main types, nucleosomes, shelterin complexes, and chromosomal transcription factors (de Lange, 2018; Kar, Willcox, & Griffith, 2016; Tardat & Dejardin, 2018).

Nucleosomes are one of the main players in telomere protection. They are involved in protein-protein and protein-DNA interactions between shelterin subunits and tandem repeat sequences (Bandaria, Qin, Berk, Chu, & Yildiz, 2016; Tardat & Dejardin, 2018). As a result of histone methylation, telomeres in higher eukaryotes are mostly heterochromatin (Chow et al., 2018; Galati, Micheli, & Cacchione, 2013). The existence of telomeres in a heterochromatin form increases genome stability (Schoeftner & Blasco, 2009). Moreover, histones play an important role in telomere capping and homologous recombination in telomeres (Chow *et al.*, 2018). In addition, heterochromatin at telomeres results in the silencing of nearby genes (Jezek & Green, 2019).

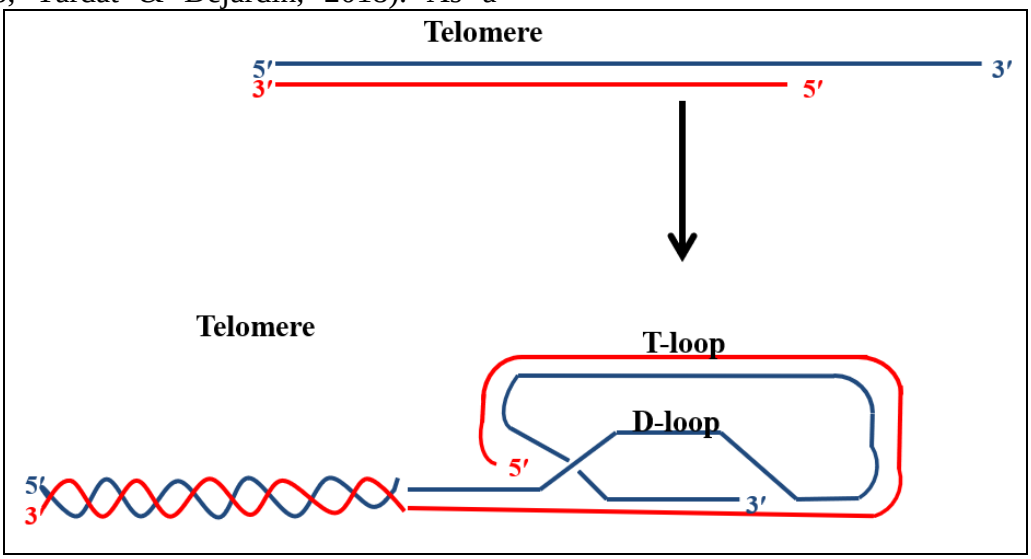

Fig. 2. T-loop structure. Single-stranded telomeric DNA folds back and invades into the double-stranded telomeric DNA providing telomere protection from exonuclease enzyme activities. Moreover, T-loop protects telomeres from end-to-end fusion.

Shelterin complex proteins are involved in telomere structure. They consist of six proteins: repressor and activator protein 1 (RAP1), telomeric-repeat-binding factor 1 and 2 (TRF1 and TRF2), protection of telomeres 1 (POT1), TRF1-interacting nuclear protein 2 (TIN2), and TIN2-interacting protein (TPP1) (Martinez & Blasco, 2011; Shay & Wright, 2019). These proteins are involved in telomere length regulation, prevention of DNA damage response (DDR) signals, and protection of telomeres from DNA damage (de Lange, 2018).

TRF1 and TRF2 proteins bind to double-stranded DNA in telomeres, whereas POT1 binds to the telomeric single-strand (Erdel *et al.,* 2017). TRF1 and TRF2 play critical roles in negatively regulating the telomere length (J. Lin *et al.,* 2013). Moreover, both TRF1 and TRF2 inhibit the non-homologous end joining (NHEJ) mechanism (Schmutz & de Lange, 2016).

