Chapter 4

The Role of Telomeres and Telomere-associated Proteins as Components of Interactome in Cell-signaling Pathways

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Additional information is available at the end of the chapter

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Abstract

Telomeres represent ends of all eukaryotic chromosomes and serve specialized biological role in maintaining genomic integrity by preventing end fusions and degradation. Various protein complexes associate with telomeres to either protect them from DNA damage machinery or maintain telomere length homeostasis. These protein complex subunits cross talk with a variety of cell-signaling components to either maintain telomere integrity or perform other functions, which are either dependent or independent of telomeres and/or their telomeric role. Mutations in these protein components lead to the development of various human diseases, such as age-related disorders, which occur mainly due to telomere dysfunction or cancer development due to telomerase reactivation. This chapter focuses on the structural and functional aspects of telomeric proteins and their importance in human diseases.

Keywords: Telomeres, shelterin, telomerase, TERT, telomere diseases, cancer

1. Introduction

Human telomeres consist of TTAGGG tandem repeats, which are generally 3–15 kbp in length [1]. The distal end of telomere has a 3′ single-stranded overhang, which is also termed a G-rich strand, and it forms a higher order structure (like a lariat) named t-loop [2]. In t-loop, both strands of the chromosome are joined to an earlier point in the double-stranded DNA by the 3′ strand end invading the strand pair to further form a D-loop. Formation of the D-loop completes the t-loop, thus establishing a capping structure, which protects chromosomes from degradation and recombination [3]. Figure 1A shows a schematic representation of telomere structure. The disruption of t-loop results in telomere dysfunction and induction of DNA

damage response (DDR) followed by cell cycle arrest [4]. Telomeres are bound by nucleosomes and a specialized complex known as shelterin, which is composed of six core protein subunits [5]. Shelterin determines the structure of telomeres. It is implicated in the formation of t-loops and also regulates the synthesis of telomeric DNA [6]. Additional proteins capable of interacting with shelterin proteins, such as DNA damage proteins, also play a role in maintaining telomere length and chromosomal stability [7].

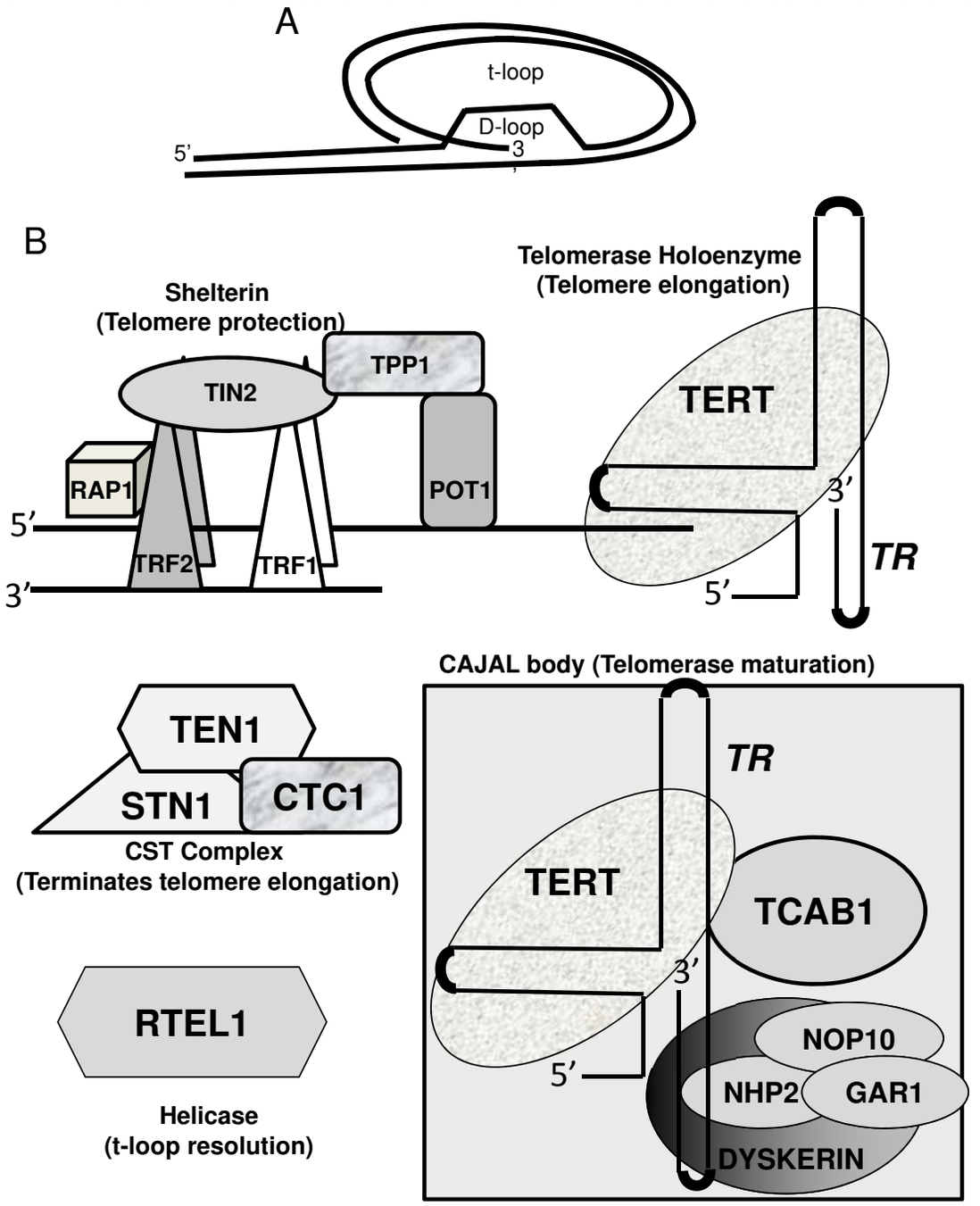

Figure 1. Schematic representation of telomere end structure (A) and Telomere associated protein complexes (B).

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Telomeres shorten with replication due to two major mechanisms: (A) end-replication problem and exonuclease-mediated resection in dividing cells, and (B) damage response to reactive oxygen species in nondividing cells [8].

DNA replication involves simultaneous duplication of antiparallel DNA strands, such that replication advances in opposite directions, across a leading strand and a lagging strand. On the leading strand, daughter strand synthesis takes place continuously in the 5′–3′ direction, whereas on the lagging strand template, DNA synthesis proceeds in the 5′–3′ direction discontinuously, leading to Okazaki fragments. The leading daughter strand is completely synthesized until DNA polymerase reaches 5′ end of the leading template. However, a primer is required for DNA replication to start. At the end of replication, RNA primer occupying the 5′ end of the daughter strand is removed, and it is not possible for the overlapping strand to be replicated. Due to this, the 5′ end of each antiparallel daughter strand becomes one primer length shorter. This is referred to as the end-replication problem, which results in chromosome shortening with each subsequent cell division. Theoretically, it should result in a loss of less than 10 bp with each replication cycle; however, the rate of loss is much higher and has been calculated to be 50–200 bp per division [9]. Exonuclease activity degrading the 5′ end is another major factor, which removes the RNA primer on the lagging strand and thus also leads to the formation of 3′-end overhang structure [10]. In vitro studies have also suggested the role of oxidative stress in telomere loss [11]. Correlative and experimental studies have also suggested links between oxidative damage and telomere loss in vivo [12]. Therefore, telomere length also serves as a biological clock and marker for chronological ageing. The solution to telomere shortening is the telomerase enzyme complex, which catalyzes de novo addition of TTAGGG repeats to chromosome ends, thus preventing telomere attrition [13].

