

Mini review

## TELOMERASE AND ITS EXTRACURRICULAR ACTIVITIES

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**Abstract:** The classical activity of telomerase is to synthesize telomeric repeats and thus maintain telomere length, which in turn ensures chromosome stability and cellular proliferation. However, there is growing evidence that implicates telomerase in many other functions that are independent of TERC being used as its template. Telomerase has an RNA-dependent RNA polymerase (RdRP) activity in the mitochondria. Other than viral RdRPs, it is the only RNA-dependent RNA polymerase that has been identified in mammals. It also plays a role in the Wnt signaling pathway by acting as a transcriptional modulator. Telomerase acts as a reverse transcriptase independent of its core subunit, TERC. Studies indicate that telomerase is also involved in apoptosis and DNA repair.

**Key words:** Telomerase, Telomere, RNA-dependent RNA polymerase, TERT, TERC, Apoptosis

## INTRODUCTION

In evolutionary terms, the multiple linear chromosomes in eukaryotes are at a great selective advantage, because they permit a high rate of recombination involving random reciprocal translocation of chromosomal DNA during sexual

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Abbreviations used: BRG1 – Brahma-related gene 1; PIP1 – POT1-interacting protein 1; PTOP – POT1- and TIN2-organizing protein; RAP1 – repressor and activator protein 1; RdRP – RNA-dependent RNA polymerase; SMARCA4 – SWI/SNF-related matrix-associated actin-dependent regulator of chromatin, subfamily a, member 4; SWI/SNF – SWItch/sucrose non-fermentable; TEP1 – telomerase-associated protein 1; TINT1 – TIN2 Interacting Protein 1; TIN2 or TIN2 – TRF1-interacting nuclear protein 2; TRF1 or TERF1 – telomeric repeat-binding factor 1; TRF2 or TERF2 – telomeric repeat-binding factor 2

reproduction. The presence of linear chromosomes has two negative aspects: the difficulty with replication of the extreme ends; and the need to protect the ends from degradation and fusion with other chromosomes. DNA-dependent DNA polymerase is unable to copy the 3' end of the template, which means that the copy strand shortens with each cycle of DNA replication, while the 3' end of leading strand is continuously synthesized by conventional DNA-dependent DNA polymerase until the end of the template. This means that the terminal DNA gets shorter through successive cycles of replication, which eventually leads to crisis and loss of cell viability [1, 2].

The ends of linear chromosomes are made up of multiple repeats of a short sequence known as the telomere, which is usually G/T-rich and heterochromatic and thus transcriptionally inactive. Telomeres are DNA-protein complexes with repeats that vary widely between species: ciliate *Oxytricha nova* has 4.5 repeats of G<sub>4</sub>T<sub>4</sub>, while *Saccharomyces cerevisiae* has around 500 bp of telomeric repeats [3]. Human telomeres are made up of 5'-TTAGGG-3' repeat sequences that are bound by a protein complex known as the shelterin complex [3]. Together with telomeric DNA, this protein complex forms a cap-like structure consisting of six proteins: (i) telomeric repeat-binding factor 1 (TRF1 or TERF1); (ii) telomeric repeat-binding factor 2 (TRF2 or TERF2); (iii) TRF1-interacting nuclear protein 2 (TIN2 or TIN2F2); (iv) TPP1 (also referred to as TINT1, PTP1 or PIP1; nomenclature provided by 3 independent research groups that each reported the protein under a different name [4-6]); (v) protection of telomerase 1 (POT1); and (vi) repressor and activator protein 1 (RAP1), which caps the end of the chromosome, protecting it from degradation and chromosomal fusion [7]. Humans have approximately 10-15 kb of telomeric DNA, while mice have 20-50 kb of telomeric repeats. Most of this telomeric DNA is double stranded but also contains a single-stranded region of several hundred base pairs which is always G-rich [8-11]. This single-stranded region basically plays two very important roles: it facilitates extension of the telomere by providing primers to telomerase, and it binds with different components of the shelterin complex to form a cap at the end of the chromosome.