POT1 binds to a single strand and protects telomeres from end fusion (Denchi & de Lange, 2007). POT1 also binds to the double-strand, but indirectly via TPP1 (Hu *et al.,* 2017). TPP1 is essential for enhancing the role of POT1 in telomeric single strands (Hu *et al.,* 2017). Additionally, TIN2 is involved with other shelterin proteins in binding single strands (Pike, Strong, Ouyang, & Greider, 2019). RAP1 as a complex with TRF2 and its Myb domain bind to telomere to suppress telomeric homologous recombination (Srinivas, Rachakonda, & Kumar, 2020).

Many other proteins are involved in telomere biology. They contribute in telomere regulation and maintenance (Arnoult & Karlseder, 2015; Pinto, Li, Nicholls, & Liu, 2011). These proteins can interact with telomeres directly or via interactions with shelterin proteins (Pinto *et al.,* 2011). CST complex proteins are conserved telomere protection component 1 (CTC1), suppressor of cdc13a (STN1), and telomeric pathway with STN1 (TEN1). These proteins bind to telomeric single strands and are involved in telomere capping and length regulation (Rice & Skordalakes, 2016). Moreover, these proteins contribute to telomere replication by interacting with DNA ploα-primase (Rice & Skordalakes, 2016). They also facilitate telomere elongation by unfolding G-quadruplex structures (Zhang *et al.,* 2019). The two subunits of the CST complex, STN1-TEN1, resolve the replication fork and participate in the telomerase-mediated extension of the telomeric single-strand (Chastain et al., 2016; Gu *et al.,* 2018).

Different additional proteins that are involved in the DDR machinery are associated with telomeres (Arnoult & Karlseder, 2015). These proteins are Werner (WRN), RecQ-family DNA helicases, and bloom (BLM). They are indirectly involved in telomere biology through their association with TRF1 and TRF2 (Zimmermann, Kibe, Kabir, & de Lange, 2014). RecQ helicase proteins contribute to the unwinding of the G-quadruplex structure and initiation of DNA replication (Higa, Fujita, & Yoshida, 2017).

Telomeres play a critical role in genome stability (Chiba *et al.,* 2017). Accordingly, short telomeres, recognised as DNA strand breaks, lead to the activation of DNA damage signals(de Lange, 2018). Additionally, reduction of telomere length may cause chromosome end-to-end fusions (T. T. Lin *et al.,* 2010; T. T. Lin *et al.,* 2014). Moreover, telomere shortening activates double-strand break repair mechanisms, non-homologous end joining (NHEJ), or homologous recombination (HR). Consequently, these mechanisms may lead to improper end-to-end chromosomal fusion. According to telomere shortening, cells undergo senescence, apoptosis, or genome instability. Therefore, telomeres play a critical role in protecting chromosome ends from degradation, DNA damage

responses, and end-to-end fusion (Fig. 3).

The T-loop and G-quadruplex structures play a major role in telomere protection. However, the flexibility of these structures is required during DNA replication to allow telomere elongation. Telomere reduction ultimately results in telomere uncapping. As a result, many types of genome aberrations can easily occur. However, histones, shelterin complex proteins, and other proteins are important players in telomere capping and protection. Telomere shortening impairs the binding of these proteins to telomeres, leading to unprotected telomeres.

