Chapter 2

Telomere and Telomerase in Cancer

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Abstract

The linear ends of chromosome are protected by specialized ribonucleoprotein (RNP) termed as telomere. These specialized terminal elements with tandem repeated sequence are the protective cap that alleviate end replication problem and cell senescence. The telomere length maintenance is essential to avoid cell death and apoptosis. Telomere shortening has been related to chronic stress due to several factors, which include not only psychological stress but also diseases such as cardiovascular diseases and cancer. Telomerase enzyme which maintains telomere length is the major factor responsible for evading cell death. Telomere length maintenance and telomerase expression put together are the prerequisite for immortality, an essential character for cancer cells. Understanding the mechanism of telomere and telomerase functions paves way for eradicating the diseases such as cancer.

Keywords: telomere length, telomerase, tumor progression, tumor immortality

1. Introduction

Telomeres are specialized DNA structures consisting of tandem arrays of hexa-nucleotide (TTAGGG) DNA sequences that cap the end of chromosomes. These structures residing near the ends of chromosomes play a very vital role in maintaining the integrity of the whole genome and prevent the loss of genetic material. As a consequence of cell division, short stretches of DNA will be lost from telomeric region and eventually lead to cellular senescence and death. There are a lot of evidences to suggest that telomere length is a better biomarker for overall health status, compared to the currently used biomarkers for the same. Few of the phenomena regarding the telomere length are unanswered till now. Till date it is unclear about the mechanistic

regarding the telomere length are unanswered till now. Till date it is unclear about the mechanistic correlation between telomere size and life span of various species. To prevent senescence of cell, the preservation of telomere length is essential, which is maintained by telomerase enzyme. Telomerase is a reverse transcriptase enzyme which has recently emerged as an attractive target for cancer as it is a crucial factor required for the tumor immortalization of a subset of cells, including cancer stem cells. Studies have proved that 80–85% of the tumor cells express telomerase whereas somatic cells lack the expression of telomerase [1]. The important paradox is that telomerase-negative normal cells have lengthier telomeres than telomerase-positive cancer cells [2]. Thus difference in telomere length and cell kinetics between normal and cancerous cells shows that targeting telomerase is a more effective way to target cancer cells. Owing to the significant role of telomere and telomerase in tumor immortalization understanding their amassed influence on cancer is crucial for cancer therapy. In this context, this book chapter is focused on disseminating the integrated impact of telomere and telomerase on cancer progression.

2. Telomere construction

The basic structure of telomere is conserved among eukaryotes and consists of short tandem DNA repeats, with G-rich sequence at the three-end referred to as the G-strand and the complementary strand is called the C-strand at the five-end (**Figure 1**) [3]. The length of telomeric DNA varies from 2 to 20 kilobase pairs, depending on factors such as tissue type and human age [4]. The telomere is conspicuous owing to the presence of G-overhang which extends beyond its complementary C-rich strand to form a single-stranded overhang, termed the G-tail. It has been proposed that the 3′ G-overhang can be sequestered into a lasso-like structure known as the T-loop (**Figure 2**) [5, 6]. The single-stranded G-overhang invades the double-stranded telomeric DNA, which displaces the bound G-strand base-pairing with the C-strand. As this displaced binding takes place at a distance from the physical end of the telomere, it generates a large duplex structure called the T-loop (**Figure 2**) [3, 5].

Figure 1. Telomeric DNA.

Telomeres can also fold into G-quadruplex DNA, an unusual DNA conformation that is based on a guanine quartet [7]. The repetitive and GC-rich nature of telomeric DNA endows it with the capability to form the higher-order DNA conformation, G-quadruplexes [8, 9]. To maintain such unusual structure of telomeres, a set of telomeric protein complex has evolved, termed shelterin. The shelterin complex consists of six individual proteins, telomeric repeat binding factor 1 (TRF1), TRF2, repressor/activator protein 1 (RAP1), TIN2 (TRF1 interacting protein 2),

TPP1 (TINT1/PIP1/PTOP 1), and protection of telomeres 1 (POT1) [6, 10]. The proteins TRF1 and TRF2 attach to double-stranded telomeric repeats facilitating the anchoring of the complex along the length of telomeres [11–14], whereas POT1 binds to the single-stranded overhang. TPP1 and TIN2 act as bridging proteins between the above DNA-binding modules and are crucial for chromosome end protection and telomere length regulation. TRF1 and TRF2 with the help of TIN2 [15] bind with POT1 via TPP1 [16–19]. TIN2 also connects TRF1 to TRF2, and this interaction contributes to the stabilization of TRF2 on telomeres [17–19]. Besides this shelterin complex, other proteins like TEN1 and Pinx1, which are not telomere specific, are also present at telomeres and carry out important functions in recruitment of telomerase [20].