## TELOMERASE STRUCTURE AND FUNCTIONS

Cells have evolved a specialized reverse transcriptase called telomerase, which solves the end replication problem by adding telomeric repeats onto the ends of newly replicated chromosomes. Greider and Blackburn were awarded the Nobel Prize in Physiology and Medicine in 2009 for their discovery of telomerase in *Tetrahymena thermophila* [12]. Almost 90% of cancerous cells have telomerase activity, while most normal differentiated somatic cells do not [13].

Telomerase is a ribonucleoprotein complex consisting of at least six subunits: human telomerase RNA component (hTERC); heat shock protein 90 (hsp90); human telomerase reverse transcriptase (hTERT); telomerase-associated protein 1 (TEP1); p23; and dyskerin [14]. hTERC and hTERT are the core subunits and

their *in vitro* reconstitution leads to telomerase activity. hTERT is a rate-limiting component of telomerase. During up- or downregulation of telomerase, only hTERT expression changes proportionately with the level of telomerase activity, while the levels of the rest of the components remain unchanged and high throughout the modulation [14].

### **TELOMERASE RNA COMPONENT (TERC)**

TERC acts as a template for telomeric DNA synthesis [15], and its size varies between species. *T. thermophila* has short TERC (159 nucleotides), while mammals have TERCs of intermediate length (murine TERC has 397 nucleotides and human TERC has 451 nucleotides) [16]. Human TERC is encoded by a single copy gene located on the long arm of chromosome 3 (3q26.3) [17]. TERC is one of the factors influencing the processivity of telomerase. It facilitates several rounds of addition of DNA after only one primer binding step [18, 19]. Telomerase RNA shows divergence of the primary sequences between species, but it also has a remarkably conserved secondary structure in a variety of vertebrate species, which indicates that RNA structure plays a very important role in telomerase activity [20].

The 3' end of TERC in vertebrates differs from that in single-cell eukaryotes – certain sequence motifs are absent in the latter [21]. This region of human TERC contains an H/ACA sequence motif that forms a specific class of non-coding RNA [22, 23] that facilitates the modification of other cellular RNA. On the basis of localization, H/ACA RNA can be divided into two groups. The H/ACA RNA that accumulates in the nucleolus is known as H/ACA small nucleolar RNA (snoRNA) [24]. It takes part in the modification of ribosomal RNA. The H/ACA RNA that accumulates in the Cajal bodies and takes part in the modification of splicing RNA is known as small Cajal body-specific RNA (scaRNA) [25]. Another sequence motif, known as the Cajal body box or CAB box, is responsible for the difference in cellular localization of H/ACA snoRNA and H/ACA scaRNA.

The conserved domain in the hTERC molecule is the binding site for many hTERC-binding proteins that recognize TERC specifically. There are many RNA-binding proteins that interact with hTERC, such as La, hnRNP C1/C1, dyskerin, hStau, L22, hGAR, hNOP10, hTERT and hNHP2 [26, 27]. These RNA protein interactions are involved in hTERC accumulation, stability and maturation, and assembly of the telomerase in a functional form. Accumulation of TERC has been seen in human cancer cells, mainly within the Cajal bodies via RNA fluorescence *in situ* hybridization [28, 29].

Of the TERC-binding proteins, dyskerin is an enzyme – a pseudouridine synthetase. It is required for H/ACA RNA stability, which it achieves by interacting with three small associated proteins: GAR1, NHP2 and NOP10. This enzyme complex is directed to its complementary RNA, i.e., ribosomal RNA and splicing RNA, by H/ACA snoRNA and H/ACA scaRNA, respectively. The

isomerization of uridine to pseudouridine is mediated by the dyskerin complex. These modifications are required for the proper function of these target RNA. The template region is included in the pseudoknot and together makes up the core domain. The core domain and the CR4/CR5 domain independently bind to hTERT. The H/ACA scaRNA domain (including the CR7 domain) binds to the RNP proteins (dyskerin, Gar1, Nop10, and Nhp2) as well as to telomerase Cajal body protein (TCAB1)/WD Repeat domain 79 [30].

