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REVIEW ARTICLE

Therapeutic Targets in Telomerase and Telomere Biology of Cancers

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Abstract Telomeres play an important role to conserve genomic integrity by protecting the ends of chromosomes in normal cells. Since, their progressive shortening during successive cell division which lead to chromosomal instability. Notably, telomere length is perpetuated by telomerase in large majority of cancers, thereby ensure indefinite cell proliferation-a hallmark of cancer-and this unique feature has provided telomerase as the preferred target for drug development in cancer therapeutics. Cancer cells have acquired the potential to have telomere length maintenance by telomerase activation- up-regulation of hTERT gene expression in tumor cells is synchronized by multiple genetic and epigenetic modification mechanisms viz hTERT structural variants, hTERT promoter mutation and epigenetic modifications through hTERT promoter methylation which have been implicated in various cancers initiation and progression. In view of these facts, strategies have been made to target the underlining molecular mechanisms involved in telomerase reactivation as well as of telomere structure with special reference to distortion of sheltrin proteins. This review is focussed on extensive understanding of telomere and telomerase biology. which will provide indispensable informations for enhancing the efficiency of rational anticancer drug design. However, there is also an urgent need for better understanding of cell signalling pathways for alternative lengthening of telomere which is present in telomerase negative cancer for therapeutic targets.

Keywords Telomere · Telomerase · ALT · Shelterin · Anticancer therapy · hTERT promoter mutations · Epigenetics

Introduction

Infinite proliferation capacity is one of the unique features of cancer cells which is accomplished by maintaining telomere length at the chromosome ends through the activation of human telomerase [hTERT] [1]. The underlying mechanism incriminated to maintain the balance between telomere length and telomerase activity is an important driving force to preserve the chromosomal and genomic stability via shielding the chromosome ends, thereby rescuing the chromosomes from degradation and fusion [2, 3]. Telomerase, a specialized ribonucleoprotein consists of a catalytic subunit telomerase reverse transcriptase [TERT], an RNA template [TERC] and accessory proteins. Recent studies have shown that an interruption in telomere length led to restriction on life span as well as tumorigenicity of human cancer cells. Notably, the telomerase activity is lacking in most somatic cells, as a result telomere shortened on successive replication cycle. Henceforth, this phenomenon is of great interest to ascertain potent telomerase and telomere therapeutic targets in the new era of anticancer strategies. Better understanding of underlying molecular mechanism involved in telomerase and telomere biology in various malignancies is pre-requisite for the development of effective targeted cancer therapies.

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In this review, prime attention is focussed on critical molecular mechanisms involved in various cancers with special reference totelomerase and telomere balance as well as its regulation. Concurrently, most critical steps involved in these phenomena which are used as therapeutic targets in various cancers are also discussed critically.

Telomerase Ribonucloprotein (RNP) Structure and Its Interactions with Telomere

Infact, Greider and Blackburn first time recognized telomerase and reported its activity as specific telomere terminal transferase which is required for telomere repeat synthesis [4, 5] The human telomere terminal transferase is a ribonucleoprotein which synthesizes TTAGGG repeat [6]. Structurally, human telomerase reverse transcriptase is a species specific protein get involved in proper functioning of telomerase [7, 8]. hTERT complex comprises of four major functional domain viz TERT N-terminal domain [TEN], TERT-RNA binding domain [TRBD], reverse transcriptase domain [RT] and TERT-C terminal (TEC) extension [8–10].

TERT Complex is generated through the physico-chemico interactions among these domains, for instances, TEN domain interacts with the single stranded telomere DNA repeats, TRBD domain interacts with multiple sites of RT, while C-terminal extension domain attached to RNA/DNA hybrid, thereby catalyes the insertion of DNA repeats onto 3′ end [11, 12]. Structure of telomerase as holoenzymes is depicted in Fig. 1.

RNA component of telomerase (hTR) comprised three major distinct domains including core domain, the CR4/CR5 domain, and the H/ACA sca RNA domain [13–15] Notably, the hTR core and CR4/CR5 domains are held together solitarily with hTERT [16, 17]. Strikingly, two sets of four H/ACARNP proteins viz Dyskerin, GAR1, NOP10 and NHP2 are attached with hTR sca RNA domain [18]. The TCAB1/WDR79 binds both the dyskerin and the CAB box which are situated at the CR7 region within the H/ACA Sca RNA domain [19].

