

Telomerase: central regulator of all of the hallmarks of cancer

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The hallmarks of cancer described by Hanahan and Weinberg are properties that cancer cells must possess for successful transformation. It is believed that each of these hallmarks is independently driven. Although elongation of telomeres is thought to be the prime function of reactivated telomerase reverse transcriptase, this activity does not account for all its effects, such as increasing cell proliferation, resistance to apoptosis, and invasion. Recent studies suggest that the telomerase subunit telomerase reverse transcriptase (TERT) has novel molecular functions including transcriptional regulation and metabolic reprogramming. We summarize these functions and discuss how they could directly regulate the various hallmarks of cancer. Finally, we suggest that therapeutics targeting noncanonical telomerase functions may work better than those that target its role in telomere extension.

Hallmarks of cancer and telomerase function

According to the Hanahan–Weinberg model [1] there are ten properties, called the hallmarks of cancer, that cancer cells must possess for successful oncogenesis: (i) sustaining proliferative signaling; (ii) evading growth suppressors; (iii) inducing angiogenesis; (iv) resisting cell death; (v) activating invasion and metastasis; (vi) tumor-promoting inflammation; (vii) evading immune destruction; (viii) reprogramming energy metabolism; (ix) genome instability; and (x) enabling replicative immortality. The hallmark enabling replicative immortality describes the ability to grow endlessly and is synonymous with reactivation of the telomerase reverse transcriptase. Unlike cancer cells, most somatic cells divide for a finite number of times that is directly proportional to the length of their telomeres [2]. Telomeres are structures at the ends of chromosomes that are composed of tandem repeats of a TTAGGG sequence. Telomeric repeats are occupied by proteins collectively termed the shelterin complex, which maintains telomere integrity [3,4]. Somatic cells stop division when their telomeres are critically short, which is known as replicative senescence [2] and is characterized by the Hayflick limit, or number of times division will occur before cell division stops [5]. Replicative senescence occurs because, during successive cell divisions, the ends of chromosomes are lost [6].

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Telomerase extends telomeres, preventing replicative senescence. Its activity is prominent in highly proliferative cells such as stem cells and germ cells, as well as in 90% of all human cancers [5,7]. Telomerase is composed of protein and RNA components; the major being the reverse transcriptase TERT and its RNA partner TERC (telomerase RNA component), which provides a template for TERT; together they constitute the telomerase reverse transcriptase activity. Besides these two components, other proteins are found to be associated with the telomerase enzyme. These include dyskerin (DKC), NOP10 ribonucleoprotein (NOP10), GAR1 ribonucleoprotein homolog (yeast) (GAR1), NHP2 ribonucleoprotein (NHP2), Reptin, and Pontin [8,9]. Unlike in germ cells or stem cells, TERT is not transcribed in most somatic cells and hence telomerase activity in these cells is low [10]. However, in cancer cells TERT is transcriptionally reactivated by the oncogenic transcription factors Myc [11], nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B) [12], and β -catenin [13]. Therefore, oncogene activation leads to telomerase expression, which overcomes replicative senescence. This newfound ability of cancer cells is entitled enabling replicative immortality in the Hanahan–Weinberg model. However, concomitant with telomerase activity, there are several new properties that cancer cells acquire when TERT is reactivated and these are exemplified by the other nine hallmarks of cancer. This review discusses these noncanonical functions of telomerase, which are largely ascribed to TERT, and suggests how it may directly regulate the various hallmarks of cancer. This understanding will be instrumental in designing drugs targeting not just cancers but also, in principle, a whole host of human ailments that are also characterized by telomerase reactivation such as atherosclerosis [14], dyskeratosis congenita [15], kidney dysfunction [16], and inflammation [17].

First evidence for telomere independent roles of telomerase components

Some of the earliest evidence for telomere-independent roles of telomerase came from mouse studies. Mice have very long telomeres (20–50 kb) compared to humans (5–10 kb), therefore, there is no need for telomerase upregulation in murine tumors to prevent telomere erosion and replicative senescence. However, it has been found that TERT is upregulated in breast [18] and skin [19] cancers in mice, suggesting that TERT could play other

roles besides maintaining telomere length. Supporting this idea, constitutive expression of TERT has been found to increase proliferation of mammary carcinomas [20] and epidermal tumors [21] in mice. Additionally, conditional induction of TERT in the mouse skin epithelium induces proliferation of hair follicle cells [22]. Overexpression of TERT in mouse thymocytes (T cells) also leads to T cell lymphomas without affecting telomere length, supporting a telomere-independent role for telomerase in mouse tumorigenesis across multiple tissue types [23].