2. Telomere-Associated Protein Complexes (TAPs)

2.1. Shelterin

Shelterin, as the name suggests, provides shelter to the ends of linear chromosomes by repressing DNA damage-signaling responses, masking telomeres from DNA repair machinery, and regulating the length of telomeres [7, 14]. Shelterin is a highly specialized complex comprising six central components namely, TTAGGG repeat-binding factors TRF1 and TRF2, TRF1-interacting protein (TIN2), protection of telomeres protein (POT1), Pot1-interacting protein (TPP1), and repressor/activator protein (RAP1) [15]. All shelterin components are ubiquitously expressed and associated with telomeres throughout the cell division cycle. The complete abrogation of all shelterin subunits (except RAP1) in mice results in embryonic lethality, thereby implicating their essential roles in development [16].

TRF1

TRF1 was the first shelterin complex subunit to be discovered that specifically associates with double-stranded telomeric DNA, mainly as a dimer through its TRF homology domain. Recently, it has been shown that TRF1 efficiently associates with telomeric DNA in nucleoso-

mal context and is capable of remodeling telomeric nucleosomal arrays [17]. TRF1 interacts with TIN2 in the shelterin complex as shown in Figure 1B. It also functions as a negative regulator of telomere length in telomerase-positive cells. In addition, some reports have demonstrated that it is essential for survival independent of its telomere length regulatory activity [18]. TRF1 genome-wide binding analysis revealed that it exclusively localizes at telomeres under normal conditions as well as under extreme telomere shortening unlike other shelterin members which have extra telomeric roles [19]. TRF1 has also been shown to assist Aurora-B recruitment to centromeres, thus contributing to appropriate chromosome segregation and maintenance of genomic integrity [20].

TRF2

TRF2 is highly similar to TRF1 in terms of protein sequence except that the N-terminal domain in TRF2 is acidic while that in TRF1 is rich in glycine and arginine residues (forming GAR domain). It possesses TRF homology domain, which mediates its dimerization. This N-terminal domain of TRF1 and TRF2 has been shown to regulate their ability to condense telomeric DNA [21]. TRF2 has been proposed to stabilize the t-loop by invasion of the upstream TTAGGG double-stranded region [22]. TRF2 has also been shown to bind at internal genomic regions, mainly at TTAGGG repeats referred to as interstitial telomeric sequences (ITSs) [23, 24]. Recent evidence suggests the role of TRF2 in the formation of novel chromosome end structures, which involve telomeres interacting with nontelomeric DNA, forming long-range chromosome loop that encompasses several megabases of chromatin and are known as interstitial telomeric loops (ITLs) [25]. Telomere-bound TRF2 is necessary to suppress the ataxia telangiectasia mutated (ATM)-dependent DNA damage response pathway [26] and the nonhomologous end joining (NHEJ) DNA repair pathway, thus playing a major role in protecting chromosome ends [9]. TRF2 also assists telomere replication by limiting resolvase activities leading to accurate repair of stalled forks [27]. It has been demonstrated that both TRF1 and TRF2 are modified post-translationally; however, the physiological relevance of these modifications is not yet completely understood [7].

TIN2

TIN2 (encoded by *TINF2*) associates with both TRF1 and TRF2, thus forming a bridge that connects the double-stranded telomeric DNA-binding proteins to those bound to single-stranded telomeric overhang region [28, 29]. A recent study by Frank et al demonstrates that TIN2 facilitates the recruitment of telomerase to telomeres [30]. In this study, the authors discovered a novel mutation in *TINF2* gene (which encodes TIN2 protein) and used novel functional assays to demonstrate a direct role for TIN2 in regulating telomere length through telomerase. This role is completely independent of its role in telomere protection.

TPP1

TPP1 interacts with TIN2 and POT1 through its carboxyl terminus and central domain, respectively [31-33]. Structurally, TPP1 possesses four domains, namely, OB (oligonucleotide/oligosaccharide-binding fold), RD (POT1 recruitment domain), S/T (serine-rich region), and TID (TIN2-interacting domain). It has been demonstrated that TPP1 recruits POT1 to telomeres [34, 35]. In addition, TPP1 has been shown to contain telomerase-interacting domain, suggest-

ing a role for TPP1 in the recruitment of telomerase to chromosome ends [36]. Interestingly, it has been shown that TPP1–POT1 interacts with N-terminal region of telomerase protein subunit TERT (telomerase reverse transcriptase), while TPP1 alone is also capable of interacting with C-terminal TERT residues through TPP1-OB domain [37]. TPP1-OB domain has been shown to contain a TEL patch (TPP1 glutamate (E)- and leucine (L)-rich patch), which mediates telomerase recruitment and processivity independent of its role in telomere capping [38]. In human stem cells, it has been further proven that TPP1 indeed mediates recruitment and activation of telomerase and telomere length homeostasis [39].

POT1

POT1 interacts with TRF1 complex via protein–protein interaction with TPP1, and this interaction is believed to affect its loading on the single-stranded TTAGGG telomeric repeat [40]. POT1 has been predicted to associate with telomeric sequences at t-loop as well as D-loop through its OB-fold domains [32]. POT1 serves a fundamental role in telomere length regulation, since it functions as a terminal transducer of telomere length control [40]. POT1–TPP1 complex has been shown to increase RAP (consecutive repeats that telomerase can add to telomere before dissociating) with minimal effect on telomerase activity [41]. Further, POT1–TPP1 has been shown to reduce the dissociation rate of telomerase from its telomeric substrate and assist in the translocation step [42].

RAP1

RAP1 associates with TRF2 and thus localizes to telomeres. As a component of the shelterin complex, it is dependent on TRF2 [43, 44]. RAP1 has been shown to be indispensable for telomere function in mouse and human systems [45, 46]. Further, RAP1 has been shown to possess extra telomeric roles that will be described in later part of this chapter.