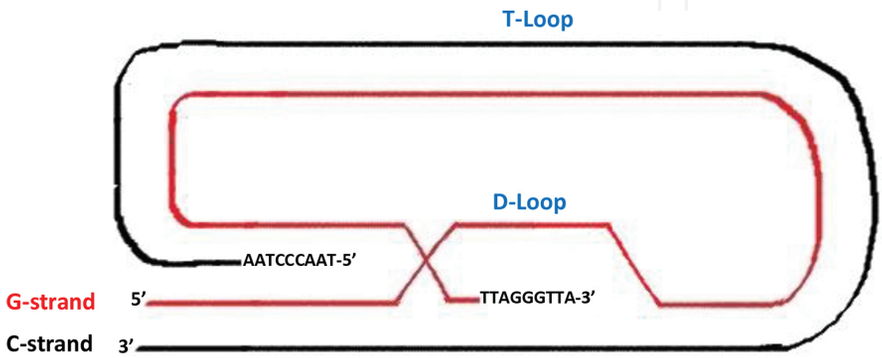

Figure 2. T-loop formation.

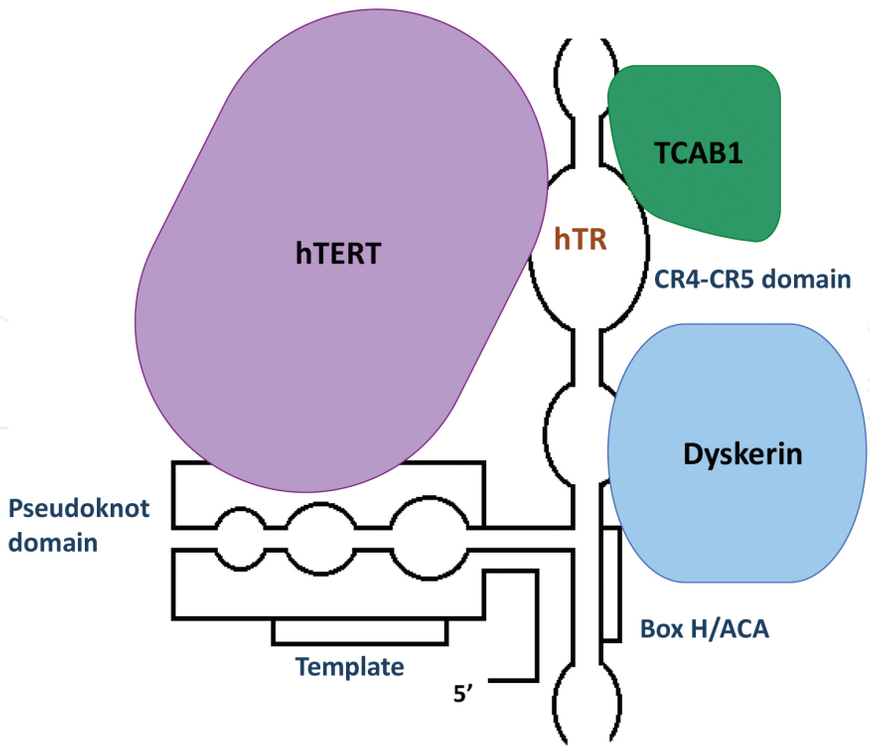

Figure 3. Telomere-telomerase assembly.