Telomeres are specialized structure on the ends of the chromosomes which consist of repeating hexanucleotide DNA sequences TTAGGG varying in the length from 2 to 25 Kb [20, 21]. It is well demonstrated that telomere formed T-loop in secondary structure through the invasion of the 3′overhang into the duplex region of the telomere which is stabilized by shelterin complex. Infact, shelterin complex consists of six proteins viz Telomere binding factor 1 (TRF1), Telomere binding factor 2 (TRF2), Repressor- activator protein 1 (RAP1), protection of telomeres protein1 (POT1), POT1-TIN 2 organizing protein(TPP1) known as tripeptidyl peptidase 1 and TERF1 interacting nuclear factor 2 (TINF2/TIN2) [22, 23]. The shelterin complex plays an important role in protection of chromosomes ends (telomere) from the DNA damage response by construction the chromosome ends using DNA repair machinery via suppression of the ATM and ATR signalling pathways [23, 24]. Two sequence-specific DNA binding proteins viz TRF1 and TRF2 binds directly to double- stranded telomere sequences in succession with the interaction of other proteins. TRF2 plays a critical role in telomere end protection and to bring about the formation of the T-loop conformation [25, 26]. Notably, TRF1 acts as a modulator of telomere length along with serving as facilitator of DNA replication via the telomere repeats [27, 28], both TRF1 and TRF2, each bind to TIN2. On the other hand, shelterin complex proteins viz POT1 directly binds to single stranded telomeric sequences and interconnected with TPP1 involve in the protection of single stranded region of telomere since loss of POT1 lessens telomere capping [29, 30]. hRAP1 is the part of shelterin complex which directly bind to TRF2 and subsequently bind to the newly synthesized double stranded telomeric DNA as a complex which in turn forming the 3′ over hang [31]. Recent studies have demonstrated that hRAP1 interects with DNA and altering the affinity of hTRF2 to telomeric DNA [32]. Diagramatic association between telomere and telomerase ribonucleoproteins (RNP) complexes are illustrated in Fig. 2.

Regulatory Mechanisms in Telomere Maintenance

T-loop are conjectured as a important domain of telomeric structure in the protection of telomeres and regulation of telomerase. Major two mechanism have been implicated in the maintenance of standard length of telomeres to abscond from genomic instability. First mechanism involves via the transcriptional activation of telomere reverse transcriptase [3]. Second mechanism, namely alternative lengthening of telomere (ALT) which maintain telomere length using DNA homologous recombination pathways [33]. Strikingly, this process is telomerase independent pathway.

Telomerase Based Molecular Mechanism

In actual fact, the cloning and complete sequencing of TERT gene [34], facilitated the field on how TERT gene expression is regulated. Multiple catalytic rounds of template copying and subsequently addition of hundreds of nucleotides to the same DNA primers is idiosyncratic ability of the telomerase catalysis. Reverse transcriptase (telomerase) copying of relatively large RNA molecules into a single molecules of complementary DNA is shown in Fig. 3.