Other evidence from human cells suggests that lengthening of telomeres may not be the only function of TERT that is necessary for transformation. For instance, the Weinberg laboratory [24] reported that human cells that could utilize a TERT-independent, alternative lengthening of telomeres (ALT) [25] pathway for maintaining telomere length were not transformed by expression of oncogenic H-Ras [24]. However, coexpression of H-Ras and a catalytically active or inactive TERT could successfully transform cells. If maintaining telomere length was the sole purpose of telomerase, then ALT should have fully substituted for this function. Further supporting a telomere-independent function of TERT, the Blackburn laboratory showed that siRNA-mediated loss of TERT, which leads to loss of telomerase activity, causes very rapid changes in cell proliferation and growth in human cancer cell lines, without any measureable change in telomere length [26]. These results suggest that the physical presence of telomerase components rather than telomerase activity also contributes to transformation. Multiple other properties of telomerase or its components have been described. For instance, TERT has been found to associate with the RNA component of mitochondrial RNA processing endoribonuclease (RMRP) and function as a RNA-dependent RNA polymerase [27]. DKC, another telomerase component, has a role in p53 biogenesis and ribosome function [28]. Besides telomerase holoenzyme assembly, Reptin and Pontin have functions in remodeling chromatin and DNA damage repair [29]. However, the molecular mechanisms by which these noncanonical activities of telomerase components contribute to oncogenesis are largely unknown. The various pieces of evidence suggest that telomerase components and TERT in particular might have other functions unrelated to their canonical role in telomere elongation, which are important in oncogenesis. We shall explore these functions in the following sections.

Telomerase and transcriptional regulation

One of the noncanonical roles of TERT is in regulation of transcription. Artandi and colleagues reported that telomerase could regulate the transcription of genes involved in cell proliferation, glycolysis, and resistance to apoptosis [30]. Park *et al.* [31] then uncovered a mechanistic link between TERT and transcription, reporting that TERT associates with the chromatin remodeler, brahma related gene (BRG)1, and that this complex activates promoters of Wnt/ β -catenin target genes such as Myc and CyclinD1 (Figure 1). Furthermore, TERT regulation of Wnt signaling was also found to be important in the development of the anterior–posterior axis in

Xenopus laevis embryos, showing that TERT exerts its noncanonical roles beyond cancer cells.

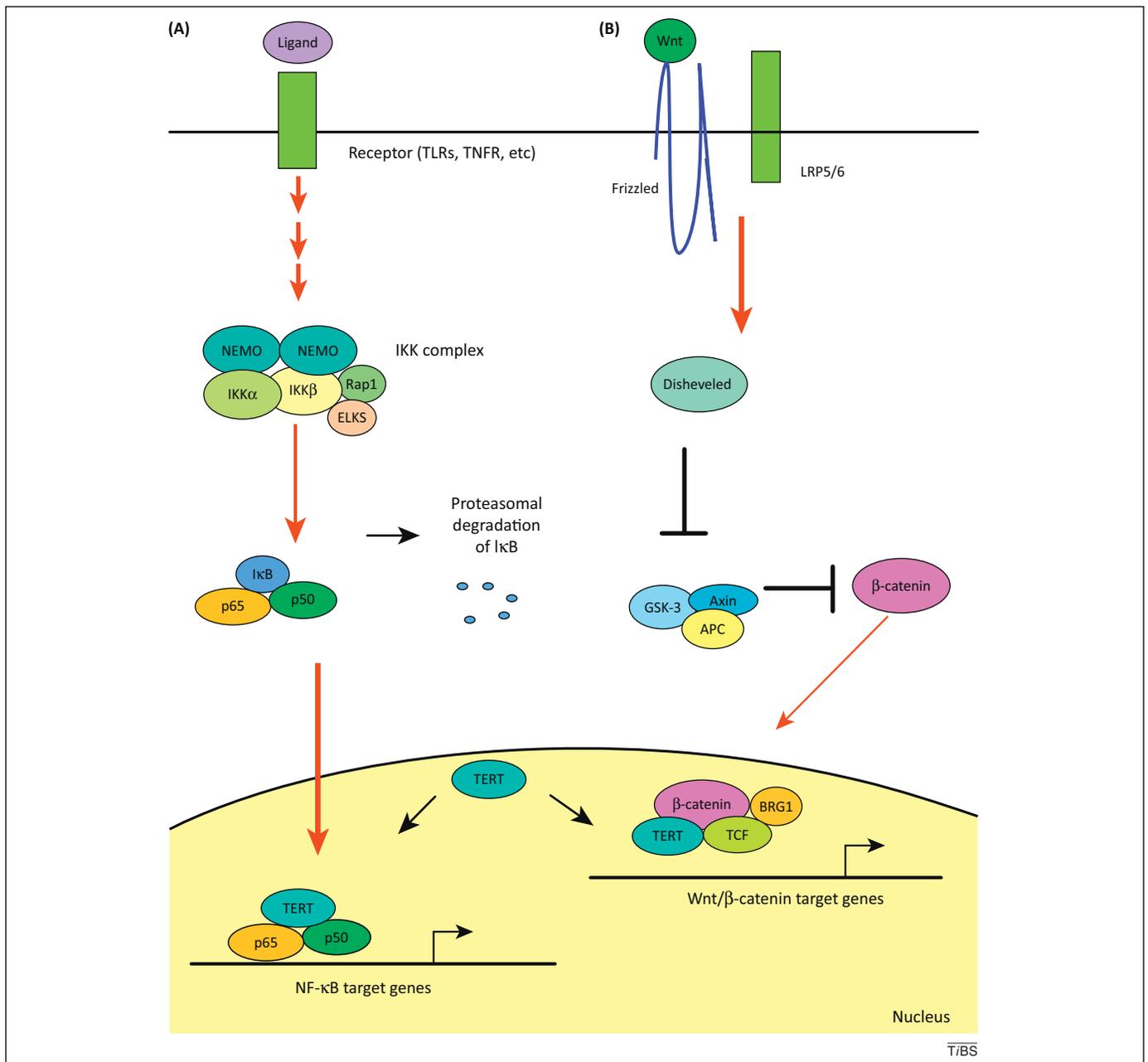
TERT can also regulate NF- κ B-dependent transcription [17]. It has been found that TERT binds the p65 subunit of NF- κ B and is recruited to and activates NF- κ B-regulated promoters such as those of interleukin (IL)-6, tumor necrosis factor (TNF) α and IL-8, which is critical for inflammation and cancer progression (Figure 1). Interestingly, NF- κ B and β -catenin are known activators of TERT expression [12,13]; and because TERT in turn regulates their transcriptional activities, a feed-forward loop therefore exists, in which the effects of these major oncogenes are amplified in a cell-autonomous manner (Figure 2). We propose that the tumor-promoting effects of TERT might therefore be a consequence of coactivation of NF- κ B and β -catenin pathways, which are master regulators of many oncogenic targets that can directly or indirectly drive various hallmarks of cancer. The NF- κ B and β -catenin pathways can also cross-talk with other oncogenic pathways such as Notch [32] and signal transducer and activator of transcription (STAT)3 [33] pathways, respectively, therefore, the effects of TERT on NF- κ B and β -catenin pathways may potentially have multiple indirect oncogenic effects. The TERT-dependent effects described are related to the various hallmarks of cancer and we explore them further in the following sections.