3. Telomere: a knight cap

During the evolvement of linear genome, the natural ends of linear chromosomes resemble DNA breaks and tend to induce DNA damage response (DDR). These natural linear ends are protected by the sequestration of the ribonucleoprotein (RNP) sequence, telomeres which mask the ends from continuous exposure to the DNA damage response (DDR). Telomeres serve as protective caps, preventing the chromosomal ends from being recognized as double-strand breaks by the DNA damage repair system and the activation of the p53 or p16INK4a pathway and the start of senescence or apoptosis. If the telomere cap is removed, genome instability is induced. Telomeres with its tightly regulated complexes consisting of repetitive G-rich DNA and specialized proteins accomplish the task of not only concealing the linear chromosome ends from detection and undesired repair, but also protect from checkpoints, homologous recombination, end-to-end fusions, or other events that normally promote repair of intra-chromosomal DNA breaks acts [21]. Telomeric proteins and their interacting factors create an environment at chromosome ends that inhibits DNA repair at that point; however, the repair machinery is also essential for proper telomere function.

The closed configuration of the T-loop of telomeric region provides a protective cap that defines the natural end of the chromosome and masks the telomere from the DDR machinery (**Figure 2**) [6]. In particular, T-loops could provide an architectural solution to the repression of the ataxia telangiectasia mutated (ATM) kinase pathway, which relies on a sensor (the MRN (Mre11/Rad50/Nbs1) complex) with DNA end-binding activity. In addition, T-loops could prevent the Ku70/80 heterodimer, a DNA repair factor that binds to DNA ends, from loading onto the telomere terminus, thereby blocking the initiation of the non-homologous end-joining (NHEJ) pathway (**Figure 4**).

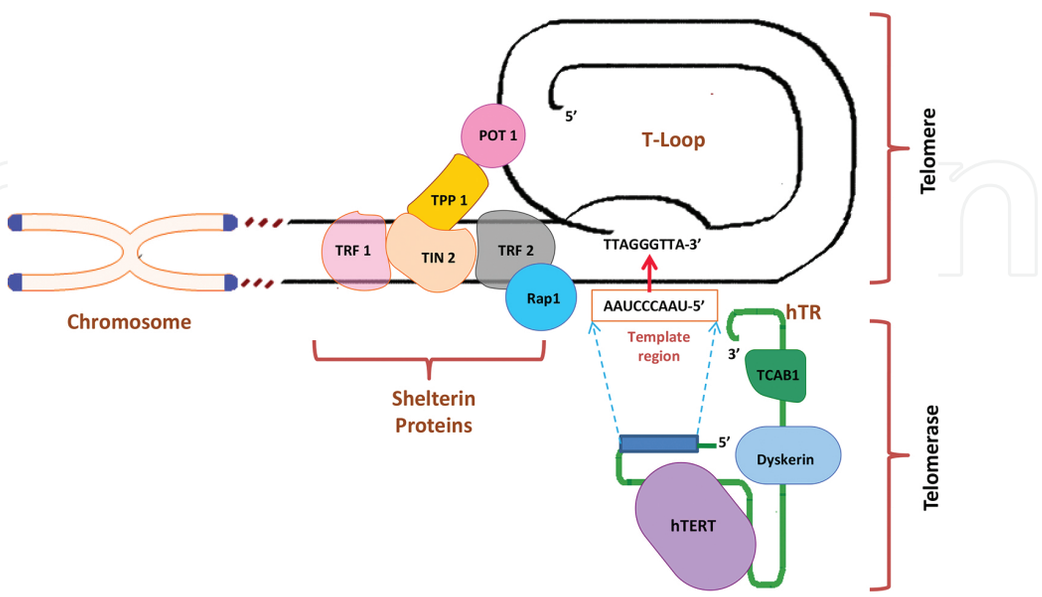

Figure 4. DNA damage response pathway.

Among the DNA-binding proteins, TRF1 has DNA remodeling activity [5, 22] and also shown to promote efficient replication of telomeres [23, 24]. On the contrary, TRF2 engages in chromosome end protection by inducing topological changes in telomeric DNA [25], T-loop assembly [26, 27] and by suppression of ATM dependent DDR and NHEJ (**Figure 4**) [28, 29]. Besides that, TRF2 plays a critical role in chromatin assembly, which was demonstrated by the observation that overexpression of TRF2 resulted in aberrant nucleosome spacing and decreased abundance of the core histones H3 and H4 at chromosome ends [30]. TRF2 lacking cells are reported to be growth arrested because of up-regulation of p53 and also show other hallmarks of ATM signaling, including the phosphorylation of ATM and Chk2 [31, 32]. Among the two DDR, the ATM kinase pathway at telomeres is repressed by TRF2 subunit, whereas POT1 is responsible for protection of telomere by suppression of ATR (ATM Rad3-related protein)-dependant DDR pathways [28, 33].