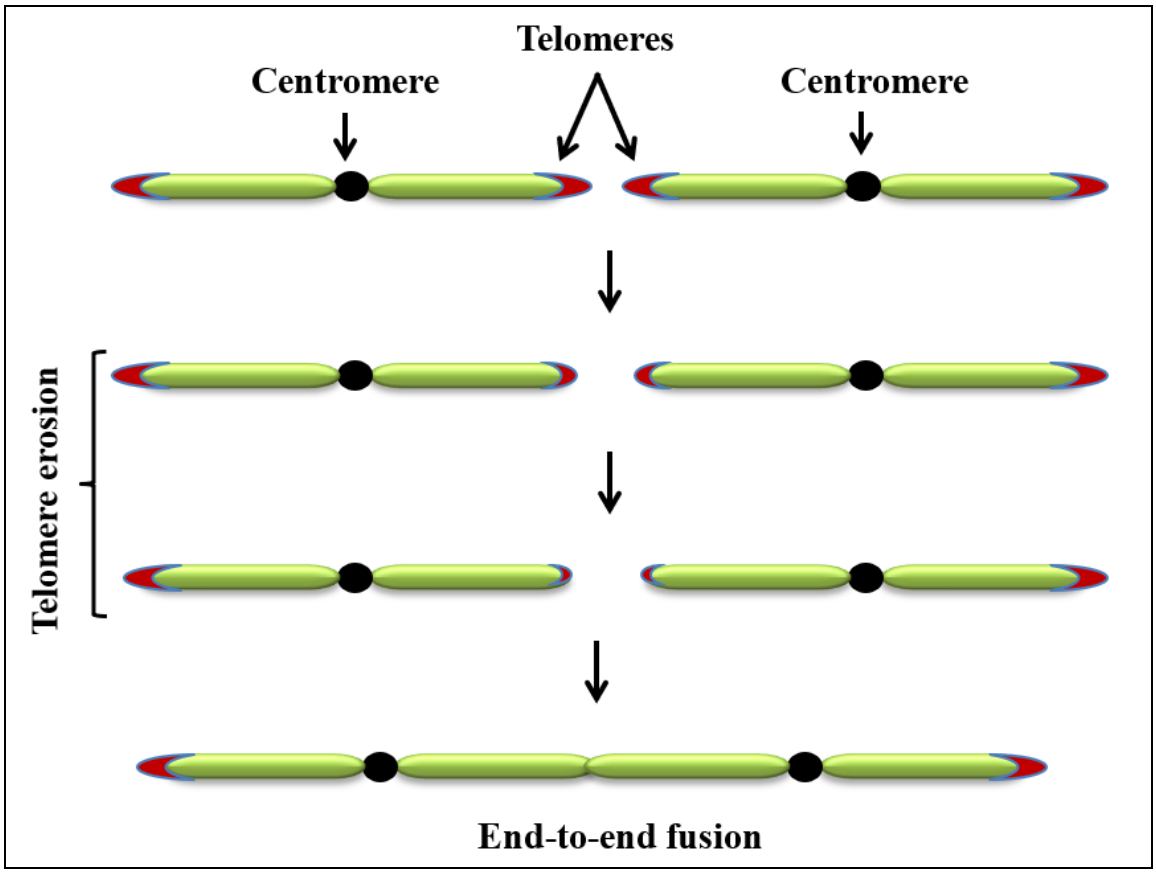

Fig. 3. End-to-end fusion. Telomere erodes with every cell cycle and can lead to critically short telomere, and then chromosomes will lose their cap protection. Unprotected telomeres contribute to genome instability, such as end-to-end fusion, which may cause chromosomal breakage.

Telomere Elongation:

In adult humans, telomerase is not active in normal somatic tissues or stem cells. Consequently, telomeres shorten with each cell division (Blasco, 2005). In contrast, telomeres in cancer cells are elongated via at least two main pathways. Approximately 90% of cancer cells are elongated via the reactivation of the telomerase enzyme, while only 10% are elongated via a homologous recombination-based mechanism called alternative lengthening of telomeres (ALT) (Gaspar *et al.*, 2018). However, the coexistence of both telomerase and ALT has been found in different types of cancers, such as peritoneal mesothelioma (Villa *et al.*, 2008), gastric carcinomas (Omori *et al.*, 2009), glioblastoma multiforme (Henson et al., 2005), soft tissue sarcomas (Yan, Benhattar, Coindre, & Guillou, 2002) like liposarcomas (Costa

et al., 2006; Montgomery, Argani, Hicks, DeMarzo, & Meeker, 2004) and fibrous histiocytomas, Wilms tumours (Venturini *et al.*, 2011), osteosarcomas (Sanders *et al.*, 2004), and adrenocortical carcinoma (Else, Giordano, & Hammer, 2008).