### TELOMERASE REVERSE TRANSCRIPTASE (TERT)

The TERT subunit of telomerase was initially isolated biochemically as p123 from *Euplotes aediculatus* [31]. TERT, an RNA-dependent DNA polymerase, is also a core subunit of telomerase, and it uses its own RNA template for DNA synthesis (Table 1). The gene that codes TERT is located on chromosome 5 (5p15.33). The cDNA and genomic sequence of hTERT (human telomerase reverse transcriptase) revealed that the hTERT gene spans more than 37 kb and contains 15 introns and 16 exons that code for 1132 amino acid residues [32]. The TERT subunit of

Table 1. Classical and extracurricular activities of telomerase

Functions	Description	Dependence on catalytic activity of TERT	Reference
Nucleus			
Regulation of gene expression	Acts as a transcriptional modulator	-	[61]
Chromatin organization	Regulates the DNA damage response pathway through its action on chromatin structure	-	[47]
Maintenance of telomere	Acts as a reverse transcriptase by using TERC as a template	+	[80]
Enhancement of cell growth	Affects the expression of growth-promoting genes	-	[81]
DNA repair	Associated with primase, a protein involved in DNA repair; increases the expression level of genes involved in DNA damage response and also physically associated with many DNA repair proteins	+	[77-79]
Mitochondria			
In apoptosis	Sensitizes cells to oxidative stress which can cause apoptotic	-	[69]
RNA-dependent RNA polymerase activity	Gene silencing	+	[65]

telomerase is conserved in humans (hTERT), *Schizosaccharomyces pombe* (Trt1), *Saccharomyces cerevisiae* (Est2) and protozoans [33, 34].

The 3D structure of the TERT protein from *Tribolium castaneum* was solved in 2008 by Emmanuel Skordelakes *et al.* at the Wistar institute in Philadelphia [35, 36]. The protein consists of four conserved domains – the RNA-binding domain (TRBD) and the palm, finger and thumb – which organize in a closed-ring tertiary structure with a larger cavity at the center. It is large enough to bind with the primer-template duplex [35].

Structurally and functionally, the TERT protein can be subdivided into three major domains: the reverse transcriptase domain, which contains the finger, palm and thumb subdomains, which may play a role in nucleotide addition and processivity; the telomerase essential N-terminal domain, consisting of 400 amino acid residues; and the TERT RNA-binding domain, which has high affinity for hTERC. The TERT subunit shows significant conservation with the reverse transcriptase enzyme from retroviruses. Inactivation of this catalytic subunit causes loss of telomerase activity.

The expression level of hTERC depends on the telomerase activity in cells and is detectable in all tissues. Cancer cells generally have five times more expression than normal cells, even though only 1 to 5 copies of TERT (mRNA) are found per cancer cell. The level of expression of TERT is low in normal cells and high in immortal cells, showing that TERT rather than TERC characterizes the immortalized cells.

### **TELOMERE-INDEPENDENT FUNCTION OF TELOMERASE**

Telomerase is a well-known enzyme that maintains the length of the telomere and the physical ends of the eukaryotic chromosome in embryonic stem cells and cancer cells. Telomerase activation extends the lifespan of cells in culture by maintaining the length of the telomere. It has now become clear that the role of telomerase is much more complex than just telomere lengthening. Telomerase influences normal cellular physiology, even in cells that contain long telomeres. Due to its key role in telomere lengthening, alteration in telomerase expression is associated with many degenerative diseases, aging and cancer-related functions. The role of telomerase in cellular immortalization and that of telomere shortening in cellular senescence has been demonstrated by cloning and expression of the TERT gene [37]. Indeed, increased incidence of spontaneous tumors has been found in many independent TERT-transgenic mouse models with constitutive expression of telomerase [38, 39]. With oncogenic stress, the proliferative rate of the cells is increased many fold, and the length of the telomere is a factor which limits cell division capacity. The telomere is shorter in cancer cells than it is in normal cells [40].

Replicative senescence (also known as the Hayflick limit or mortality stage I) is the first cellular response to occur at the time of telomere attrition, and its induction needs the proper action of p53 and RB tumor suppressor pathways [41].

Inactivation of these two tumor suppressor pathways extends the replicative potential of the cell, ultimately leading to continuous telomere erosion and loss of telomere capping. Uncapped telomeres are highly recombinogenic, which leads to the formation of dicentric chromosomes and breakage at the time of cell division. They are also prone to a high degree of genomic instability and loss of cell viability during this period of crisis [42].