The high affinity of POT1 for single-stranded telomeric DNA makes it a G-strand binding component displaced from the T-loop and forms a closed configuration locking in the structure (**Figure 2**). Earlier report models suggest that POT1 and TPP1 compete with telomerase for access to the overhang [33]. Contrarily, direct interaction between TPP1 and telomerase bolsters telomerase processivity [34, 35] whereas increased loading of POT1 along the overhang block telomerase accessibility to the 3′-OH substrate. The role of RAP1 is obscure and its function has recently been elucidated. The presence of RAP1 at telomeres appears as a backup mechanism to prevent NHEJ when topology-mediated telomere protection is impaired [36]. In case of mutation in TRF2 which wraps the DNA, RAP1 has been implicated in the inhibition of NHEJ [37, 38].

Thus physically taken together, the shelterin complex and G-overhang of telomere are the protective cap that have an immensely complex role in convergence of end protection and telomere length maintenance mechanisms.

4. Telomerase activity

With each cell division, telomere length is reduced by ~50 to 200 bp [39] primarily because the lagging strand of DNA synthesis is unable to replicate the extreme 3′ end of the chromosome which is denoted as end replication problem [40, 41]. When telomeres become sufficiently short, cells enter an irreversible growth arrest called cellular senescence. In most eukaryotes, telomeres are stabilized, and the shortening telomeric DNA is replenished, by the action of the RNP reverse transcriptase telomerase. Progressive telomere loss has been experimentally demonstrated using non-immortalized cells in culture that lack detectable telomerase [42, 43].

In cells with active telomerase, such as cancer cells, the telomere length is continually being built up and shortened in a regulated way that maintains telomere length homeostasis and retains telomere functionality. Shortening of telomeres occur due to nucleolytic degradation and incomplete DNA replication. On the contrary, lengthening is primarily accomplished by the action of a specialized reverse transcriptase called telomerase [44] and occasionally by homologous recombination (HR) [45]. Telomerase uses the 3′ G-rich strand of a chromosome

as primer to elongate chromosome end by reverse-transcribing the template region of its tightly associated RNA moiety and coordinative action with the DNA replication machinery [44, 46]. For lengthening activity, telomerase requires not only hTERT catalytic subunit and RNA template (hTR) but also other factors [47, 48].

The 3′ half of the hTR resembling the box H/ACA family of small nucleolar RNAs (snoRNAs) [49, 50] is essential for proper 3′-end processing, stability and nucleolar targeting *in vivo* [44]. The 5′ end of hTR not only acts as template for the telomere extension at chromosome ends [5, 51] but also serves as a pseudoknot that is likely to be important for telomerase function (5, 49). A 6 bp U-rich sequence at the 5′ end of hTR also interacts directly with hnRNPs C1 and C2 (**Figure 3**) [52]. Even though hTR is highly expressed in all tissues regardless of telomerase activity [53], in cancer cells hTR is generally expressed fivefold higher than normal cells [54]. However, the expression (mRNA) of the telomeric catalytic component hTERT which is closely associated with telomerase activity is estimated to be less than one to five copies per cell [54]. hTERT is generally repressed in normal cells and up-regulated in immortal cells, suggesting that hTERT is the primary determinant for the enzyme activity.