Telomerase and the hallmarks of cancer

Regulation of cell growth and proliferation

Expression of TERT in some human and murine cell types can cause rapid cell proliferation, which occurs without measurable changes in telomere elongation [22,30,34,35]. It has been found that human fibroblasts immortalized by overexpression of TERT secrete epiregulin, a growth factor belonging to the epidermal growth factor (EGF) family. Importantly, epiregulin neutralization reduces growth of these cells [36]. Although it was not directly tested, NF- κ B target genes such as IL-1 β and TNF- α have been shown to regulate epiregulin expression, therefore, epiregulin induction by TERT might be a consequence of NF- κ B activation [37].

Activation of EGFR signaling might also be a mechanism through which TERT stimulates proliferation. Specifically, Smith *et al.* [34] found that ectopic expression of TERT induced proliferation in human mammary epithelial cells (HMECs) through increased EGFR signaling. Interestingly, there is some evidence that the ability to elongate telomeres is not required for induction of proliferation by TERT. For instance, telomerase induction can cause proliferation of hair follicle cells in mice and this does not require TERC/telomerase activity [22]. Supporting and expanding on these data, Choi *et al.* [30] more recently found that a catalytically inactive TERT could promote epithelial proliferation in hair follicle and skin cells through the Wnt and Myc signaling pathways. Related to these studies, Hrdličková *et al.* [35] found that alternatively spliced variants of TERT that cannot elongate telomeres, but are able to stimulate Wnt signaling, can increase human cell proliferation. Overall, these studies show that TERT can regulate cell growth and proliferation, independent of its role on telomeres.



TIBS

Figure 1. TERT and transcriptional regulation. **(A)** TERT cooperates with nuclear factor (NF)- κ B to regulate its target genes. In canonical NF- κ B signaling, a ligand (e.g., lipopolysaccharide or TNF- α) binds to its cognate receptor which, through a series of steps, leads to activation of the IKK complex. The IKK complex consists of the IKK1/2 kinases, NEMO the chaperone, and adapters such as ELKS and Rap1. Activated IKK phosphorylates I κ B, leading to its degradation by the proteasome, which allows the NF- κ B dimer (p65-p50) to enter the nucleus and activate transcription of its target genes. TERT has been shown to bind p65 subunit of NF- κ B in the nucleus and enhance the transcription of its target genes. **(B)** TERT cooperates with Wnt to regulate its target genes. In the canonical Wnt signaling, Wnt binds Frizzled and LRP5/6, which activates Dishevelled. Dishevelled inhibits a complex containing GSK-3, Axin and APC, allowing β -catenin to be able to enter the nucleus and activate the Wnt target genes. TERT has been shown to bind BRG1, a chromatin remodeler and enhance the transcription of Wnt target genes. Abbreviations: APC, Adenomatous polyposis coli; BRG1, brahma related gene 1; ELKS, a protein rich in protein rich in glutamate (E), leucine (L), lysine (K), and serine (S); GSK-3, glycogen synthase kinase 3; I κ B, inhibitor of κ B; IKK, inhibitor of NF- κ B; LRP, low-density lipoprotein receptor-related protein; NEMO, NF- κ B essential modulator; NF- κ B, nuclear factor κ -light-chain-enhancer of activated B cells; Rap1, repressor-activator protein 1; TERT, telomerase reverse transcriptase; TNF- α , tumor necrosis factor- α .