Of all the cells that undergo crisis, only the  $10^{-7}$  to  $10^{-5}$  fraction emerges from crisis [43], perhaps accompanying enforced expression of hTERT and activation of telomerase, which helps to avoid both senescence and crisis in primary cultured cells. It also causes transformation of primary human cells by its coexpression with SV40 early genes and H-RAS [44, 45]. Thus, activating telomerase averts the crisis by capping the telomere and reducing the frequency of dicentric and abnormal chromosomes [44]. Without performing telomere lengthening, stabilization of the telomere can also occur in a TERT-mediated, telomere capping-dependent manner, which increases cellular lifespan [44, 46].

Telomerase can play a role in modulation of chromatin structure and response to DNA damage [47]. TERT is also known to induce the expression of pro-proliferative genes and inhibit that of anti-proliferative genes. This promotes cell growth and proliferation independent of telomere elongation [48, 49].

#### **REGULATION OF GENE EXPRESSION BY TELOMERASE**

TERT depletion in mouse skin results in a genome-wide transcriptional response in genes involved in signal transduction, epithelial development, the cytoskeleton and adhesion [50 and our unpublished results]. This resembles the transcriptional program regulated by Wnt, a well known player in stem cell maintenance, cellular transformation and proliferation [51-55]. TERT acts as a transcription factor in  $\beta$ -catenin complexes. It is only directly involved in the modulation of the canonical Wnt pathway; non-canonical Wnt pathways that do not involve the  $\beta$ -catenin complex are not regulated by TERT. Wnt ligands bind with Wnt receptor(s) (the Frizzled family of transmembrane receptors) [55] and the LRP5/6 coreceptor (a low-density lipoprotein receptor-related protein) (Fig. 1) [56]. The coreceptor facilitates the interaction between the Wnt receptor and its ligand. The interaction leads to activation of a cytoplasmic phosphoprotein, Dishevelled (Dvl), which inhibits the activity of glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ), which degrades the  $\beta$ -catenin. Wnt signaling allows accumulation of  $\beta$ -catenin, which then translocates into the nucleus and forms a complex with TERT-BRG1 (Fig. 1). The BRG1 is also known as SMARCA4, a SWI/SNF-related chromatin-remodeling protein that binds to the  $\beta$ -catenin and takes part in Wnt signaling [57]. The  $\beta$ -catenin complex binds at the TCF/LEF site in the promoter/enhancer regions of target genes like Axin2, LEF1, WNT4 and WNT11, and enhances their expression in the canonical pathway (Fig. 1) [58-60].