Telomerase reactivation in cancers occurs through various genetic and epigenetic mechanisms. These mechanisms lead to the amplification of telomerase reverse transcriptase (TERT) and an RNA component (TERC). Moreover, mutations within the TERT promoter and genomic rearrangement of TERT lead to telomerase reactivation. Additionally, epigenetic modifications through TERT promoter methylation have been found to cause telomerase reactivation (Barthel *et al.*, 2017; Gaspar *et al.*, 2018).

ALT is a mechanism used by cancer cells to elongate telomeres in the absence of telomerase (Fig. 4). Cells use a homologous recombination mechanism to maintain telomeres in the absence of telomerase. ALT is a RAD52 dependent mechanism. Telomere lengths in cancer cells that use ALT mechanism are heterogeneous, they can be extremely long (>50 kb) or short (<5 kb) (Bryan, Englezou, Gupta, Bacchetti, & Reddel, 1995; Xu, Li, & Stohr, 2013).

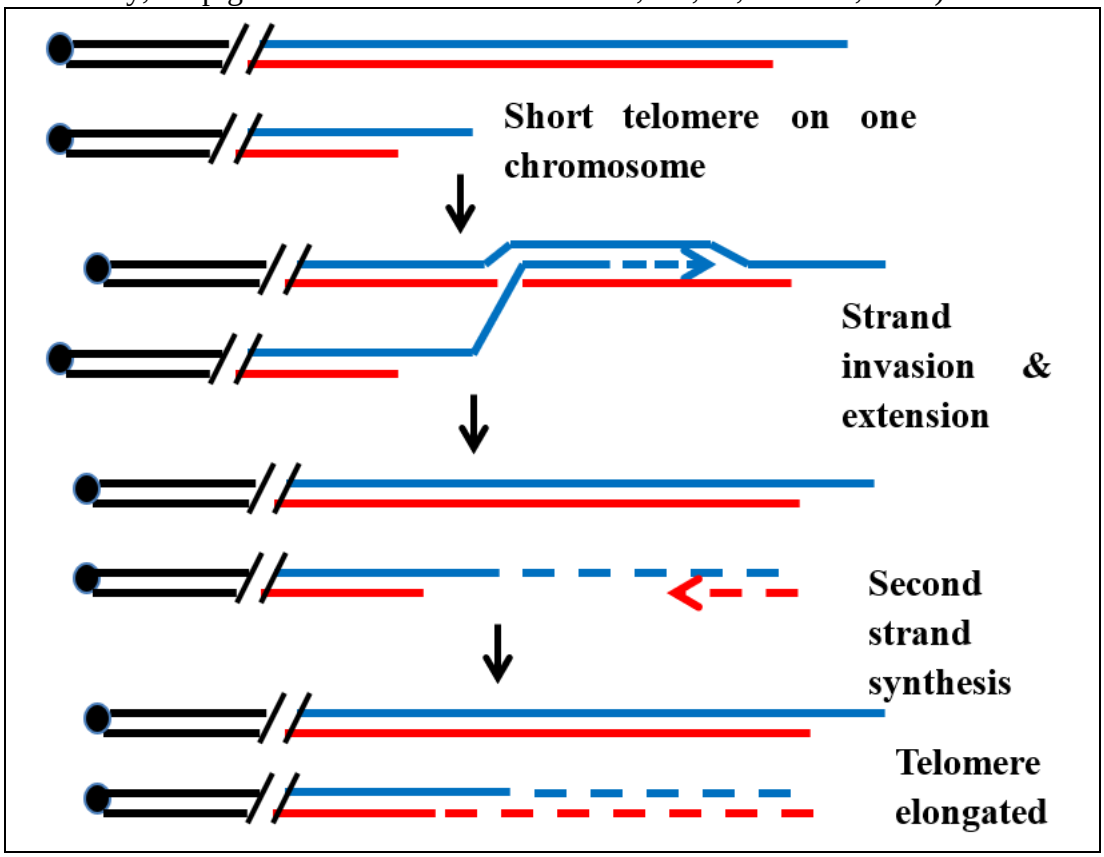

Fig. 4. Alternative lengthening of telomeres (ALT) mechanism. In the absence of telomerase, telomeres in cancer cells can be elongated by homologues recombination-based mechanism that is called ALT. Single strand in short telomere invades into a homologous long telomere of another chromosome and extends to the end of this telomere. Then, the second strand of the short telomere undergoes synthesis, eventually leading to the elongation of the short telomeres.

Telomeres in Cancer Cells:

Telomere length is an obvious sign of cancer. Cancer cell association with short or long telomeres has been widely documented.

Cancers that are initiated in proliferative tissues mostly contain short telomeres (Shay & Wright, 2011).

Additionally, cancer occurrence is more frequent in older people than young people; furthermore, it is well known that older people's somatic cells display shorter telomeres compared to young people. Telomeres that are critically short are more likely to cause a high risk of cancer (Ma *et al.,* 2011). Oncogenic changes and short telomeres are the main reasons for genomic instability, which eventually leads to the occurrence of many cancers (Maser & DePinho, 2002). However, short telomeres can increase the occurrence of epithelial cancers as a result of non-reciprocal translocations (Artandi *et al.,* 2000). Moreover, bladder cancer has been found to be significantly associated with short telomeres (Broberg, Bjork, Paulsson, Hoglund, & Albin, 