Resistance to antigrowth signals

Transforming growth factor (TGF)- β is a cytokine that inhibits the growth of nonmalignant epithelial cells and hematopoietic precursor cells, but many malignant cells become refractory to its antigrowth effects [38]. Ectopic expression of TERT in HMECs and mouse embryonic fibroblasts (MEFs) antagonizes TGF- β -dependent growth suppression [39,40]. Furthermore, ectopic expression of TERT in HMECs also downregulates the expression of antigrowth factors such as IL-1 receptor (IL-1R) antagonist and parathyroid hormone-related peptide, suggesting that

TERT might help cancer cells bypass antigrowth signals through a general mechanism [34].

TERT might also confer resistance to antigrowth signals by causing cells to bypass differentiation, another strategy that cancer cells utilize to continue to proliferate [41]. Oncogenic Myc and Wnt/ β -catenin signaling, which TERT can positively regulate, can bypass differentiation [42,43]. Indeed, telomerase-mediated activation of Wnt signaling [31] was shown to suppress differentiation and induce proliferation. Furthermore, β -catenin could transcriptionally upregulate Myc expression by binding to β -catenin

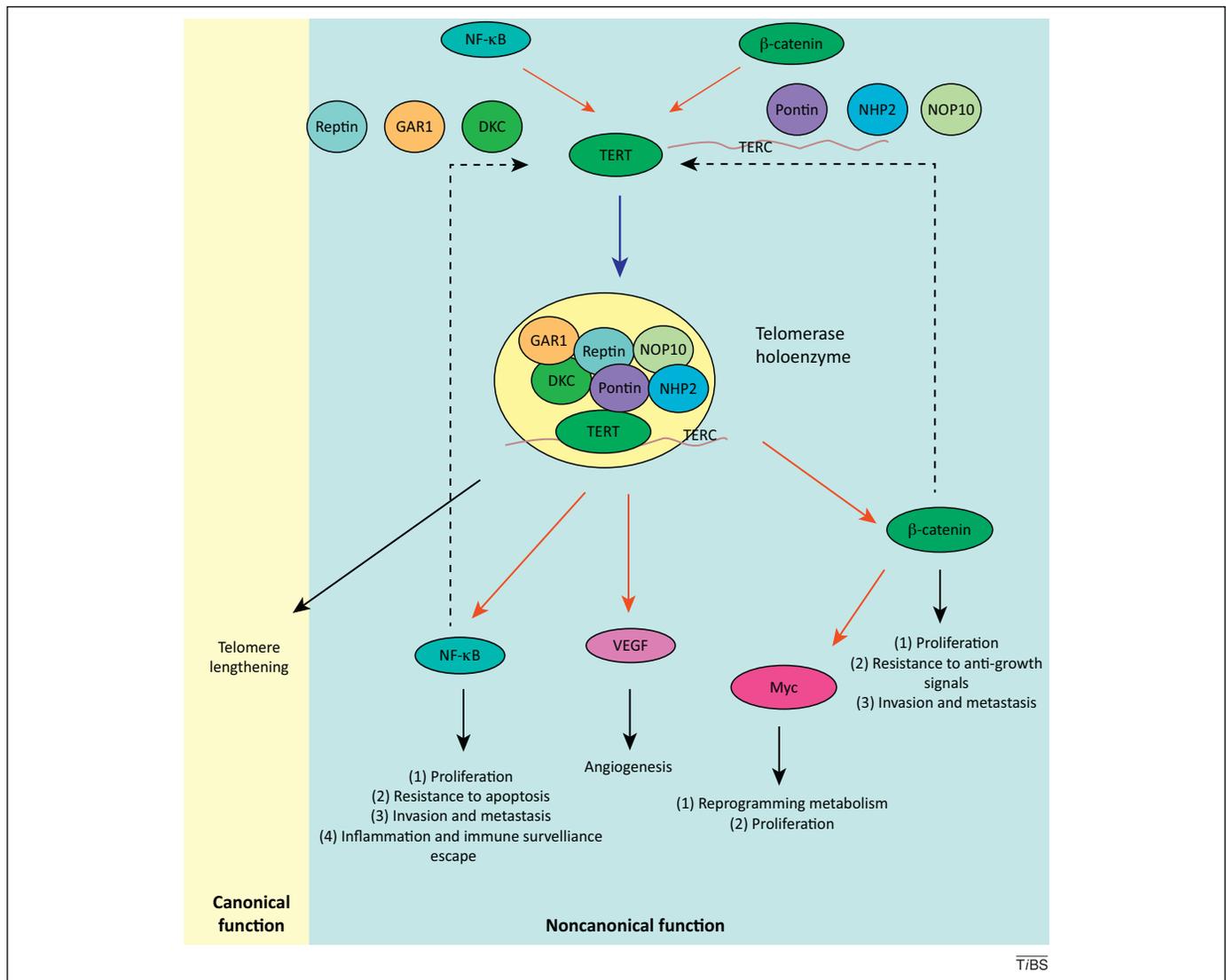


Figure 2. Feed forward loop of TERT expression. NF- κ B and β -catenin regulate TERT expression. Reconstitution of TERT along with other telomerase components reconstitutes the telomerase reverse transcriptase activity but this complex, or members of this complex, also show other noncanonical activities as depicted. TERT in turn regulates the expression of NF- κ B and β -catenin target genes to maintain itself, forming a feed forward loop. Other oncogenes of these pathways are induced as a byproduct of this feed forward loop. Key: unbroken red arrow: activation; unbroken blue arrow: complex formation; unbroken black arrow: induction of hallmark of cancer; broken black arrow: feedback loop to induce TERT. Abbreviations: DKC, dyskerin; GAR1, GAR 1 ribonucleoprotein homolog (yeast); NF- κ B, nuclear factor κ -light-chain-enhancer of activated B cells; NHP2, NHP2 ribonucleoprotein; NOP10, NOP10 ribonucleoprotein; TERT, telomerase reverse transcriptase; TERC, telomerase RNA component; VEGF, vascular endothelial growth factor.

binding sites in the Myc promoter, potentially amplifying TERT-dependent suppression of differentiation [44]. Importantly, overexpression of an enzymatically inactive TERT was shown to inhibit differentiation and induce proliferation of glioma cells through upregulation of EGFR and basic fibroblast growth factor (bFGF), supporting the hypothesis that this is a telomere-independent effect of TERT [45]. Taken together, TERT seems to favor tumor growth by inhibiting antigrowth signals and suppressing differentiation, perhaps independent of its role on telomeres.

Angiogenesis

TERT has been reported to modulate angiogenesis as well. TERT knockdown reduces, whereas TERT upregulation enhances, the formation of microvessels in a mouse model of angiogenesis [46]. Compared to controls, xenografted glioblastoma cells transfected with siRNA against TERT show reduced production of proangiogenic factors such as vascular endothelial growth factor (VEGF) and bFGF, and