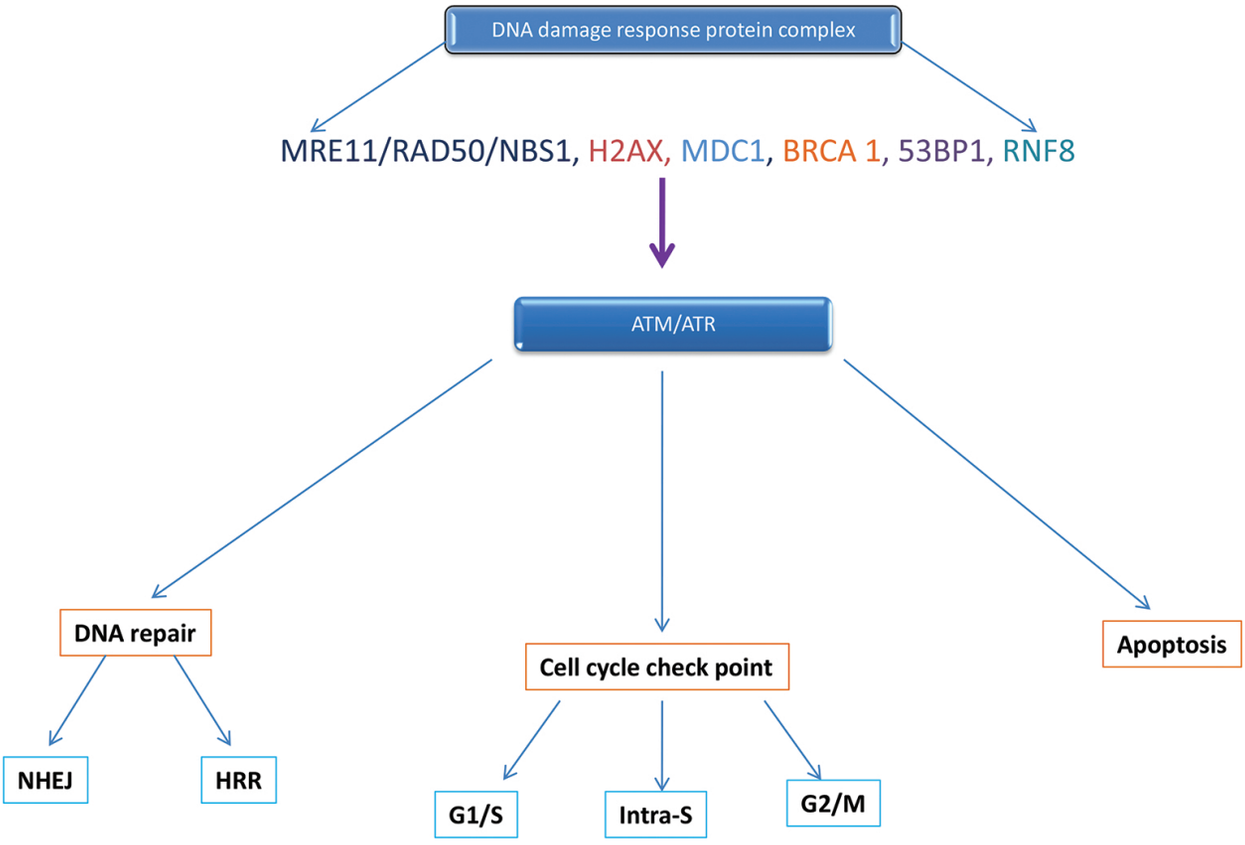

Figure 5. Telomerase complex.

It has been suggested that in addition to telomere elongation another aspect of telomerase RNP function is to allow even short telomeres to remain functional, which in the absence of telomerase would have caused cells to stop dividing or led to telomere–telomere fusions [55].

In other words, telomerase permits cell proliferation by stabilizing short telomeres that would be unstable in the absence of functional telomerase. In recent years, evidence has accumulated that telomerase, and in particular its catalytic subunit TERT, is involved in various non-telomere-related functions such as regulation of gene expression, growth factors and cell proliferation [56–61]. It has been reported that the telomerase has a role in modulation of Wnt/β-catenin pathway [60]. TERT has been demonstrated to bind to TBE-containing promoter elements, the specific chromatin sites of Wnt/β-catenin target genes, forming a part of the β-catenin transcriptional complex, which was facilitated by interaction with BRG1. These data endorsed the precipitous role for telomerase as a transcriptional modulator of Wnt/β-catenin signaling pathway involved in progenitor cell regulation.

In addition, various groups have shown that TERT shuttles from the nucleus and translocates to mitochondria upon exogenous stress [62–67]. Singhapol and his coworkers have demonstrated that mitochondrial telomerase localization specifically decreases mitochondrial ROS generation and cellular oxidative stress after induction of exogenous stress generated by H_2O_2 or irradiation in cancer cells and might thereby prevent damage to nuclear DNA [68]. Thus the presence of telomerase not only maintains telomere length imparting immortality but also play multifarious role in tumorigenesis via non-telomere-dependent mechanism which demonstrated the imperative ubiquity of telomerase in cancer cells.