2.2. Telomerase complex

Telomeres in human somatic cells shorten with each replication cycle due to end-replication problem consequently leading to genomic instability and cell death. Telomerase elongates telomeres and thus maintain their replicative potential. The minimal components of telomerase are a catalytic protein subunit termed as telomerase reverse transcriptase (TERT) and an RNA template known as *Terc* (*TR*) [47]. Telomerase catalyzes the addition of TTAGGG sequences to the ends of the chromosomes during each replication cycle, thus preventing telomere attrition and maintaining genomic integrity. Telomerase activity is detected predominantly in stem cells and cancer cells [48]. In normal somatic cells, telomerase activity is almost undetectable, consequently leading to shortened telomeres, which limit their life span. Furthermore, while *TR* is highly expressed in all the cell types, the levels of TERT are highly regulated at transcriptional level [49]. In somatic cells, TERT is transcriptionally turned off while stem cells display high expression of TERT, resulting in high telomerase activity. Ninety percent of cancer cells reactivate TERT expression either by mutation in *TERT* promoter or by activating oncogenic transcription factors such as NF-κB, MYC, and β-catenin that are known to activate TERT transcription [50]. This reactivation of TERT confers cancer cells with unlimited replicative potential. Distinct from its telomeric function, TERT has also been shown

to cross talk with various signaling pathways and impart several additional functions to cancer cells [51].

Terc (TR)

Mature human *TR* is a small noncoding RNA consisting of 451 nucleotides and serves as a template for de novo telomeric repeat synthesis by telomerase. Structurally, it is subdivided into four domains namely the pseudoknot domain, the CR4-CR5 domain, the CR7, and H/ACA domain [52]. These domains perform various functions including RNA binding, dimerization, and recruitment of telomerase to telomeres. The pseudoknot domain and the CR4/5 domain along with TERT constitute the minimal requirement for reconstituted in vitro telomerase activity [53, 54]. The CR7 and H/ACA domains are required for stability and localization of telomerase [55]. H/ACA motif consists of two hairpins connected by a short single-stranded stretch, the H box, and a terminal ACA region [56] which is found in small nucleolar and small Cajal body (CB) RNAs (snoRNAs and scaRNAs). H/ACA small nucleolar ribonucleoprotein complex (H/ACA snoRNP) catalyzes pseudouridylation of small RNAs like ribosomal RNA, which may serve to stabilize their conformation [57]. The H/ACA domain of *TR* is essential for assembly into ribonucleoprotein (RNP) with four member H/ACA–RNP complex which include the core heterotrimer (Dyskerin, NHP2, and NOP10) and a fourth factor, GAR1 [56]. Dyskerin represents the catalytic subunit of H/ACA complex [57]. Major function for the association of *TR* with dyskerin is its stabilization and nuclear retention. However, no pseudouridylation of *TR* has been reported [58]. Structurally, dyskerin contains a TruB domain that functions in eukaryotic ribosomal RNA processing. The TruB domain consists of two motifs, TruB I and TruB II. In addition, it has two nuclear localization (NL) signals, N-terminal and C-terminal, and the PUA, pseudouridine synthase and archaeosine transglycosylase, domain involved in RNA modification [59]. Dyskerin and NOP10 form the stable core to which GAR1 and NHP2 subsequently bind [60]. Mutations in these proteins affect the stability of *TR* consequently affecting telomere synthesis and result in various human diseases discussed in a later section of the chapter.

Although *TR* is transcribed by RNA polymerase II, it is not polyadenylated; instead, its 3′ end is formed by exonucleolytic cleavage up to the boundary element formed by the H/ACA domain, where further cleavage is prevented by dyskerin associated with RNA [61]. The tetrameric complex of dyskerin, NOP10, NHP2, and the chaperone NAF1 associates with *TR* cotranscriptionally and is essential for its accumulation [62]. The significance of this complex formation is highlighted by a number of telomere length-associated disorders with mutations in these factors which results in reduced levels of *TR* and thus telomerase activity [63].

TR also harbors specialized sequence elements in the terminal loop of its 3′ hairpin namely BIO box, which stimulates *TR* stability by H/ACA RNP formation, and CAB box, which it shares with the scaRNAs. CAB box is required for trafficking *TR* to the CB, where it receives its 2,2,7-trimethyl guanosine (TMG) cap, and NAF1 is replaced by GAR1 [57, 64, 65].

TERT

Human TERT is a large protein consisting of 1133 amino acids and it is subdivided into four distinct domains: (a) N-terminal extension (TEN), (b) TERT RNA-binding domain (TRBD), (c)

the reverse transcriptase domain (RT), and (d) the C-terminal extension (CTE) [66]. TEN domain is essential for telomerase activity and functions in proper localization and correct positioning of its catalytic site on telomeric DNA [67]. The TRBD domain functions in telomerase RNP assembly as well as RNA binding [68]. The RT domain of TERT forms the catalytic center in telomerase complex and consists of seven universally conserved RT motifs [69–71]. RT domain can be divided into two putative subdomains namely the fingers and the palm domains where fingers domain interacts with nucleic acid substrate while the palm domain contains the catalytic site [72]. CTE possesses the thumb domain and is sequentially not conserved among species [66]. CTE serves a critical role in catalytic activity and processivity of telomerase [73].

TERT interacts with chaperones HSP90 and p23 as well as with AAA^+ ATPases pontin and reptin [74, 75]. HSP90 and p23 interact with active telomerase; however, reptin and pontin are reported to interact with a pool of TERT, which is not assembled into active complex suggesting their role in telomerase assembly [75]. In addition, reptin and pontin are known to interact with dyskerin and are necessary for H/ACA RNP assembly, which is an essential step in TR stability [75, 76].

TERT transcription and telomerase activity is highest in the S phase of the cell cycle [77], and telomerase recruitment to telomeres has been shown to be restricted to the S phase [78, 79].

TERT interacts with two TR elements. The TRBD associates with CR4/5 region of TR and RT domain of TERT associates with pseudoknot region of TR [53, 80]. The human telomerase RNP purified from HEK293T cells overexpressing TERT and TR has been shown to be a dimeric structure, which is around 28 nm in length [81]. Although many studies have suggested the existence of multiple copies of dyskerin, NOP10, NHP2, and GAR1 with human telomerase RNP, the presence of two catalytically active TERT has been controversial since its biological significance is not clearly understood [82].

Telomerase activity in cells is limited by the levels of TERT protein (reported to be around 600 molecules/cell). All the other components of telomerase RNP are abundant [77, 83]. In normal somatic cells, TERT expression is repressed epigenetically or due to lack of activating transcription factors such as MYC, NF-κB, NFAT, RAS/RAF pathway, Ets factor steroids, and HIF [84]. Thus, transcriptional reactivation of TERT represents one of the major mechanisms responsible for activating telomerase and thus achieving replicative immortality in cancers. Recently, many cancers have been reported to harbor *TERT* promoter mutations resulting in high TERT expression and telomerase activity [85]. This is further discussed in later sections of the chapter.

2.3. Accessory proteins/complexes/factors for proper telomere maintenance

Regulator of telomere elongation helicase 1 (RTEL1)

RTEL1 is a DNA helicase, which contains N-terminal helicase domains and a C-terminal extension [86]. TRF2 recruits RTEL1 to telomeres during S phase of cell cycle to assist t-loop disassembly. RTEL1 is required for stability, protection, and elongation of telomeres [87].

RTEL1 has also been shown to interact with proliferating cell nuclear antigen (PCNA) and this interaction is important to prevent telomere fragility [87].