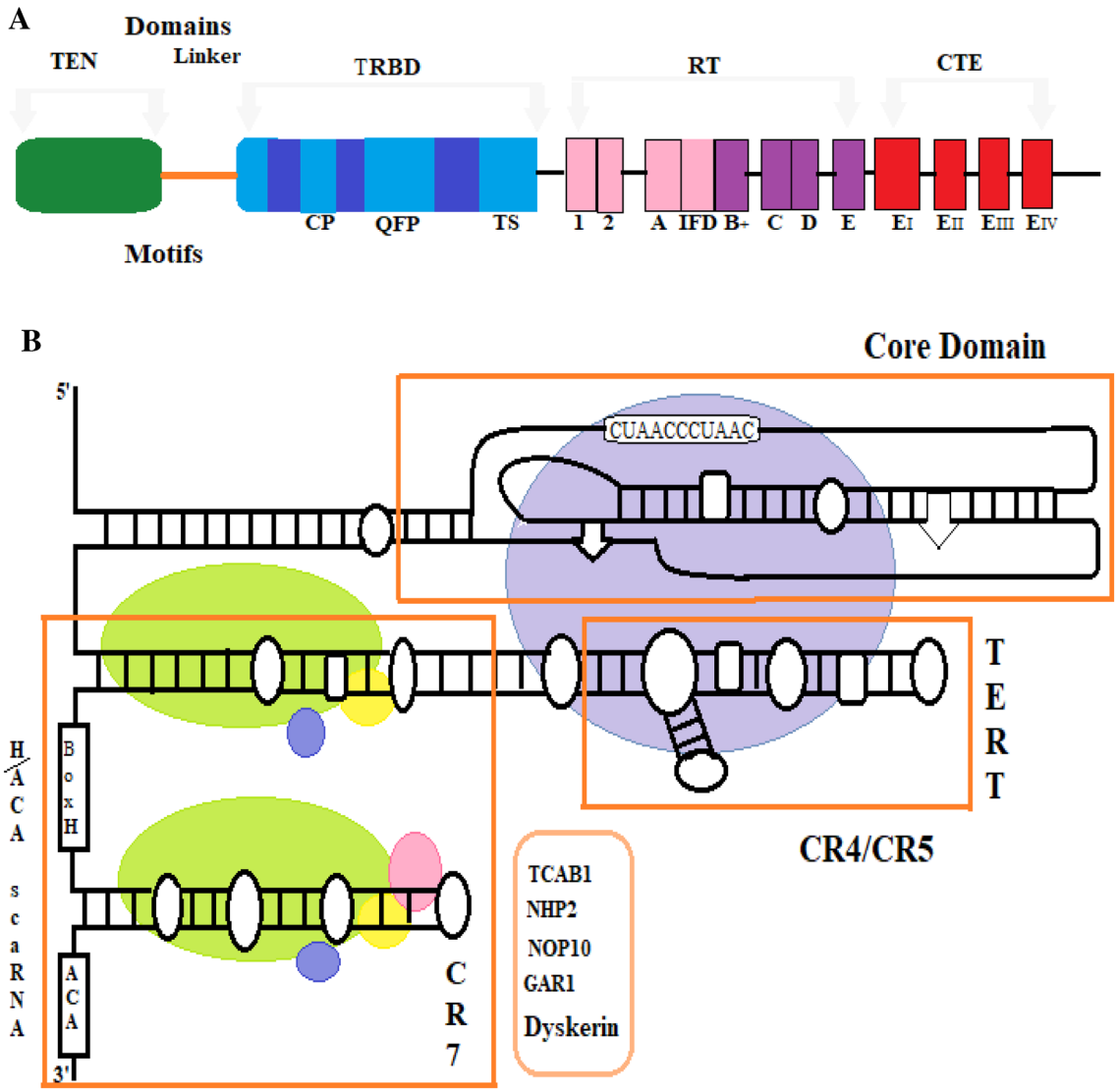

Fig. 1 Systemic organization of hTERT. **a** Schematic domains of human reverse transcriptase (hTERT) which consists of four viz the TERT essential N-terminal (TEN) domain, the TERT high affinity RNA binding domain (TRBD) reverse transcriptase (RT) domain and c-terminal extension (CTE) and telomerase-specific motifs CP, QFP and TS represents TRBD. Seven evolutionary conserved motifs in the RT domain (1, 2, A, B, C, D, E) and IFD. The CTE contains four blocks of conserved amino acids (E-i, E-ii, E-iii, E-iv). B. Secondary structure of human telomerase holoenzyme: It consists of three structural domains viz a Core domain, CR4/CR5 domain and H/ACA sca RNA domain. The hTR core and CR4/CR5 domains bind independently to hTERT. The hTR sca RNA domain interacts two sets of the four H/ACA RNP proteins dykerin, GARI, NOP10. NHP2. The protein TCAB1/WDR79 binds both the dyskerin and the CAB are situated at the CR7 region within the H/AG Acasca RNA domain

Telomerase reaction begins with primer recognition and binding. Here RNA template is placed on the DNA primer. In second event, telomere synthesis starts with the sequential addition of deoxynucleotide triphosphates (dNTPs) to the free 3′-hydroxyl group of the telomeric ssDNA primer which is thought to be the natural primer for telomerase-mediated DNA synthesis [7]. When the dNTPs addition reaches to the 5′-template boundary element, after that a translocation step bring the same template, to the 3′-end of DNA in former position for a second round of telomere synthesis which is known as repeat addition processively. Telomerase- specific residues that mediate processivity are located in RT domain [35], N-terminal extension [36] and C-terminal extension [37]. Interestingly, mutations in these regions led to defects in telomere length maintenance in humans [12, 38, 39]. More over, recent evidence suggests that telomere associated protein viz TPPI-POTI heterodimer play on important role in the activity and processivity of human telomerase in vitro [40, 41]. The cloning and complete sequencing of TERT gene was reported by [34], after that an extensive efforts were made on how TERT expression is regulated. Recent

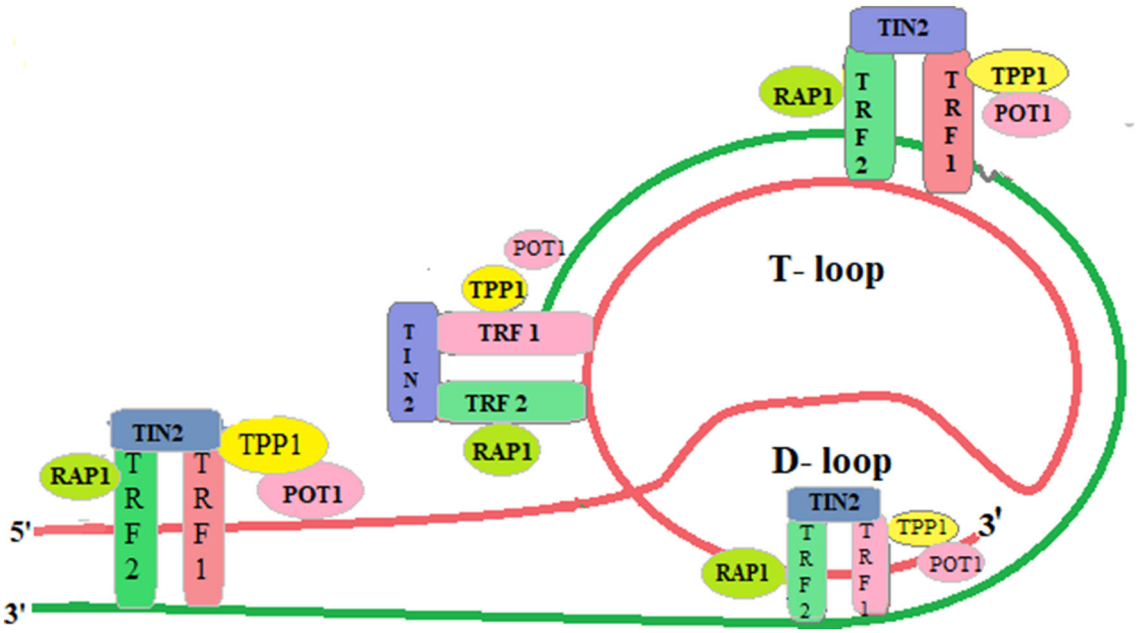

Fig. 2 Telomere structure with shelterin. Telomeres are TTAGGG double-stranded DNA repeats and 150–200 nucleotide long single stranded DNA overhang of G-rich strand. The G-strand overhang invades the telomeric double-stranded DNA region to form a protective T-loop with a displacement to form a protective T-loop with a displacement D-loop of the invasion site. The shelterin complex comprises of the telomeric repeat binding factor 1 (TRF1), (TRF2,) repressor activator protein1 (RAP1), and POT1 bind directly to the telomeric DNA repeats, with TRF1 and TRF2 binding to telomeric double stranded DNA and POT1 to the 3-single standed overhang. TIN2 binds TRF1 and TRF2 through independent domains and recruits the TPP1-POT1 complex which provide a bridge among the different shelterin components