show reduced formation of microvessels [47,48]. This effect of TERT, which is independent of its catalytic activity, could be due to direct activation of VEGF transcription [49]. Furthermore, VEGF and acidic/basic fibroblast growth factor (a/bFGF) have been shown to activate the TERT promoter via the phosphoinositide 3-kinase (PI3K/Akt), nitric oxide [50], and extracellular signal-regulated kinase (Erk)1/2 pathways [51]. Taken together, these studies suggest an autocrine feedback loop by which TERT promotes angiogenesis by inducing the production of angiogenic factors such as VEGF and a/bFGF, which in turn can upregulate the transcription of TERT and reinforce angiogenic factor expression.

Resistance to apoptosis

Cancer cells with reactivated TERT gain resistance to death-inducing stimuli through activation of NF- κ B, which is a master regulator of survival genes [17,52]. Furthermore, TERT can localize to mitochondria [53]

and inhibit caspase-mediated apoptosis in cancer cells [54] independent of its function in telomere elongation [55]. It has also been suggested that mitochondrial TERT can increase intracellular reduced glutathione/oxidized glutathione ratios, thus increasing the cellular antioxidant buffering capacity and reducing intracellular reactive oxygen species (ROS)-induced apoptosis [56]. These disparate studies suggest that TERT can inhibit apoptosis through various mechanisms, while activating cell proliferation and inhibiting antigrowth signals to have a net pro-growth effect.

Invasion and metastasis

Liu *et al.* [57] have shown that TERT overexpression promotes the epithelial–mesenchymal transition (EMT), whereas its inhibition decreases it. This is achieved through TERT-mediated Wnt/ β -catenin signaling, which then upregulates Snail-1 (snail family zinc finger 1) and vimentin; important EMT markers. This study shows that TERT binds to β -catenin directly and localizes to vimentin promoters, reiterating its role as a transcriptional coactivator. Other indirect evidence for TERT involvement in invasion and metastasis is through its enhancement of NF- κ B signaling. NF- κ B has been shown to be critical for EMT [58] and it also upregulates matrix metalloproteinase (MMP)-9, which is important for metastasis [59]. Taken together these results suggest that TERT could play a role in invasion and metastasis as well.

Inflammation and immune surveillance

Key inflammatory cytokines such as IL-6, IL-8, and TNF- α have been shown to be direct targets of TERT [17]. Inflammation can upregulate growth factors, survival factors, promote angiogenesis, and induce EMT [1]. IL-6 has been shown to be important in promoting various aspects of cancer progression [60–62] and in the development of chemoresistant niches [63]. IL-8 and TNF- α have also been implicated in proliferation, invasion, metastasis, and inhibition of apoptosis in cervical [64] as well as in breast [65,66] cancers. These and other cytokines thus link inflammation to various hallmarks of cancer [67–69]. However, chronic inflammation can also lead to the formation of an immunosuppressive environment [70–72]. In fact, IL-6 is a key cytokine in the chronic inflammatory environment that can cause immunosuppressive effects [73]. Importantly, as mentioned earlier, these cytokines activate NF- κ B, which is both a positive regulator of TERT and is positively regulated by TERT, suggesting multiple TERT-dependent mechanisms may reinforce inflammatory signaling [17,74,75]. Based on this evidence, TERT might directly and indirectly regulate chronic inflammation and immunosuppression.