CST complex

The human CST complex consisting of CTC1, STN1, and TEN1 proteins plays a role in telomere protection and DNA metabolism [88]. Each telomerase RNP is believed to add 50–60 nucleotides to most telomeres following a single initiation event [89]. The CST complex has been proposed to set the upper limit of telomere elongation by binding to telomeric single-stranded DNA (ssDNA) and displacing telomerase, once telomeric overhang has reached certain length [90]. Study reported by Chen et al showed that CST competes with POT1–TPP1 for telomeric DNA [90]. It terminates telomerase activity through primer sequestration and physical association with the POT1–TPP1 subunits, which functions as a telomerase processivity factor [91]. CST–telomeric-DNA binding increases during late S/G2 phase following telomerase activity, concurrently with telomerase turn-off. Attenuation of CST enables excessive telomerase activity, fostering telomere elongation. It is suggested that through binding of the telomerase-extended telomere, CST limits telomerase activity at individual telomeres to approximately one binding and extension event per cell cycle.

Telomerase Cajal body protein 1 (TCAB1)

TCAB1 (encoded by gene *WDR79*) contains a proline-rich region and WD40 motif and is localized in CBs. CBs have been shown to accumulate telomerase as well as associate with telomeres [78]. TCAB1 has been demonstrated to be a component of active telomerase and is necessary for the telomerase holoenzyme to accumulate in CB and thus regulate telomere elongation [92]. TCAB1 interacts with CAB box motif of *TR* and functions in telomerase assembly by driving telomerase to CB. Subsequent report by Stern et al showed that TCAB1 and CB are required for telomerase recruitment to telomeres independent of each other [93].

TERRA

TERRA functions as a negative regulator of telomere length. It is transcribed from subtelomeric regions of telomeres that consist of UUAGGG repeats. TERRA may inhibit telomerase in cis by directly binding to telomerase [94, 95].

3. Cross talk of telomere-associated proteins with cellular signaling pathways

3.1. TAPs and DNA damage response

When the genomic DNA undergoes any damage such as single-strand DNA breaks, double-strand breaks (DSBs), nicks, or chromosome fusions, cells activate DNA repair pathway depending on the type of damage. During this process, cells seize to grow and initiate the repair, and once the repair is completed growth resumes; otherwise, they undergo apoptosis. Telomeres can also be sensed as breaks by cellular machinery. However, telomerase and

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shelterin complex cap the telomeres and thus maintain telomere integrity by inhibiting DNA-damage-response pathway at telomeres. Further, some of these DNA repair proteins play an essential role in telomere maintenance by directly associating with various TAPs. Recently, these TAPs have been shown to play a role in DDR at locations distinct from telomeres. TRF1, which is predominantly localized to telomeres, has been shown to interact with proteins implicated in DNA damage response such as ATM and Mre11/Rad50/Nbs1 [96]. Further, it has been shown that TRF1 is phosphorylated by Cdk1 and this form is incapable of binding to telomeres [97]. It has been shown that this phosphorylated TRF1 in a telomere-independent way facilitates end resection and homology repair (HR), activates G2/M checkpoint, and enables cell survival following double-strand break induction [98]. TRF2 is known to prevent activation of ATM-dependent DDR pathway at telomeres [26, 99, 100]. TRF2 has also been shown to localize at DNA double-strand breaks during early stages (within 2 s) of cellular response to DSBs and leaves those sites during repair processing [101]. Further reports suggested that TRF2 may also participate in HR of the extra telomeric damaged DNA [102].

It has been shown that telomerase localizes to mitochondria and protects cancer cells from nuclear DNA damage and apoptosis [103]. Further, TERT has also been shown to function in DNA damage response pathway and in regulating histone-dependent chromatin remodeling [104]. *TR* knockout mice display impaired DDR in response to damaging agents; however, the effects are evident clearly in late-generation TR null mice, which show significant telomere shortening and dysfunction [105, 106].

3.2. TAPs and NF-κB pathway

NF-κB transcription factors are key mediators of various cellular, inflammatory, and development pathways [107]. NF-κB family consists of five transcription factors namely RelA (p65), RelB, c-Rel, and also includes p50 and p52 (processed from p105 and p100, respectively). Rel family proteins possess REL homology domain, which harbors DNA-binding domain, dimerization, and IκB-binding domain. NF-κB family members are held inactive in cytoplasm by IκB family of proteins. Upon stimulation, IkB proteins are phosphorylated by IκB kinase (IKK) complex and thus degraded or processed to produce active dimers, which can enter the nucleus and activate the transcription response [107]. TAFs, which have been shown to modulate NF-κB signaling, independent of their role at telomeres, include shelterin complex protein RAP1 and telomerase complex protein TERT [108, 109].

A fraction of cytosolic RAP1 associates with functional IKK complex. RAP1 increases the efficiency of IKK complex in phosphorylating p65 subunit at serine 536; however, it has no effect on the degradation of IκBa inhibitory protein, thus functioning as an adaptor in the IKK complex [110]. In line with this, it was recently shown that RAP1 regulates cytokine levels followed by fine healing of corneal injury by effective modulation of NF-κB signaling [111]. Since RAP1 is also localized in cytoplasm, there are possibilities that it might function as an adaptor in various other complexes under different stimuli.

TERT has also been shown to associate with p65 in the nuclear compartment, thus directly regulating its transcriptional response. Akiyama et al demonstrated that TERT associates with p65 to mediate its translocation to nucleus in multiple myeloma cells [112]. Recently, our group

showed that TERT associates with p65 to directly affect its transcriptional output [113]. We showed that *TR*-knockout mice are more resistant to inflammatory agent lipopolysaccharide (LPS), which majorly functions by activating NF-κB pathway. Ectopic expression of TERT led to increased proliferation of cancer cells as well as xenograft model, which could be abrogated by inhibiting p65. Subsequently, another group showed that TERT regulates matrix metalloproteinase (MMP) expression independently of telomerase activity via activation of NF-κB-dependent transcription [114].

3.3. TAPs and WNT/β-catenin pathway

WNT pathway plays a key role in development processes like cell-fate determination, progenitor cell proliferation, and cell polarity [115]. In canonical WNT pathway, WNT ligand binds to its receptor leading to stabilization of β-catenin in the cytoplasm. Stabilized β-catenin then enters nucleus to activate transcription through its interaction with TCF/Lef family members. It further recruits chromatin remodelers like BRG1 to facilitate transcription.