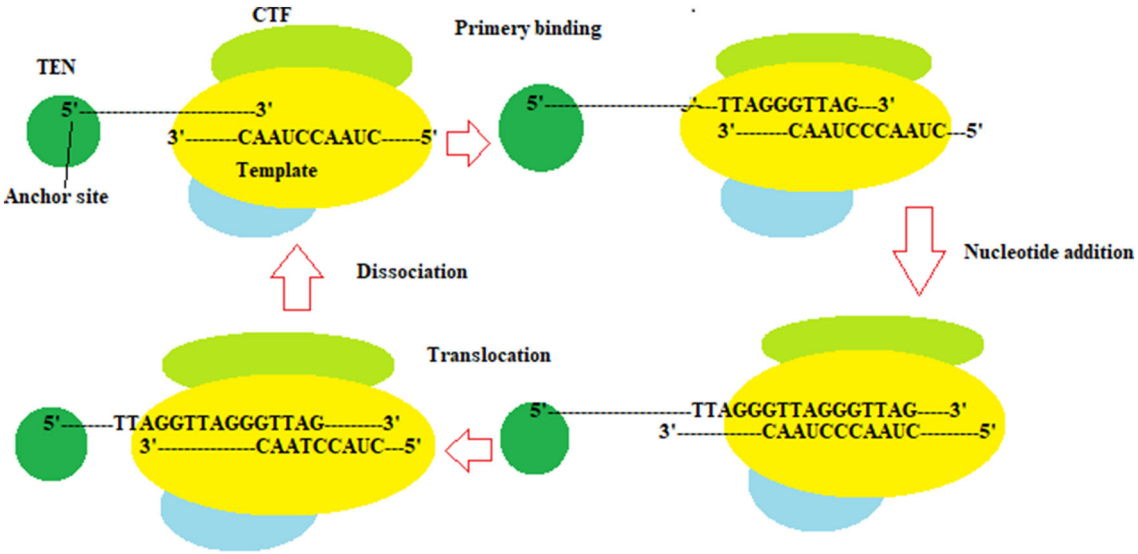

Fig. 3 Schematic diagram of telomerase cycle: this diagram elucidates mechanistic flow of telomerase cycle which is carried out in four step: 1. Primer recognition and binding ii Nucleotide addition iii Translocation iv Dissociation. The TR provide the template for telomerase synthesis. DNA synthesis is catalyzed by TERT which consists of TEN domain, TRBD, RT domain and CTE domain. Ten domain contains a ss DNA binding region referred as telomerase anchor site. Telomerase binds the ssDNA in such a way that 3'end is aligned with the TR template in the active site. The 5-end is positioned within the telomerase anchor site. Tolomerase reverse transcribes the template region. Nucleotide addition occurs by 1nt at a time until reaching the 5'template boundary element. At this juncture, a translocation step repositions the new DNA. 3'end within the template for second round of telomere synthesis. Conformational changes within the telomerase holoenzyme are believed to facilitate nucleotide and repeat addition processivity

advances on hTERT promoter mutations provided new insights in better understanding of molecular mechanism involved in hTERT regulation [42].

hTERT gene is positioned on short arm of chromosome 5 and designated as 5p15.33 which spans across 40 kb of human genome [43]. hTERT gene consists of 16 exons and 15 introns. It is of great interest that it has 22 splicing variants and splicing variants which can act as dominant negative variants [44]. hTERT promoter consists of 260 bp of proximal region which has multiple binding motifs for various transcription factors, thereby it undergo composite regulatory dynamics. TERT promoter is distinctly different since it is lacking typical transcription regulatory elements for instances TATA and CAT boxes whilst it has E-boxes and GC boxes.

hTERT promoter core is critical in the expression of hTERT gene. It has at least five GC boxes which is interacted with zinc finger transcription factor SP1. Importantly, promoter core has enhances binding proteins such as MAX/MXD1/MYC family as well as USF1/2 at positions − 165 and + 44 nucleotide. Additionally, E boxes also has binding sites to MADI and USF1 to concillate hTERT suppression. The transcription start site (TSS) has the potential to bind multifunctional transcription factor THF1. It is noteworthy here that c-MYC, H1FI-1, E-twenty-six (ETS) family members, NF-κB, AP-2 and HIFI-1 play a role in the upregulation of hTERT transcription, conversely transcriptional factors, WT1, MZF-2 and S1P1 and have been reported in down regulation of hTERT transcription (Fig. 4).