Reprogramming energy metabolism

Several genes in the glycolytic pathway were shown to be downregulated by TERT knockdown [76]. There was a corresponding decrease in glucose consumption and lactate production, which are characteristics of the Warburg effect, upon TERT knockdown, showing that TERT can directly regulate metabolism in cancer cells. TERT might also regulate energy metabolism via β -catenin, which in turn regulates Myc [44]. Myc is well known to be a major

player in reprogramming energy metabolism in cancer cells [77,78]. In fact, it was found that the TERT transcriptional response is highly similar to that of Wnt and Myc in follicular cells [30]. Lastly, it was found that NF- κ B can upregulate glucose uptake via Glut-3 and increase the rate of aerobic glycolysis [79]. TERT can regulate NF- κ B expression, therefore, this could possibly link TERT to glycolysis via the NF- κ B pathway.

Genome instability

Although TERT enhances the other nine hallmarks of cancer, which are all protumorigenic, TERT suppresses the hallmark of genome instability and reduces mutation, which can be viewed as both a tumor-suppressive effect as well as an oncogenic effect. TERT promotes genome stability through multiple means. First, the canonical function of telomerase is to maintain telomere ends, without which telomere erosion ensues. This then leads to unstable chromosomes, which increases the rate of deletions or amplifications of chromosomal segments [80]. Second, it has been found that TERT can regulate DNA damage signaling through ataxia telangiectasia mutated (ATM), breast cancer 1 (BRCA1), and γ -H2A histone family, member X (γ -H2AX) [81]; thus, knock-down of TERT alters histone modifications and decreases heterochromatin, stifling the DNA damage response and resulting in genome instability. Third, TERT localization to the mitochondria can protect against DNA damage in the nucleus by decreasing mitochondrial ROS production,

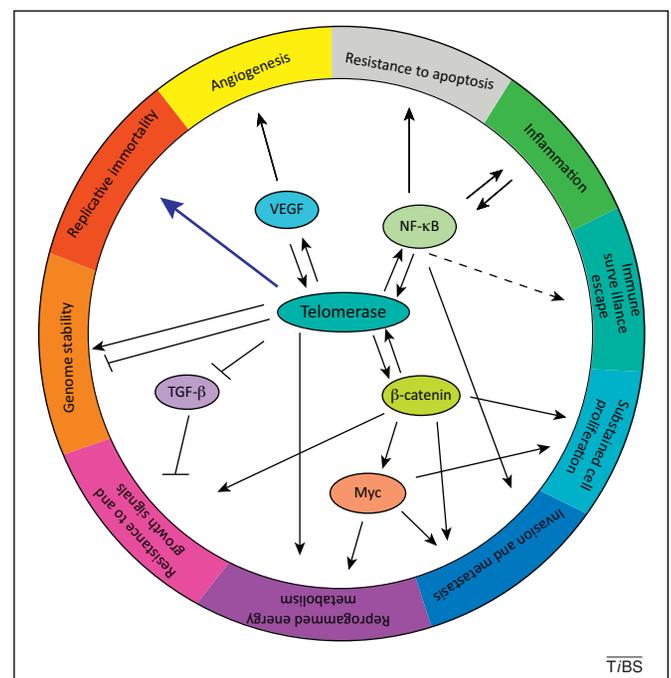


Figure 3. Regulation of the hallmarks of cancer by telomerase. TERT regulates the various hallmarks of cancer through its enhancement of transcription of VEGF, NF- κ B and β -catenin target genes. β -Catenin also activates Myc. TERT also suppresses TGF- β -dependent growth suppression. Telomerase also antagonizes or suppresses genome instability, in contrast to the other effects, and that this might be through a transcription-independent mechanism (please see text for details). Arrows not drawn to scale. NF- κ B, nuclear factor κ -light-chain-enhancer of activated B cells; TGF- β , transforming growth factor- β ; VEGF, vascular endothelial growth factor. (—>: Direct activation ———>: Indirect activation. —|: Inhibition. <—>: Two-way activation. —>: Canonical telomerase activity.)

which is one of the telomere-independent functions of TERT [53]. Thus, telomerase can act as a tumor suppressor by ensuring genome stability and inhibiting mutations.

However, maintaining genome stability can be oncogenic as well. This is because telomere shortening due to TERT inhibition leads to apoptosis [82] through a p53-dependent pathway [83]. Thus, maintaining genome stability can be viewed as an equilibrium where too little mutation inhibits tumor progression, whereas too much mutation causes genome catastrophe, leading to cell cycle arrest or cell death. Figure 3 summarizes the various potential mechanisms by which the telomerase component TERT in particular could regulate the hallmarks of cancer. It is not known if TERT acts alone in these functions or if the telomerase holoenzyme could participate in some of these functions assayed for and ascribed to TERT.