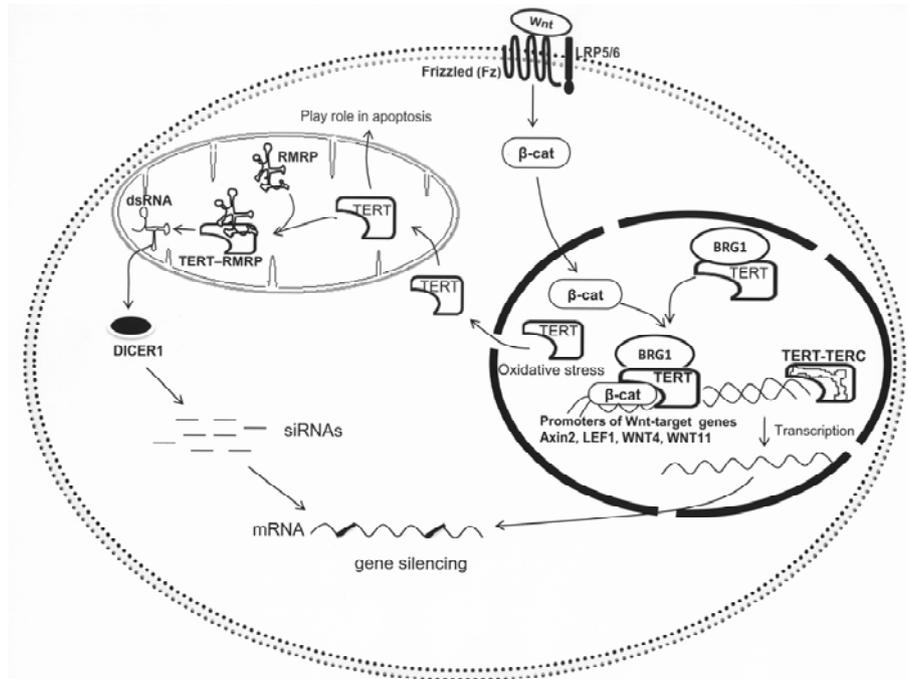


Fig. 1. Multiple functions of telomerase. TERT is directly involved in the modulation of the canonical Wnt pathway, in which it acts as a transcription factor in  $\beta$ -catenin complexes. Stimulation of Wnt receptor(s) after binding with Wnt on the plasma membrane causes binding of TERT with Wnt transcription factor BRG1 and forms a complex, which then binds to the promoters of Wnt-target genes and regulates their expression. TERT also associates with the RNA component of mitochondrial RNA processed into endoribonuclease RMRP. This complex has RdRP activity, which produces a double-stranded RMRP molecule that is further processed into 22 nucleotide siRNA by dicer and RISC (RNA-induced silencing complex). These siRNA suppress the expression of RMRP. As a result, TERT-RMRP-RDRP regulates the level of RMRP by a negative-feedback control mechanism. These siRNA-mediated suppression pathways mediate control of gene expression by TERT. With oxidative stress, TERT translocates from the nucleus to the mitochondria. Recent results also show that TERT also regulates apoptosis in the mitochondria.

Furthermore, TERT was found to bind with promoters responsive to Wnt signaling and to promoter elements recognized by BRG1 and  $\beta$ -catenin [54, 61]. Studies also indicate a role for TERT in Wnt signaling in collapsing glomerulopathies (characterized by the proliferation of glomerular differentiated epithelial cells, the podocytes) [54, 62]. Further analysis has shown that this effect of TERT on kidney cells is independent of its catalytic activity: it is coupled to its Wnt signaling stimulation, with increased expression and nuclear localization of  $\beta$ -catenin.

However, increasing evidence implies a bidirectional connection between the Wnt pathway and TERT in both embryonic stem cells and cancer [63, 64]. It has

been experimentally shown that embryonic stem cells expressing an activated  $\beta$ -catenin show high telomerase activity and have longer telomeres, while in mice lacking  $\beta$ -catenin, the length of the telomere is short and telomerase activity is also low. Zhang *et al.* found the same results in human cancer cell lines by inducing or repressing  $\beta$ -catenin expression [64]. In fact, in embryonic stem cells,  $\beta$ -catenin binds with Klf4, a transcription factor expressed by pluripotent cells, and regulates the TERT expression, whereas in human cancer cells TERT appears as a direct target of  $\beta$ -catenin/TCF4-mediated transcription. Therefore, during transformation, the Wnt pathway also participates in some carcinogenic processes via the stabilization of the telomere and stimulation of telomerase activity.

### **RNA-DEPENDENT RNA POLYMERASE ACTIVITY OF TELOMERASE**

TERT is known for its RNA-dependent DNA polymerase activity in association with TERC. Studies also indicate the role of RNA-dependent RNA polymerase activity of TERT in post-transcriptional gene silencing, which is independent of TERC (Table 1). TERT is the only RdRP identified in mammals [65]. This function of TERT depends on a mitochondrial non-coding RNA: mitochondrial RNA-processing endoribonuclease (RMRP). Further analysis shows that TERT is associated with two types of RNA in HeLa cells (which overexpress TERT): TERC and RMRP [66].

RMRP is a non-coding RNA, the mutations of which lead to cartilage-hair hypoplasia, an inherited pleiotropic syndrome that is characterized by premature multi-organ failure, mainly in highly proliferative organs, and that involves stem cell dysfunction [67]. The TERT-RMRP complex has RdRP activity, which produces a double-stranded RMRP molecule [54] that is processed into 21 nucleotide siRNA by dicer and RISC (Fig. 1). These siRNA suppress the expression of RMRP. As a result, TERT-RMRP-RDRP regulates the level of RMRP by a negative-feedback control mechanism. The siRNA-mediated suppression pathway demonstrates control of gene expression by TERT. In the same way, the TERT-RMRP complex may amplify other small non-coding RNA and thereby regulate the expression of other genes by producing specific siRNA. It has been experimentally shown that TERT has a role in the control of cellular proliferation. TERT is known to increase cellular proliferation by increasing cell division and decreasing apoptosis in TERT-transductant human mammary epithelial cells (HMECs) [68]. Further analysis shows that the effect of TERT on proliferation of cells is connected with alterations in cyclin D1, A2, E2F and pRB, which are all cell cycle regulatory proteins, and requires the catalytic activity of telomerase rather than activation of Wnt signaling by TERT [68]. Mukherjee *et al.* found a reduction in RMRP levels in TERT-transduced HMECs because dicer and RISC process the double-stranded RMRP molecules into 22 nt siRNA that control the level of RMRP. They also showed a connection between the enhancement of cellular proliferation and a decrease in RMRP