The mechanism of reactivation of telomerase has been incriminated in human malignancies, notwithstanding underlying mechanism are not comprehensibly understood. Recently, germ line and somatic mutation are found in high frequency in hTERT promoter region in various cancers [45, 46]. These mutations are transpired in two hot spots locations for instances − 124 and − 146 bp upstream from the ATG start site (− 124G→A and − 146 G > A, C > T on opposite strand) which are fraternized with augmented TERT promoter activity. These mutations have been implicated in various tumor types viz glioblastoma, bladder and upper urinary tract, thyroid cancer, melanoma, hepatocellular carcinoma [46–49]. It is of particular interest that activation of telomerase was not noticed in all the cancers, not with standing some pre malignant lesions exhibited the TERT promoter mutations [50]. Which is indicative of initiation of oncogenic process [51, 52] strikingly, recent studies have suggested that in fact TERT mutations are responsible for healing the shortest telomere and later when telomere critically short resulted in genomic instability and subsequently reactivation [53].

However, TERT promoter mutations have been implicated as initiator genetic event in various cancers viz bladder cancer [49] HCC [54] thyroid cancer [48] cutaneous melanoma [50, 55] basal cell and squomous cell carcinoma [56] and oligodendroglioma [57]. Strikingly, hepatic cellular carcinoma is associated with the activation of an oncogenic pathway for instance WBT signalling whilst MAPK signalling in melanoma which are thought owing to second genetic event [54]. hTERT gene amplification is an underlying mechanism for the genesis of tumorigenesis. Since, augmented level of h TERT gene copy number is linked with upregulation of hTERT expression owing to telomere dysfunction as well as formation of chromosomal fusions [58].

Second mechanism involves in the maintenance of telomere length via the stimulation of alternative lengthening (ALT). This process is telomerase independent which is regulated by DNA homologous recombination repair pathway [34]. Immortalized human cell lines avail oneself of ALT show various phenotype characteristics which are in accordance to the hypothesis that ALT utilize homologous recombination-mediated DNA copying of a telomere DNA template [34]. These characteristics include telomere length heterogeneity and extra chromosomal linear and circular telomere DNA [40, 59, 60], an elevated frequency of telomere-sister chromatid exchange events [61] and the presence of a specific subclass of promyelocytic leukemia (PML) nuclear bodies containing telomeric DNA, shelterin proteins and HR factors viz MreII-Rad 50 – Nbsi (MRN) referred as ALT associated PML bodies [62]. The template for synthesis of new telomeric DNA can be the telomere of a non homologous chromosome [34] or telomeric sequence elsewhere in the same telomere or the telomere of a sister chromatid [63], is suggestive of extra chromosomal telomeric DNA may also act as copy template [64].

ALT in Cancers

ALT positive cases are found in various tumors viz bladder, cervix, endometrium, oesophagus, gall bladder, kidney, liver, and lung [65]. Most of the carcinoma subtypes presented with considerable frequencies except in some epithelial malignancies. Recently, we have demonstrated very high frequencies of ALT positive cases of RCC [66]. However, the presence of ALT positivity was observed in all the grades of tumors as well as subtypes of RCC. Further studies are needed to clarify the clinical and biological significance of ALT in high stage stages of tumors as well as its utility as a predictive and/or therapeutic target other studies have shown that ALT positive cases of liposarcomas are associated with malignant tumors and unfavourable disease outcome.