5. Skewed expression of telomerase

Telomerase, the RNA-dependent DNA polymerase by preventing the shortening of telomeric DNA sequences, accouters unlimited proliferation. As per the telomere hypothesis of cancer cell immortalization, telomere shortening limits the life span of telomerase-negative normal cells, whereas telomerase activation in cancer cells extends their life span [4]. In normal human cells, telomerase activity is quenched during embryonic differentiation [69]. On the contrary in some tissues, like male germ cells, activated lymphocytes, and certain types of stem cell populations, the telomerase activity is induced [15, 70]. Owing to its diverse activity, the telomerase [71] which was established to be absent in most of the normal human somatic cells is recorded to be expressed in more than 90% of cancerous cells and in vitro-immortalized cells [15, 70]. A study showed that while most of the glioma tissues possess increased telomerase activity, only few (10%) anaplastic astrocytomas are reported to be telomerase positive [72–74]. In contrast to most cancerous cells, the telomerase expression is present in only 50% of glioblastoma and retinoblastoma samples, and activity is even rarely found in meningiomas and astrocytomas [75, 76].

Induction of telomerase activity in primary human keratinocytes and mammary epithelial cells has been attributed to the effect of human papillomavirus 16 E6 protein [77]. Similarly, during the menstrual cycle involving the proliferation of endometrial cells, telomerase activity is detected in normal human endometrium [78, 79]. These reports emphasis that telomerase might be the reason for tumorigenesis in hormone-dependent cancers.

It has been suggested that up-regulated expression of telomerase is contributed by the increased copy number of hTERT which was demonstrated by the report that while hTERT protein expression was strongly positive in tumor cells, the expression of hTERT in non-neoplastic mucosal cells as well as stromal elements (except lymphocytes) was weak or negative [80]. In most cases, hTERT expression is closely correlated not only with telomerase activity but also with cancer initiation and progression. In head and neck squamous cell carcinoma and human glioma cell lines, there was decrease in telomerase activity which has been correlated with overexpression of p53, E2F, p16, p21, and p15 individually [81, 82]. In malignant and nonmalignant human hematopoietic cell lines, primary leukemic cells, and normal T lymphocytes, IFN-α is reported to inhibit telomerase activity by suppressing hTERT transcription [83]. In addition to growth and differentiation-related regulation, telomerase activity is subject to regulation by other external and intracellular factors such as UV irradiation [84]. The telomerase having influence over several signaling pathways that determine cell proliferative or death responses when overexpressed might abrogate anti-proliferative or cell death signals. Thus cancer cells with high levels of telomerase might gain a selective growth advantage.

6. Telomerase as biomarker of cancer

Advent of latest cancer biomarkers has increased opportunities for improving cancer diagnostics by enhancing the quickness of detection and efficacy of treatment. In relation to the practice of new therapeutic interventions, proficient biomarkers are helpful in detection and prediction of remission or relapse of cancer at both gross and molecular levels. Telomerase activity is a hallmark of most cancer biopsies, but not generally detected in premalignant lesions and in normal tissue samples except germ cells and hematopoietic stem cells. Thus telomerase activity can be a promising biomarker for diagnosis of malignancies and a target for chemotherapy or gene therapy. Extent of telomerase activity in tumor tissues may be prognostic indicators of patient outcome. Thus, at the present time telomerase is being studied in anticipation of clinical usage. Many clinical trials for telomerase assay in cancer diagnosis are under trial. Fresh or fresh-frozen biopsies, fluids, and secretions are subject for these trials.

Other components of telomerase enzyme complex have also been utilized as biomarkers for telomerase activity. The expression of the RNA subunit of the telomerase complex (hTR) is also regarded as a diagnostic marker [85]. But the expression of hTR does not always correlate with telomerase protein expression in that particular cell type. hTR can be constitutively expressed in certain cell types in which even telomerase activity is not present [86]. Apart from this, mutation in genes of telomerase and associated proteins are considered as a diagnostic and prognostic marker for many genetic abnormalities collectively termed as telomeropathies. Early-onset melanoma tumor syndrome with multiple co-morbid cancers can be predicted from telomerase gene promoter mutation analysis. In this disorder, the mutation in promoter of telomerase gene introduces an erythroblast transformation-specific transcription factor-binding site, resulting in approximately twofold up-regulation of telomerase [87].