Therapeutic targeting of telomerase

The preceding sections have summarized how telomerase regulates the various hallmarks of cancer; that is, mainly through TERT acting as a cofactor to regulate NF- κ B and β -catenin signaling pathways. These findings have therapeutic implications for the design of inhibitors that can target human ailments such as cancers, which show

reactivation of TERT. For this we would need to delineate which noncanonical activities of telomerase require which components of the telomerase complex besides TERT. Such a molecular dissection will require precise genetic and biochemical assays and the understanding that TERT reactivation not only induces telomerase activity, but can also regulate other signaling cascades that directly or indirectly regulate all of the hallmarks of cancer.

An important strategy to develop effective therapeutics would be to test the drugability of the interactions between TERT and its partner transcription and growth factors because TERT is not the only crucial regulator controlling these hallmarks. A putative drug that blocks such an interaction would in principle simultaneously target many of the hallmarks of cancer. Furthermore, because such a drug will only block sustained oncogenic signaling in cancer cells, and not normal signaling in noncancerous cells, such a drug will be preferable to those that simply block these master oncogenic regulators that have been unsuccessful due to obvious toxicity issues. Numerous chemical inhibitors of telomere extension have been developed, such as the telomerase inhibitors MST-312 [84] and BIBR1532, a non-nucleoside analog [85]; G-quadruplex stabilizers such as β -rubromycin [86], PIPER {N,N'-bis [2-(1-piperidino)ethyl]-3,4,9,10-tetracarboxylic diimide} [87], and TmPyP4 [88]; and general reverse transcriptase inhibitors