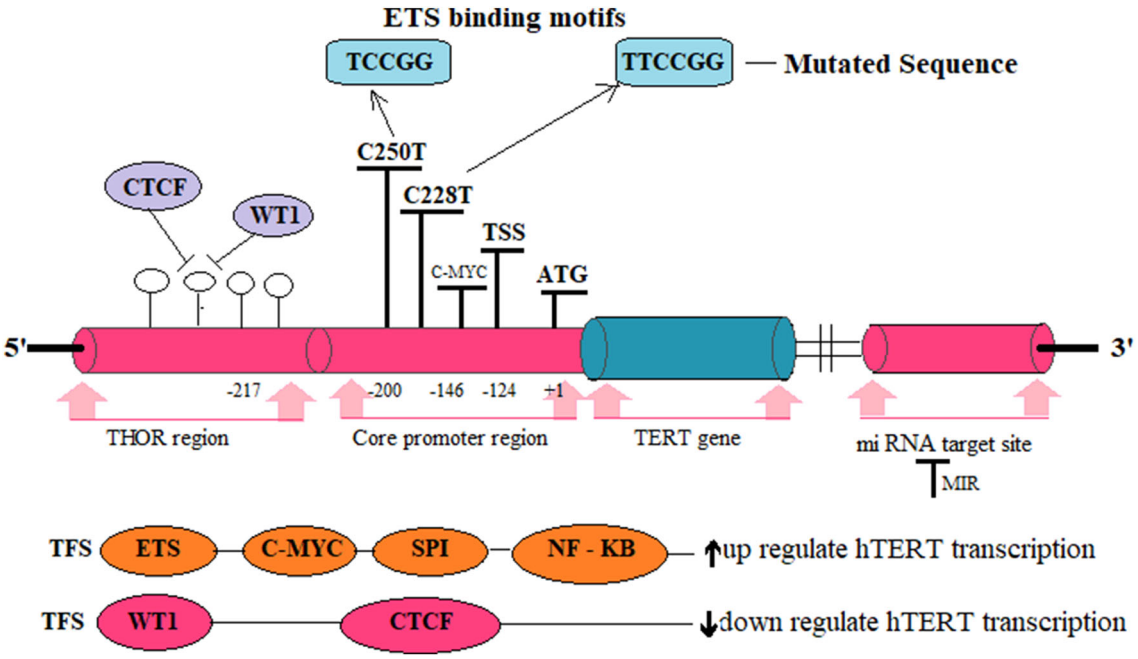

Fig. 4 Implications of hTERT promoter mutations and its expression in cancers. Transcription factors binding sites and the location of both hTERT promoter mutations (C228T: C250T) hypermetheylated region upstream to TSS (THOR) and TERT-mirna are depicted. The hTERT gene is highly suppressed in almost all normal cells and tissues. Specific hTERT promoter mutations involved in cancer progressing owing to increased transcription of hTERT gene. The hTERT gene expression is regulated by a series of transcription factor (TFs) hTERT promoter mutation at − 124 and − 146 positions generate ETS binding motifs. Each mutation create a new ETS/TCF binding site. Upregulating TFS such as ETS, CMYC, SPI and NF-κB bind to their respective site and can enhance hTERT transcription whilst WTI and CTCF act as a repressor of hTERT gene transcription not with standing binding of TFs is essential for hTERT transcription, inoveover a permissive chromatin micoenvironment is needed mi-RNAs targeting the 3′UTR promotes translation suppression of hTERT

Role of Epigenetics in hTERT and ALT Activity

Epigenetics also has been implicated in both hTERT regulation as well as telomeric recombination and ALT activity. The modification of histones in telomeric chromatin involve in regulation of transcriptional activity [2]. These modification occurs at the hetero chromatin marks [H3K9me3 and H4K20me3 [trimethylation of histone H3 at lysine 9 and histone H4 at lysine 20], hypoacetylation and accumulation of several isoforms of heterochromatin protein 1(Hp1) [67–69]. On other hand, chromatin modifications also occur which lead to repression of ALT activity at enriched at sub telomeric repeats [70] which seems paradoxical to sub-telomeric transcriptional origin of telomeric repeat containing RNA (TERRA) [71, 72]. In fact, TERRA has been incriminated in the regulation of telomere length and telomeric chromatin structure via facilitating heterochromatin formation at telomere through the recruitinenl of H3k9me3 and HP1 and negative feed back regulation of its own transcription [73, 74].

DNA methylation pattern via the epigenetic process is utmost important in regulation of gene expression activity which play a pivotal role in cancer development and aggressiveness [75, 76]. The correlation among hTERT methylation with hTERT mRNA as well as telomerase activity clearly infer that hTERT promoter intricates in the regulation of telomerase via epigenetic modification. Notably, numerous transcription factors such as cMyc, SP1, MAD1 and HIF are invisaged in the recruitment of either histone acetyl transferase or deacetylase to the hTERT promoter in regulation of hTERT expression [77, 78]. DNA methylation pattern could possibly responsible for alteration in chromatin conformation which may result in gene expression activity by exposing DNA for its binding to transcription factors at the promoter sites [76]. Therefore specific conformational alterations owing to hTERT promoter methylation could be resulted in differential recruitment of transcription factors which may lead to drive hTERT expression in cancer [79]. Recently, we have documented increased levels of telomerase activity and hTERT gene expression in ccRCC [80]. Moreover there was a good correlation found between augmented levels of telomerase activity and clinico pathologic features of tumors with special reference to stage and grades.

Telomerase as Therapeutic Targets in Cancers

Telomerase is considered as a prime target in the development of a potent therapeutics against the cancers. There are few solid reasons for it. Firstly telomerase is expressed in the majority of cancer, cancer types/subtypes as well as in cancer stem or stem like cells. Secondly normal humans cells including stem cells which are associated with lower telomerase activity, even through these cells sustain telomere length at reasonable length to that of cancer cells. These characteristic provide an advantage that ensures minimal risk for possible telomere shortening in normal cells. Main impetus of anti-telomerase therapeutics is to induce apoptosis and cell death selectively in cancer cells whilst it has minimal effects on normal cells due to minimal or non-existent expression of telomerase in somatic cells [81, 82]. Major effective therapeutics against telomerase and telomere have been shown in Fig. 5. Multiple approaches have been adopted to achieves this goal via the development of vaccines, antisense oligonucleotides and small nucleotide inhibitor targeting hTERT [83].

Targeting Telomeric Cap

Telomeric cap consist of group of six proteins TRF1, TRF2, RAP1, POT1, TPP1 and TIN2. Many studies have reported upregulation of TRF1 and TRF2 in several cancers. TRF1 and TRF2 binds to the telomeric double-stranded 5′-TTAGGG-3′ repeat and protects chromosomes from end to end fusion [84]. Hence, TRF1 and TRF 2 are important for genomic stability, So targeting of TRF1 and TRF 2 can be of greater significance to induce genomic instability in cancerous cell that leads to cell death. In view of this, we have shown that targeting TRF1 and TRF2 in RCC leads to cell cycle arrest and apoptosis [85]. The possible mechanism involved is activation of DNA ligase IV-dependent nonhomologous end joining and activation of the ATM kinase/p53 DNA damage response pathway, leading to cell death in vitro models [86]. Also Maria Garcia-Beccaria group has shown that targeting TRF1 abrogation cause *marked* reduction in the number and the size of malignant lung carcinoma [87]. POT1 is another essential protein that bounds to single strand overhang of telomeric sequence with an exceptionally high sequence specificity end and stabilize telomeric structure [88]. Role of POT1 in telomere protection was ascertained in Breast cancer where breast cancer cells were treated with anti-POT1 siRNAs. Treatment with POT1 siRNA leads to increased expression of p53 and pro-apoptotic protein-Bax, telomere dysfunction and induction of apoptosis [88]. Also, In our lab we have observed increased expression of POT1 in renal cell carcinoma RCC. A putative role of POT1 in carcinogenesis has been further suggested by findings that POT1 gene deletion in mice resulted in telomere end fusion and chromosomal instability [89]. Further a study have shown that POT 1 deletion in leads to transient DNA damage response at chromosomal ends [90]. It has also