TERT is the only TAP shown to modulate WNT pathway independent of telomeres and telomerase catalytic activity. The first evidence demonstrating a link between TERT and WNT pathway came from Choi et al who showed that knock in of catalytically inactive TERT in hair follicle stem cells led to their proliferation [116]. Changes in gene expression as analyzed by microarray, revealed differential expression of genes involved in development/morphogenesis, signal transduction, and cytoskeleton/cell adhesion signaling pathways. Modulated gene expression pattern strongly correlated with transcriptional program of MYC and WNT, suggesting existence of a potential association of TERT with the WNT and MYC pathways. Subsequently, Park et al demonstrated the first evidence of the direct regulation of Wnt/β-catenin signaling by telomerase in mouse embryonic stem cells and *Xenopus laevis* embryos. The study reported that TERT functions as a cofactor in the β-catenin transcriptional complex through its interaction with Brg1, a chromatin-remodeling factor [117]. Ectopic expression of TERT or catalytically inactive TERT led to the activation of WNT-dependent reporters in vitro and in vivo, while chromatin immunoprecipitation assays uncovered TERT localization at WNT target gene promoters. It was shown that TERT null mice display partially penetrant homeotic transformation of vertebrae, due to the loss of 13th rib of one or both the vertebrae. Recently, another group reported that TERT forms a complex with Brg1, together with nucleostemin (NS), a nucleolar GTP-binding protein and/or its family member GNL3L and is essential for maintenance of the tumor-initiating cell phenotype in human cancer cells [118].

3.4. TAPs and MYC

Recently, we reported that TERT regulates MYC transcription [119]. It is well known that MYC directly regulates TERT transcription [120]. However, our study illuminated the existence of a feed-forward loop between TERT and MYC in MYC-driven cancers such as lymphomas. Using genetic and biochemical approaches, we showed that the absence of TERT delayed MYC-dependent lymphomagenesis and strikingly, this effect was not observed when the RNA component of telomerase, *TR* was removed. Using in vivo and in vitro approaches, we established that TERT stabilizes MYC and thus results in increased MYC-dependent tran-

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scriptional output. Furthermore, we showed that this effect of TERT on MYC stability was independent of its catalytic activity. Mechanistically, we showed that TERT associates with MYC, preventing its proteasomal degradation, thus stabilizing its protein levels [119].

3.5. TAPs and mitochondria

Among TAPs, TERT and TIN2 have been shown to be involved in regulating mitochondrial activity. It has been shown that TERT translocates to mitochondria under certain stress conditions [121–123]. Mitochondrial TERT binds to and protects mitochondrial DNA from hydrogen peroxide-induced oxidative damage [103, 124]. Overexpression and knockdown studies involving TERT in cancer cells have shown that the role of TERT in mitochondrial pathway of apoptosis is independent of its catalytic activity [122, 125]. Interestingly, it has also been shown that TERT functions as a reverse transcriptase in mitochondria using mitochondrial tRNA as a template [126]. Furthermore, it has been shown that TERT can interact with RNA component of mitochondrial RNA-processing endoribonuclease (*RMRP*) to form a complex similar to RNA-dependent RNA polymerase (RdRP). This complex then affects gene silencing at the post-transcriptional level [126].

TIN2 has also been shown to localize to mitochondria, where it results in altered mitochondrial structure. The group showed that the reduction of TIN2 levels led to augmented mitochondrial oxidative phosphorylation and reduced aerobic glycolysis in cancer cells [127].

3.6. TAPs and miscellaneous associations

Apart from the above-described associations of TAPs with cellular machinery, there are various reports about many more interacting partners. TRF2 has been shown to function as a transcriptional activator by directly binding to promoter of the angiogenic tyrosine kinase platelet-derived growth factor receptor β (PDGFRβ). This study highlighted the angiogenic role of TRF2 uncoupled from its telomere-capping role [128]. Telomerase was shown to regulate rDNA transcription by directly associating with RNA polymerase I upon hyperproliferative stimuli such as during liver regeneration and Ras-induced hyperproliferation [129].

Figure 2 summarizes the role of telomere-interacting proteins in cross talk with cellular signaling pathways.

4. Telomeres and TAPs in human diseases: Telomeropathies

Telomeres shorten with each cell division. When telomeres become excessively short, they lose their protective role and activate a DNA damaging signal response resulting in genomic instability, cell cycle arrest, and senescence. TAPs play an essential role in maintaining telomere length, and genetic mutations affecting their activity can result in telomere dysfunction. This manifests into a wide variety of diseases collectively named as “telomeropathies” or “telomere syndromes”, which exhibit impaired telomere maintenance.

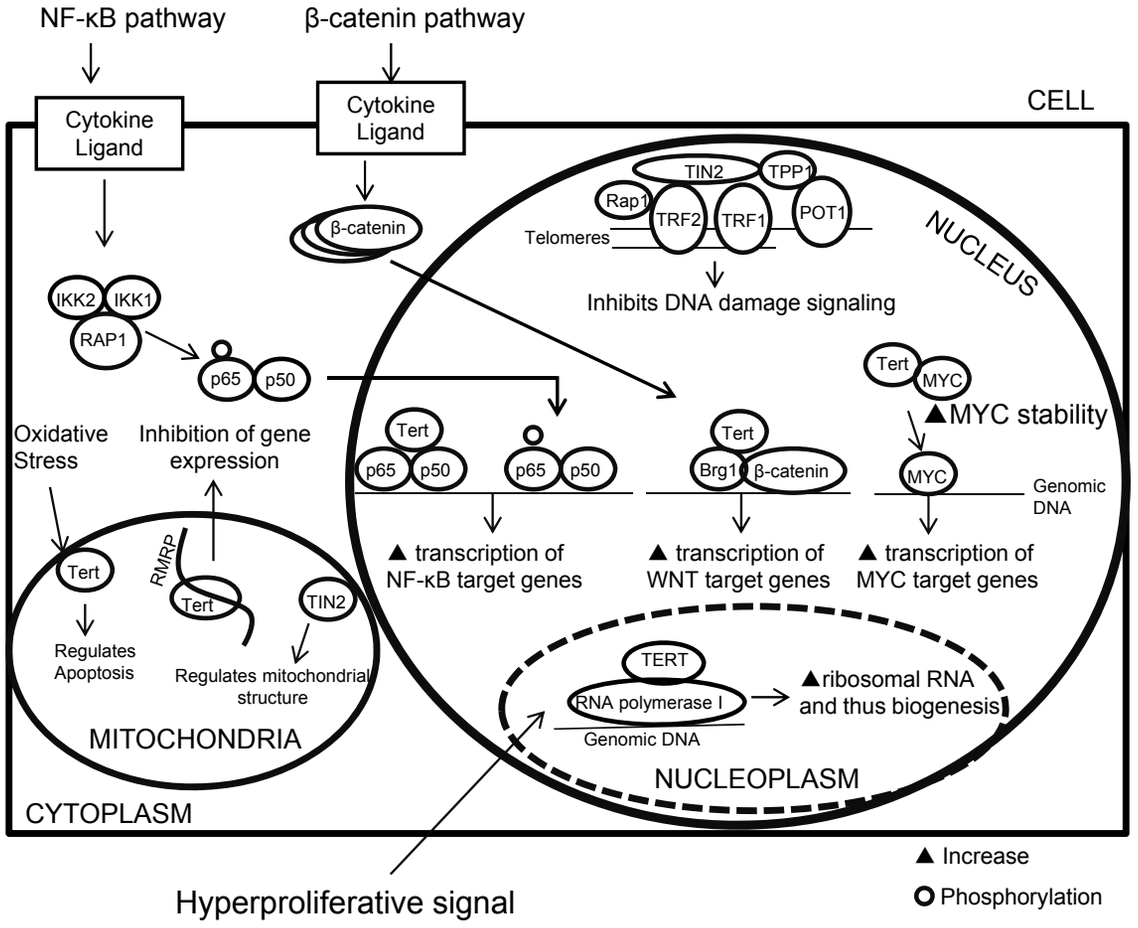

Figure 2. Schematic representation showing Telomere associated proteins interacting with several cell-signaling pathways.