levels [68]. Knockdown of RMRP using shRNA (short hairpin RNA) results in proliferation of HMECs, which means that both results are comparable in enhancing cellular proliferation and lowering the RMRP levels. Together, these data indicate that TERT has an RNA-dependent RNA polymerase activity that enhances cellular proliferation via small interfering RNA.

### RNA-DEPENDENT DNA POLYMERASE ACTIVITY OF TELOMERASE

TERT also has RNA-dependent DNA polymerase activity independent of TERC (Table1). TERT is present in the mitochondria and the nucleus (Fig. 2). TERT has an N-terminal mitochondrial targeting signal [69] that helps it to migrate into the mitochondria, probably through the protein complexes known as translocases, which are present on the outer and inner mitochondrial membranes. Translocation of TERT from the nucleus to mitochondria occurs following oxidative stress [54, 69-71] and involves the improvement of mitochondrial function and stress resistance, independent of its telomeric function, which finally leads to the survival of tumor cells. It has been experimentally shown that TERC is not present in the mitochondria, which also supports the idea that TERT reverse transcriptase activity is independent of TERC [72].

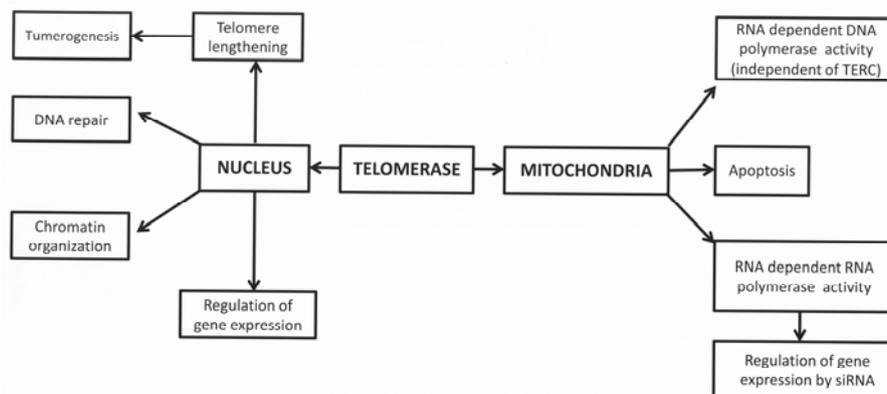


Fig. 2. A schematic representation of the classical and extracurricular activities of telomerase. Telomerase is found to be active in the mitochondria and the nucleus. In the mitochondria, telomerase shows RNA-dependent DNA polymerase activity independently of TERC and uses tRNA as a template. It also shows RNA-dependent RNA polymerase activity, and is the only RNA-dependent RNA polymerase known in mammals. It has been experimentally shown that telomerase plays a role in the regulation of apoptosis. Telomerase sensitizes the DNA of mitochondria to  $H_2O_2$ , which causes oxidative damage to mt-DNA perhaps through the modulation of metal homeostasis [69]. In the nucleus, telomerase maintains telomere length. Telomerase is found to be directly involved in the modulation of the canonical Wnt pathway, in which it acts as a transcription factor in  $\beta$ -catenin complexes. Telomerase is also found to be involved in chromatin organization.