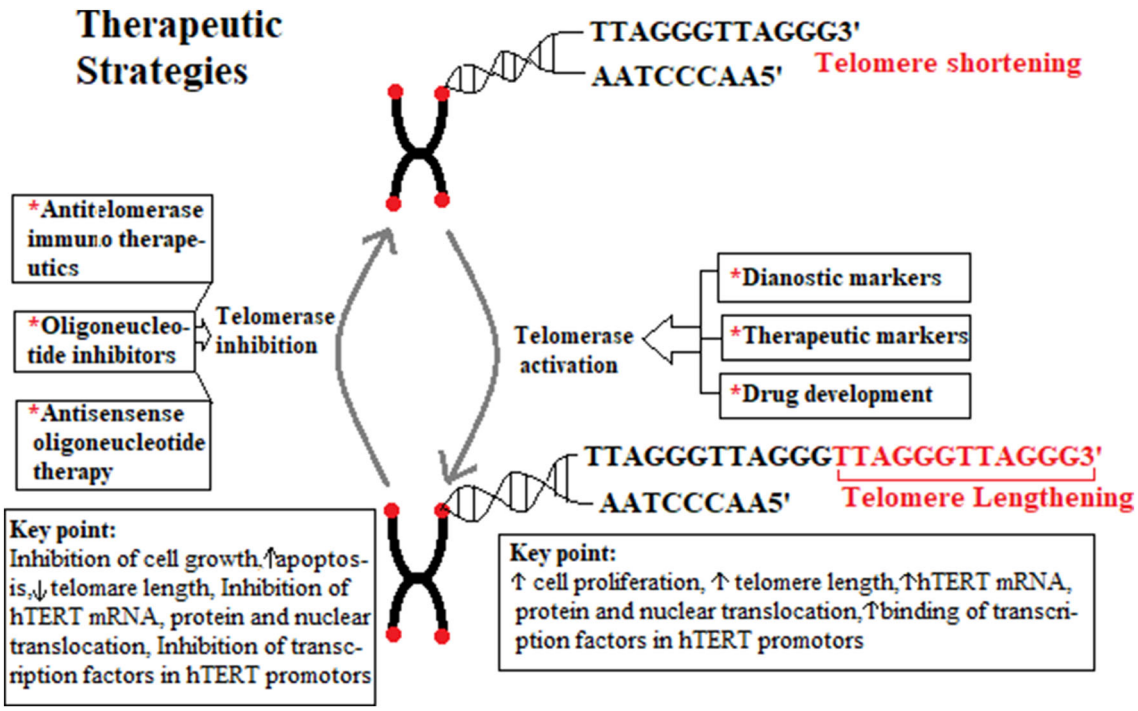

Fig. 5 Telomerase associated anticancer strategies

been observed that targeting TPP1 or TIN2 has also shown cytotoxicity at least in some cells [91]. These studies have paved the pathway for the further development of pharmacological agents that disrupt shelterin complex structure and induce DNA damage response in cancer cells.

Targeting G-Quadruplex

Telomeric DNA consist of guanine rich DNA sequence 5′-GGGTTA-3′ at the end. Under physiological ionic conditions, G-rich sequence form variety of four-stranded structures containing G tetrads called G-quadruplex [92]. The G-quadruplex protects the telomeric 3′-overhang from being accessed by telomerase enzyme. Evidence from the experiments suggest that G-quadruplex structure exist both invitro and in vivo where it plays role in 3′ end protection [93]. Importance of G-quadruplex structure in telomere stabilization prompt development of molecules that stabilize G-quadruplex structure. In view of this a study by [94] reported that a trisubstituted acridine BRACO-19 (3,6,9-trisubstituted acridine 9-[4-(N,N-dimethylamino)phenylamino]-3,6-bis(3-pyrrolodinopropionamido) acridine, which is a G-quadreplex ligand restrict telomerase access and induce telomere uncapping along with telomeric fusion. Another G4 ligand RHPS4 RHPS4 (3,11-difluoro-6,8,13-trimethyl-8*H*-quino[4,3,2-*kl*]acridinium methosulfate) is a pentacyclic acridine that may possess antitumor activity. RHPS4 treatment induced DNA damage signalling, telomere dysfunction and toxicity in melanoma cells but not in normal fibroblasts [95]. A G-quadruplex stabilizing compound teloestatin isolated from Streptomyces anulatus 3533-SV4 also showed telomere shortening and apoptosis [96].