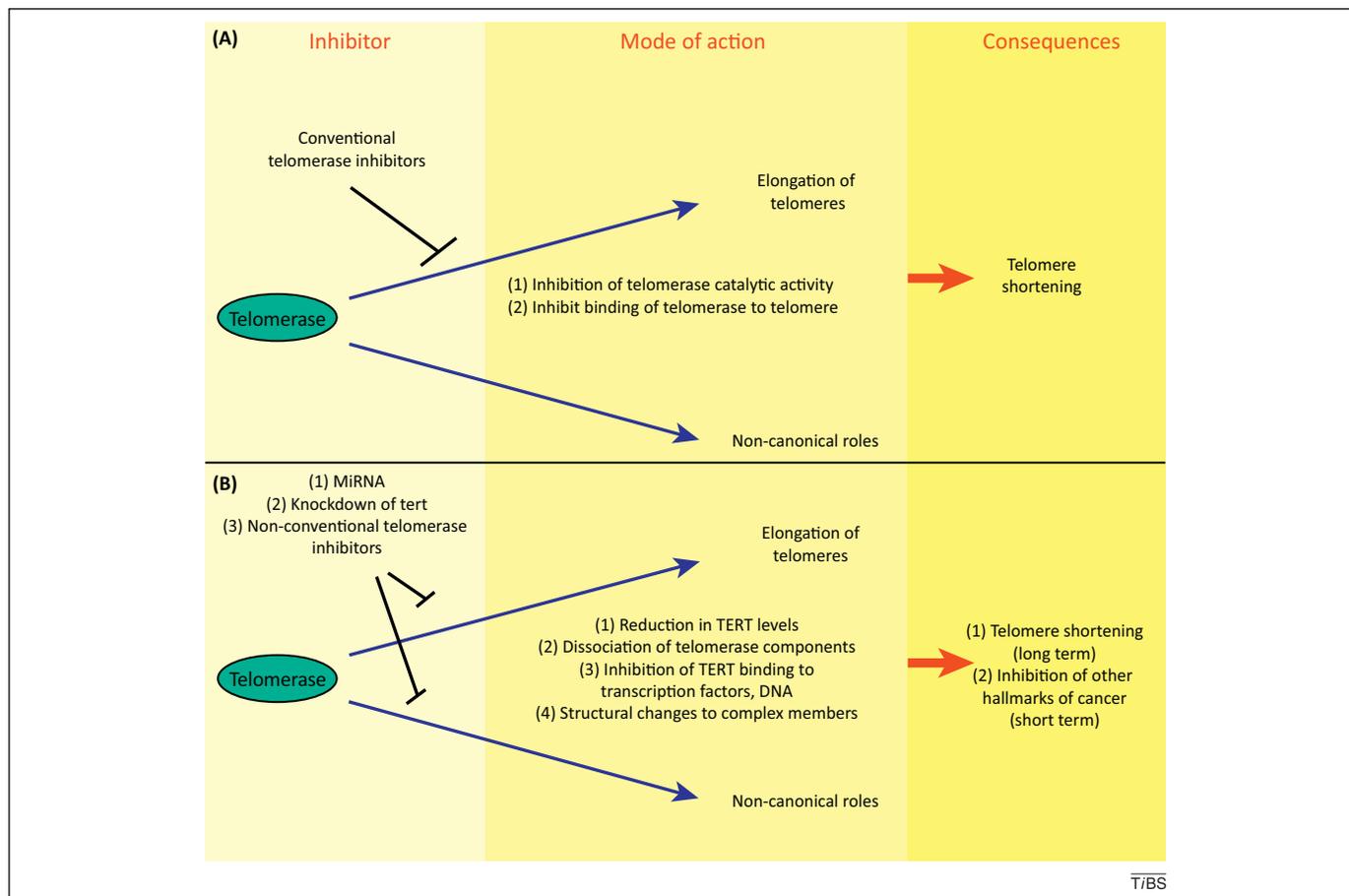


Figure 4. Therapeutics against telomerase. (A) Conventional telomerase inhibitors inhibit the canonical roles of telomerase by negating its catalytic activity or inhibiting its binding to telomere ends, leading to shortening of telomeres, but do not inhibit its noncanonical roles. (B) Cell proliferation, metastasis, and telomere lengthening might be better controlled through the use of miRNAs that downregulate TERT concurrently with other oncogenes, nonconventional telomerase inhibitors that affect its noncanonical roles, and/or drugs that knockdown telomerase levels. These inhibitors downregulate TERT levels, dissociate telomerase complex or inhibit its interaction with transcription factors.

such as AZT (azidothymidine), which acts on the reverse transcriptase activity of TERT [89]. Another way to inhibit telomerase function is to reduce levels of TERT transcripts. In fact, one such telomerase inhibitor, imetelstat (GRN163L), demonstrated activity in various cancers and has entered Phase I and II clinical trials [90]. It also depleted cancer stem cells in breast and pancreatic cancer cell lines without affecting telomere length, suggesting that imetelstat can target noncanonical functions of telomerase [91]. Supporting this strategy, siRNA against TERT can induce apoptosis and cause growth inhibition, independent of p53 or telomere elongation [26]. This was subsequently found to be due to suppression of target genes involved in angiogenesis and metastasis, showing that siRNA against TERT can inhibit its noncanonical functions [92]. Also, expression of ribozymes that target TERT can cause cell death within a short time of treatment, independently of telomere shortening, showing that ribozymes most probably inhibit the noncanonical functions of TERT [93]. miRNAs have been found that can target telomerase as well as other oncogenes, thus miRNA delivery might be another means of reducing telomerase expression. Pharmacological approaches have been developed that can deliver miRNAs such as miR-138 and 196b, which target telomerase, to cells [94–97]. Finally, human interacting protein X1 (PinX1) was found to be a potent tumor suppressor that can downregulate the transcript levels of TERT [98]. In summary, several approaches of either single or combinatorial treatment could be refined and developed further to target TERT in particular and telomerase components in general for treatment of various human ailments.

Immunotherapy is also an attractive therapeutic option because telomerase is primarily active in cancer cells. To generate a telomerase-targeted immunotherapy, TERT is presented as a HLA class I antigen to cytotoxic T cells (CD8⁺) to activate them, which leads to lysis of cancer cells. Through this method, up to 26 different TERT peptides have been generated so far that could elicit an effective antitumor response [90]. Besides cytotoxic CD8⁺ T cells, CD4⁺ T cells have been shown to play an important role in telomerase-based immunotherapy [99]. Lastly, dendritic cells (DCs) can be transfected with mRNAs encoding telomerase component proteins, which can then stimulate CD8⁺ and CD4⁺ T cell responses to kill cancer cells [100]. Pitfalls of these strategies would be that stem cells and germ cells that express TERT could be affected, because these express TERT as well.

Taken together, simultaneous targeting of both the canonical and noncanonical functions of telomerase might be more effective against cancer than targeting only the canonical function of telomerase (Figure 4). It is clear that telomerase can directly regulate the various hallmarks of cancer through its canonical and noncanonical functions. Further understanding of the telomerase complex would prove valuable in designing therapeutics that could selectively disrupt its oncogenic signaling, while leaving its activity intact in normal stem cells and germ cells, which require continuous telomere replenishment. Although making stem cell and germ cell telomerase activities refractory to these inhibitors will

pose a challenge, such inhibitors will inevitably possess fewer toxic side effects compared to current telomerase inhibitors.

Concluding remarks

In this review, we have summarized the existing literature that suggests how telomerase in general and its TERT subunit in particular can regulate the various hallmarks of cancer. We suggest that co-regulation of NF- κ B and β -catenin signaling pathways by TERT could be the key mechanistic basis for the noncanonical functions of TERT, which impart its ability to regulate the hallmarks of cancer. It is important to note that the regulation of the various hallmarks of cancer by TERT should not be viewed as the sole function of either its canonical or its noncanonical activities. This is because telomere lengthening by telomerase is also critical for overcoming senescence, whereas the other noncanonical functions enable cancer cells to survive, proliferate, spread, and metabolically reprogram in the cancer milieu. Thus, telomerase inhibitors that target the canonical and noncanonical roles of TERT could prove useful in a combinatorial drug treatment because these would target many hallmarks of cancer at once, besides just affecting only the replicative immortality hallmark.

Acknowledgments

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