4.1. Telomere-shortening syndromes

Inherited mutations, which hamper telomerase or telomere maintenance genes, result in progressive shortening of telomeres. Telomere shortening has major impact on highly proliferating tissues, such as bone marrow, where stem cells reach senescence stage and organ failure might ensue. Clinical conditions associated with shortened telomeres may be very different. This may be partly due to genetic anticipation since telomere length is inherited [63].

4.1.1. Dyskeratosis congenita

Dyskeratosis congenita (DC) arises primarily due to bone marrow failure and is associated with a diagnostic triad of oral leukoplakia, skin hyperpigmentation, nail dystrophy, and other manifestations. Dyskerin (encoded by *DKC1*), which is an essential component of telomerase enzyme in vivo, was the first gene identified as a cause of DC, and was thus named after this syndrome. DC is a heterogeneous disease showing all modes of inheritance. To date, 11 genes have been associated with DC. These include genes encoding products involved in telomere elongating enzyme, telomerase components (TERT and *TR*), telomerase stability (dyskerin,

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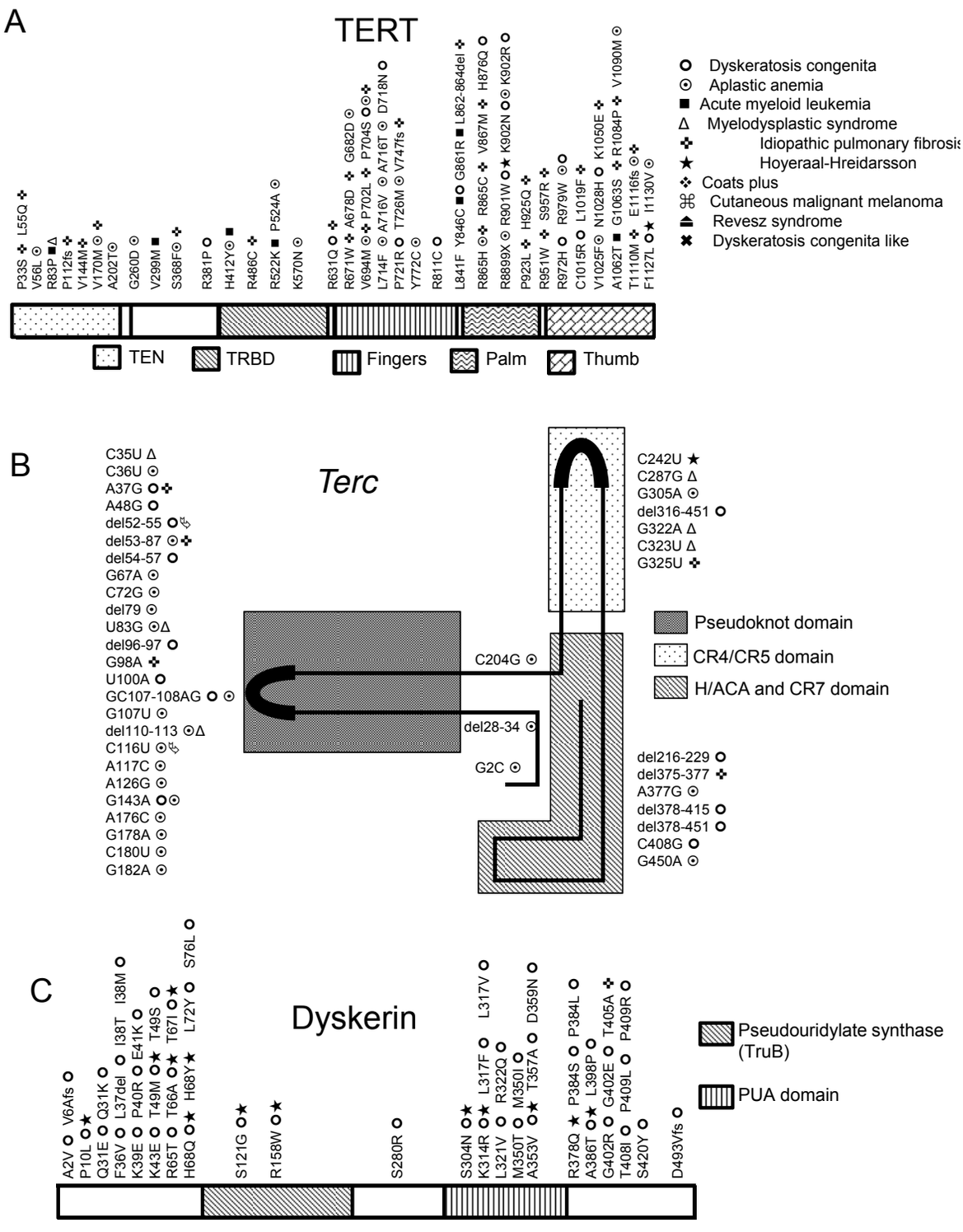
NOP10, NHP2), telomerase recruitment (TIN2 and TPP1), telomerase trafficking (TCAB1), telomerase docking (CTC1), and telomere replication (RTEL1) [130].

Figure 3 shows schematic representation of telomere-interacting proteins with domains and positions of reported germ-line mutations, which result in various forms of DC.