Anti Telomerase Immune Therapeutics

Foremost concept of anticancer immune therapeutic approach is to target telomerase positive tumors by using hTERT specific T lymphocytes. Infact, the telomerase degradation using proteasomes leads to formation of proteins fragments of telomerase which are expressed on the tumor cell surface as antigens by the human leukocyte antigen (HLA) class-1 pathway [97, 98]. Thereby antigenic epitopes of telomerase can be targeted by cytotoxic T cells to destroy the tumor cells [99]. Telomerase-specific epitopes include CD^{4+} or CD^{8+} cytotoxic T-lymphocyte responses or stimulate antigen presenting cells are able to attack tumor cells [100]. This therapy sensitize the immune system to the tumor cell expresenting hTERT peptides to activate and generate h TERT specific CD^{8+} cells to produce enhanced anticancer effects. Interestingly three major vaccines GV1001L, Vx001 and GRNVACI are being used to evoke anti telomerase immune responses in cancer patients [101]. Interestingly, vaccination with autologous dendritic cells transfected with hTERT mRNA also triggers CD^{4+} and CD $^{8+}$ T responses in mice and Humans [102].

Development of Oligonucleotide Inhibitor Imetestat

Imetestat is a competitive inhibitor of telomerase activity. It contains a 13 mer N3′–P5′ thiophosphoramide oligonucleotide that is covalently attached to a palmitoyl moiety through a 5′- thio-phosphate group. The thiophosphonate back bone of imeteblast has many physico chemical properties viz high aqueous solubility metabolic stability, resistance to the action of nucleases and stability to form RNA duplexes [103]. Imetelstat's sequence (5′-palmitate-TAGGGTTAGACAA-NH2- 3′)binds to a complementary 13- nucleotide region of h TR which has high affinity and specificity at the active site of the telomerase activity and specificity at the active site of the telomerase. Imeteblast has been undergoing clinical trails in USA, Europe and Asia.

Anti-sense Oligonucleotide Therapy

The principle of the mechanism is based on the specificity of the synthetic nucleic acids to bind to the mRNA of hTERT and then degrade it. It includes two strategies which are antisense oligodeoxynucleotides (AS-ODNs) and small-interfering RNAs (siRNA). Single-stranded AS-ODNs function by interfering with the translation of hTERT which can lead to degradation of hTERT mRNA via RNase H-mediated cleavage [104]. The antisense oligonucleotides (AS-ODNs) approach for targeting telomerase was first described using AS-ODNs to block the translation of mRNA with a sequence complementary to sense RNA [105–107]. AS-ODNs is used to target the catalytic component of telomerase(hTERT) and are composed of short single-stranded DNA (SS-DNA) sequences that inhibits telomerase activity by complementary binding to the RNA template. AS-ODNs have been studied intensively and their structure has been modified and significantly improved over the past decade. One of the advantages of this approach is that AS-ODNs do not promote multidrug resistance mechanisms [108, 109]. Currently the most successful AS-ODNs isGRN163L. Treatment of human bladder cancer cells through AS-ODNs targeted to hTERT in vitro leads to inhibition of the proliferation of these cells [110]. siRNA is based on the ability of short double-stranded RNA molecules to formthe

RNA-induced silencing complex (RISC) which can then hybridize with specificmRNA and cleave it, thereby silencing expression [111]. This technique is especially effective for short-term hTERT knockdown because the dsRNA is degraded in the cells [112]. RNAi of hTERT has also been successful with the use of plasmid constructs that exogenously express short hairpin RNA Sequences to the hTERT transcript. This technique serves as an alternative approach to gene therapy using viral vectors and allows long-term and permanent gene knockdown [113]. The use of retroviral vectors that express short hairpin RNA specific to a segment of the hTERT transcript is also effective for long-Term knockdown of hTERT. These RNAi-based techniques can provide effective Knockdown of hTERT and involves incorporation of the anti-telomerase sequence into the host genome [114].

Inhibition of Signalling Pathways

Signalling pathways such as those carried out by MAP kinase can result in stimulation of the hTERT gene. For example, EtS and AP-1 may play a role in MAP kinase signaling to the hTERT gene, and inhibition of this pathway could be a novel approach to reducing hTERT expression and telomerase activity [115]. Our lab has also reported hTERT inhibition induced apoptosis in RCC cells (A498 and ACHN) by inhibiting MAP kinase signalling [116].

Mechanisms and Future Perspectives

Cellular self renewal is a hallmark of cancer which is regulated by telomerase reactivation. Molecular understanding of the underlying mechanism in telomere and telomerase biology along with ALT mechanism in majority of the cancers has improved remarkably in recent years. Recent insights into the regulation of telomerase activity via telomerase-shelterin interactions and by recruitment of substrate at telomerase active site, provided the opportunities for the development of effective anticancer therapeutics. There is an urgent need to better understanding of underlining molecular mechanisms regulated by hTERT activation through various genetic and epigenetic mechanism which are involved in initiation and progression of cancers targeting of these underlying molecular mechanisms will provide a ground for the development of precise, therapeutics for treatment of cancer.

Notwithstanding, hTERT promoter mutation, hTERT methylation and miRNAs targeting have gained special attention as mechanisms associated with telomerase reactivation which have been considered potentially useful for cancer therapies. Moreover, clinical trials with telomerase inhibitors certainly have established as a viable target. Therefore, there is a major challenge is to develop telomerase inhibitors that can kill rapidly telomerase positive tumor cells whilst sparing normal telomerase positive cells.

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