Introduction of telomeric repeat amplification protocol (TRAP) assay has facilitated the detection of telomerase activity in tumor biopsy samples as well as cell lines [88]. Specificity of telomerase activity in malignant phenotype further enforces the reliability of this assay. The most important advantage of TRAP assay is its low detectable limit. TRAP assay has allowed the analysis of minimal tissue samples, such as fine-needle aspirates of the breast and thyroid, cervical smears, oral washings, and urine [89, 90]. Telomerase also has been used to detect circulating tumor cells also [85]. Newly emerged technique, droplet digital TRAP assay can detect telomerase activity even in a single cell [91]. However, the positive ratios of detection of telomerase vary in sedimented cells obtained from secretion, washing, brushing samples, etc. Electrochemical telomerase assay (ECTA) is another newly emerged technique to detect telomerase activity in biological samples [92]. It is comparatively simple and rapid PCR-free method. ECTA consists of a TS primer-immobilized electrode and ferrocenyl naphthalene diimide derivative as a tetraplex binder. This method has shown a high efficiency of telomerase detection in oral cancer biopsies [93]. Taken in account of all these reports, telomerase and its functionality can be utilized as a promising diagnostic and prognostic method in cancer.

7. Telomeres in prognosis

Better understanding of telomere structure and its dynamics focused the research on telomeres as biomarkers for several diseases especially in early detection and prognosis of cancers. A reduced telomere length in human hematopoietic tumors predicts a reduced survival time in patients suffering from myeloid leucaemia [94], chronic lymphocytic leucaemia [95], and myelodysplastic syndromes [96]. Although telomere length in solid tumors is suggested as a potential prognostic marker, patient survival rates vary with different cancer types. For example, a short telomere length in prostate cancer correlates with short disease-free interval and shorter overall survival time [97]. Analysis of telomere length of blood cells is also considered as predictive markers for pulmonary and esophageal neoplasia as well as of lymphoma in humans [98]. It has been suggested that the reduced TL in these patients reflects the effects of increased oxidative stress which correlates with cancer risk. Advent of latest technologies to measure relative and absolute telomere lengths has paved the way to use telomere length as diagnostic and prognostic markers. Telomere restriction fragment assay, qFISH, flow FISH, qPCR assay, single telomere length analysis (STELA), and dot-blot telomere assay are the currently available assay methods for telomere length [99].

8. Telomerase as drug target

Telomerase enzyme has recently emerged as an attractive target for cancer as it is a crucial factor required for the tumor immortalization of a subset of cells, including cancer stem cells. Studies have shown that 80–85% of the tumor cells express telomerase, whereas somatic cells lack the expression of telomerase [1]. In the present scenario, the major concern about the

chemotherapeutic approaches is the specificity of action and side effects of drugs on normal cells. Difference in telomere length and cell kinetics between normal and cancerous cells shows that targeting telomerase is an effective system to target cancer cells specifically [100]. Although telomerase is not considered as an oncogene, the expression of telomerase is the major reason for the transformation of a normal cell to cancer cell [80]. Compared to most other cancer targets, telomerase antagonists are advantageous due to the wide expression of this enzyme in cancer types [1]. Telomerase also possesses extra telomeric functions which are very crucial for tumor survival and homeostasis [101]. Studies have shown that telomerase-based cancer therapies are less likely to develop resistance against the drugs compared to drugs which target growth factor receptors or signal-transducing enzymes in cancer cells [2]. This ensures that cancer drugs based on telomerase inhibition are non-cytotoxic anticancer approach and have a broad therapeutic value.

9. Conclusion

Maintenance of telomere length in cancer cells is a critical factor in imparting the ability to undergo uncontrolled multiplication and thus immortality to the cells. It is imperative to discern the factors involved in telomere length preservation. Understanding the influence of telomerase and other factors in sustaining telomere length in cancer cells paves way for perceiving the theranostic role of telomere and telomerase in cancer treatment. Besides the theranostic effect, the possible side effects could be determined leading to precautionary methods to nullify the negative impact.

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