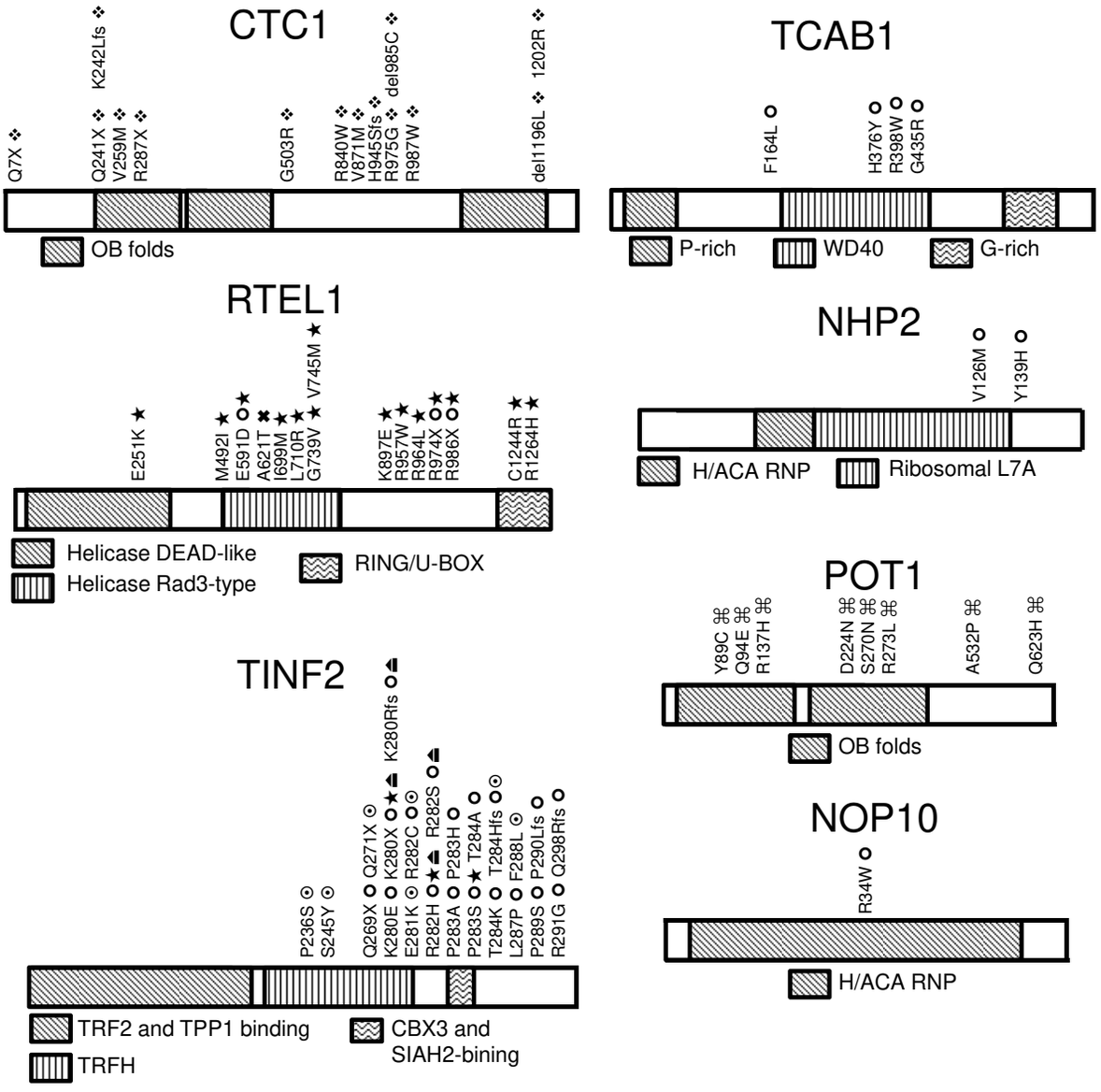


Figure 3. Schematic representation showing protein structure and localization of reported mutations in telomere associated proteins. The information is adapted from Espinoza et al [144].

Recently poly(A)-specific ribonuclease (*PARN*) gene mutations have been reported in a subgroup of patients with DC wherein *PARN* deficiency results in reduced stability of several key TAPs (dyskerin, TRF1, RTEL1, and *TR*) and specifically leads to telomere attrition [131]. Almost all modes of inheritance have been reported in DC, which include autosomal recessive, autosomal dominant, and X-linked. Based on functional relevance of mutated proteins and their penetrance, clinically diverse variant manifestations of DC are reported.

Calado et al reported a study of five families who were suffering from liver disease (familial liver cirrhosis) in combination with hematologic and autoimmune disorders [132]. They analyzed the mutations associated with the disease and found rare loss of function mutations in *TERT* or *TR* (3.7% vs 0.8%). Hoyeraal–Hreidarsson syndrome (HHS) is associated with

intrauterine growth retardation (IUGR), microcephaly, cerebellar hypoplasia, and thrombocytopenia along with various nonspecific enteropathies. HHS patients are also found to harbor DC mucocutaneous triad in adulthood. Detailed analysis revealed mutations in *DKC1*, *TIN2* along with some cases showing autosomal recessive mutations in *TERT*, *NHP2*, *NOP10*, *TPP1,* and *RTEL1* genes [133, 134]. Revesz syndrome (RS) is associated with various disease manifestations mainly bilateral exudative retinopathy. Other symptoms reported include IUGR, intracranial calcifications, developmental delay, and nail dystrophy in different cases, which were highly overlapping with DC symptoms. It was discovered that RS patients have short telomeres and harbor germ-line mutations in *TINF2* gene [135]. Coats plus syndrome (CCS) is a rare recessive disorder that is characterized by intracranial calcifications, hematological abnormalities, and retinal vascular defects. CCS patients display shortened telomeres indicating telomere dysfunction as a major cause. Missense mutations in *CTC1* gene whose protein is a part of CST complex has been reported to occur in CCS patients [136]. HHS, RS, and CCS represent severe forms of DC.

About 10% of DC patients develop cancer at a very young age. Various DC families display an increased incidence of acute myeloid leukemia and myelodysplastic syndrome [137]. Spontaneous reversion to the functional *TR* allele in hematopoietic stem cells of haploinsufficient DC patients has been observed predisposing them to hematological disorders. The mechanism behind high cancer incidence, in spite of short telomeres that should have cancer-protecting effect, remains largely unexplained. The only proposed mechanism is genomic instability due to fusion of chromosome ends by NHEJ as has been observed in mutation carriers and in *TR*-knockout mice [138].

4.1.2. Pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) disease is characterized by progressive lung scarring and fibrotic changes. The disease is associated with abnormal telomere maintenance and is an attenuated form where fibrosis develops with cumulative age-related changes. This disease arises from mutations in genes encoding *TERT* and *TR* leading to reduced telomerase activity and subsequently shorter telomeres, resulting in impaired growth of lung stem cells [139]. Surprisingly, short telomeres have been detected in IPF patients with intact telomerase genes, indicating that IPF may develop in people who have short telomere lengths [140]. This study also showed the development of liver cirrhosis in 3% of sporadic IPF patients, demonstrating a complication of telomere-mediated disease outside the lung even in the absence of telomerase mutations. Also, increased incidence of insulin-dependent diabetes is detected in IPF patients [141]. Short telomeres have been shown to cause insulin secretion defects and glucose intolerance in telomerase-deficient mice [142].

4.1.3. Bone marrow failure

Many bone marrow failure disease cases have been linked to telomere biology. Mutations in telomeric proteins can lead to accelerated telomere attrition in hematopoietic compartment leading to bone marrow failure. The most common gene associated with bone marrow failure is *TERT*, which generally harbors point mutations in its gene [143, 144].

4.2. Role of TAPs in cancer

The role of TAPs in cancer development is well known. People with long telomeres are at a greater genetic risk of developing cancers [145]. Thus, examining the role of telomere proteins in cancer holds immense prognostic, diagnostic, and therapeutic value.