2005). Additionally, short telomeres have been found in hereditary breast cancers (Martinez-Delgado *et al.,* 2013). A study on zebrafish concluded that telomere shortening plays a critical role in the incidence of cancer (Lex *et al.,* 2020). Short telomeres lose the binding sites of shelterin proteins. Consequently, telomeres are uncapped. Uncapped telomeres cause many types of genome instability, such as end-to-end fusion, chromosome breakage, and translocations. Accumulation of these genetic changes can easily initiate cancer cell types. Telomere shortening leads to cell senescence, which is mostly followed by cancer initiation.

Associations between various types of cancer and long telomeres have been widely documented. These cancers include basal cell carcinoma, lung cancers, melanoma, tumours of the urogenital system, glioma, and lymphoma (Haycock *et al.,* 2017; McNally, Luncsford, & Armanios, 2019; Rode, Nordestgaard, & Bojesen, 2016). Additionally, different studies have found an association between cancers and long telomeres, such as B-cell lymphoma, neuroblastoma, renal cell carcinoma, melanoma, osteosarcoma, adult glioma, lung adenocarcinoma, and meningioma (Hosnijeh *et al.,* 2014; Pierce, Kraft, & Zhang, 2018). Telomere elongation via telomerase reactivation or ALT mechanisms plays a critical role in allowing cancer cells to duplicate many times with long telomeres. Thus, long telomeres allow cancer cells to divide rapidly multiple times.

Conclusion

Telomeres play a critical role in the protection of chromosome ends. In addition, they play important roles in maintaining genome stability. Telomeres are elongated by the telomerase enzyme; however, telomerase is not active in normal somatic cells. On the other hand, telomerase is activated in most cancer cells and is utilised to elongate telomeres. A small fraction of cancer cells elongates their telomeres via ALT mechanisms. Associations between cancer and long telomeres have been widely documented (Haycock *et al.,* 2017; McNally *et al.,* 2019). Cancer cells benefit from long telomeres to replicate their chromosomes many times before stopping when telomeres become short. To summarise, both short and long telomeres are involved in the occurrence of cancer. Short telomeres clearly participate in cancer initiation, while long telomeres are implicated in the continuation of cancer cell growth.

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