4.2.1. Shelterin proteins and cancer

The shelterin complex member POT1 was found to be somatically inactivated in chronic lymphocytic leukemia where it led to telomere deprotection and length extension [146]. Recently, two studies reported occurrence of rare, germ-line variants in *POT1*, making them susceptible to the development of familial melanoma [145]. In these cohorts, carrier individuals displayed significantly longer and more fragile telomeres than controls, and in some cases developed cancer in other tissues along with melanoma. Molecular and functional analysis showed that some of the variants abrogate the binding of POT1 to ssDNA, thus raising the possibility that carriers are predisposed to malignancy via telomere uncapping and a more permissive extension of chromosome ends. However, the exact biological mechanism needs further investigation. Mutation in *RAP1*, another shelterin protein member was reported in a melanoma cohort. RAP1 is involved in negative regulation of telomere length and functions by repressing homology-directed repair [147]. Mutations were reported to occur in TRF1-interacting region of RAP1. This loss of interaction with shelterin may increase the risk of cancer development.

Germ-line mutations affecting other proteins that interact with shelterin complex members and increase cancer risk have also been reported. For example, ku80, which interacts with RAP1 and PARP1, which in turn interacts with TRF2, has been found to be associated with diffuse large B-cell lymphomas.

4.2.2. Telomerase and cancer

Telomerase activity is essential for immortalization. Thus, targeting telomerase activity represents an attractive approach for both cancer diagnosis and treatment [148, 149]. As described previously, TERT is the limiting factor for telomerase activity. Therefore, its reactivation mechanisms hold great significance in understanding the development of cancer and thus designing targeted therapies.

Two hot-spot mutations in the *TERT* promoter, -228 C>T and -250 C>T, were recently reported to occur at high frequency in several solid tumors, for example: melanoma, gliomas, carcinoma of bladder, urothelial cancer, thyroid and squamous cell carcinoma of the tongue, as well as in liposarcomas and hepatocellular carcinomas, which have relatively low rates of self-renewal [85, 150–153]. It was recently shown that *TERT* promoter mutations create novel binding sites for GABP, which belongs to Ets family of transcription factors [154]. These mutations have strong clinical implications with worse prognosis and poor survival, and thus may represent a novel therapeutic target [153].

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TERT promoter mutation in skin cancers

Stem cells differentiate into normal somatic cells and as a consequence repress TERT transcription. Upon subsequent cell division, progressive telomere shortening occurs due to lack of telomerase activity. This acts as a barrier for tumor development and progression. Skin epidermal cells are highly differentiated cells, possess short telomeres, and are thus capable of undergoing limited proliferation [155]. However, in melanoma, increased telomerase activity is reported and this has been associated with high proliferation rate and early metastasis [156, 157].

High frequency of *TERT* promoter mutations has been reported in familial and sporadic melanoma (about 29–73%) [150, 151]. In primary cutaneous melanoma, *TERT* promoter mutations were found to be associated with BRAFV600E mutations, worse prognostic features, and shorter disease-free and overall survival [158, 159]. *TERT* promoter mutations have also been reported to be common in nonmelanoma skin cancer ranging from 39 to 74% in sporadic basal cell carcinoma and up to 50% mutation frequency in squamous cell carcinoma [158, 160, 161]. Various studies have assessed the association between telomere length and risk of developing skin cancer [162]. Some reports suggest no association between telomere length in peripheral blood leukocytes (PBL) and risk of nonmelanoma skin cancer [163]. On the contrary, other authors have reported that longer telomeres in PBL are protective for certain skin cancer types [162].

TERT promoter mutations in central nervous system (CNS) tumors

Within CNS tumors, gliomas have been shown to possess the highest frequency of *TERT* promoter mutations, while medulloblastoma and meningioma show lower frequencies [164]. Within gliomas, the percentage of cases with *TERT* promoter mutations varies depending on the histopathological type of tumor. *TERT* promoter mutations are reported in a large number of cases of glioblastoma multiforme (GBM), which is the most frequent and aggressive form of glioma, and in oligodendrogliomas, in contrast to astrocytoma and ependymoma, where only a small percentage of the tumors possess such mutations [159, 164]. Furthermore, the frequency of *TERT* promoter mutations in oligoastrocytomas, gliomas with a mixed origin, is in between that of oligodendrogliomas and astrocytomas [152]. These findings are consistent with the reported data on telomerase activity in gliomas, which is significantly higher in GBM (50–89%) and oligodendrogliomas (75–100%) than in astrocytomas (0–45%) [165–167].

Some studies also reported an association between single-nucleotide polymorphisms (SNPs) in the *TERT* gene and an increased risk of glioma development [168, 169].

TERT promoter mutations in other cancers

Telomerase role in bladder carcinoma (BC) has been reported. In majority of BC tumor samples, telomerase activity was detected, while it was absent in the respective normal parallel samples [170, 171]. In some reports, telomerase activity was associated with lower grade and lower stage BC [170, 172]. Other studies showed that both telomerase activity and telomerase expression are associated with more advanced and higher grade of cancers [171, 173]. Preliminary evidence obtained in cell lines suggests that BC might have *TERT* promoter mutations

[150]. *TERT* promoter mutations are also frequently detected in BC cell lines, with a prevalence ranging from 47 to 85%. These results have clearly shown that *TERT* promoter mutations represent one of the most common genetic events, perhaps the most frequent, in BC [85].

TERT promoter mutations also occur at high frequency in other cancer types, for example: hepatocellular carcinoma (56%), several soft tissue tumors histotypes (e.g., 93% in atypical fibroxanthoma, 79% in myxoid liposarcoma, and 76% in pleomorphic dermal sarcoma) and carcinoma of the renal pelvis (64%). Tumor histotypes with intermediate frequencies of *TERT* promoter mutations comprise laryngeal carcinoma (27%) and clear cell carcinoma of the ovary (16%) [174]. *TERT* promoter mutations are not frequently found in leukemias and colorectal cancers [174].

The high prevalence of *TERT* promoter mutations suggests the significance of telomere maintenance in cancers. Clinically, *TERT* promoter mutations represent a potential biomarker in cancer prognosis. Furthermore, *TERT* promoter mutations also serve as an attractive therapeutic target since they occur specifically in cancer cells and are absent in surrounding healthy tissues.

5. Conclusion

Telomeres are organized into highly specialized structures at chromosome ends. Telomerase and shelterin plays a role in telomere homeostasis. Along with telomere maintenance, telomere-associated proteins also play a significant role in various cell-signaling pathways. The significance and implication of telomerase and shelterin in human diseases have also been firmly established in various models of degenerative diseases. In cancer, telomerase dysfunction has been identified as a critical step for immortalization, although the underlying mechanisms are unclear. The recent identification of telomerase promoter mutations has stimulated research, following which numerous studies have reported similar alterations in various cancer models. In several relevant cancer types, telomerase promoter mutations seem to represent a new biomarker for prognosis with potential applications in presurgical diagnosis and in the monitoring of patients. Mechanisms regulating telomerase promoter mutations also hold immense therapeutic value since they occur specifically in cancers.

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