In the mitochondria, TERT uses tRNA as a template to synthesize cDNA [72]. Human VA13 cells that do not have TERC and use a recombination-based method of telomere lengthening known as alternative lengthening of telomeres (ALT) were transfected with wild-type TERT. This showed that TERT performs its mitochondrial function. Furthermore, in the same cells, a dominant negative form of the enzyme is inactive [72]. RNA-dependent DNA polymerase activity of TERT based on the TRAP assay has also been shown in rabbit reticulocyte lysates (RRLs), in which translation of TERT in the presence of added TERC results in telomeric-DNA synthesis *in vitro* [72]. Addition of total cellular RNA from TERC-negative VA13 cells or from TERC-positive HeLa cells in the reaction mixture, along with random hexamers to prime the reactions followed by PCR with primers for different mt-tRNA genes demonstrated the synthesis of cDNA in the absence of TERC. It can be concluded that mt-TERT uses tRNA rather than TERC for cDNA synthesis [72]. In the absence of TERT, no products were observed, clearly demonstrating that mt-TERT can act as a reverse transcriptase by using mt-tRNA rather than TERC as a template.

#### **ROLE OF TELOMERASE IN APOPTOSIS**

It has been experimentally shown that telomerase plays a role in the regulation of apoptosis (Fig. 2). This role is independent of its conventional function of telomere lengthening. Further analysis has shown that telomerase sensitizes the DNA of mitochondria to H<sub>2</sub>O<sub>2</sub>, which causes oxidative damage to mt-DNA, perhaps through the modulation of metal homeostasis [69]. The N-terminal leader sequence of TERT contains a mitochondrial localization signal that targets TERT to the mitochondria. Mutation in this region of TERT causes loss of mitochondrial targeting, and cells with mutated hTERT show decreased levels of mt-DNA damage [71]. These observations suggest proapoptotic activity of hTERT in the mitochondria and the roles of TERT in the mitochondria are consistent with reports showing that oxidative stress triggers nuclear export of hTERT [73].

By contrast, it has been shown that hTERT overexpression renders cells resistant to apoptosis. This anti-apoptotic effect of TERT occurs at a pre-mitochondrial step before the release of cytochrome c and apoptosis-inducing factor [74]. The siRNA-mediated downregulation of hTERT triggers the apoptotic pathway devoid of obvious involvement of telomere erosion, but by post-translational activation of BAX, which induces a CD90-independent mitochondrial pathway of apoptosis [75]. In addition, a recent report indicated that TERT enhances cellular and organism viability independently of its telomerase activity. Cultured cells and a transgenic mouse model expressing wild-type TERT were treated with staurosporin and *N*-methyl-*D*-aspartic acid, which both promote apoptosis. Increased resistance against apoptosis was observed in both the cultured cells and the transgenic mice, and this effect of TERT is again independent of telomerase activity [76]. Even though the exact mechanism as to how TERT

regulates apoptosis in mitochondria is unknown, TERT may exhibit discrete functions in apoptosis regulation by promoting apoptosis via alteration of the mitochondrial membrane potential or metal homeostasis in mitochondria.

### **ROLE OF TELOMERASE IN DNA REPAIR**

Telomerase may also play a role in DNA repair independently of its telomere-lengthening function (Fig. 2). It has been experimentally shown that hTERT is also associated with primase [77], a well known protein involved in replication and DNA repair, which indicates a role for telomerase in DNA repair. In addition, studies indicate that ectopic expression of hTERT causes an increase in the expression level of genes involved in the DNA damage response, and this is thought to be associated with a decrease in spontaneous chromosome damage in G1 cells and improvement in the DNA repair kinetics [78]. Moreover, hTERT is also found to be associated physically with many DNA repair proteins and the telomere, thus enhancing the stability of the genome and DNA repair functions [79]. However, increasing evidence is emerging to indicate that the role of telomerase in the DNA damage response is not limited to DNA double-strand break repair but also associated with many other types of DNA repair, including via nucleotide excision [80]. These studies predict a role of telomerase in the DNA damage response independent of its classical activity of telomere length maintenance.

### **CONCLUSIONS**

Accumulating evidence indicates that the telomerase complex performs several functions that do not depend on its classical function of telomere maintenance. All of these telomere-independent roles affect normal cell physiology and promote the proliferation of cancer cells. The exact pathways by which telomerase interferes with or enhances tumorigenesis point towards possible new targets for cancer treatment. Further research is needed to clarify the role of TERT as a mitochondrial RNA-dependent DNA polymerase. It is very necessary to develop the tools that would help to study functions of TERT in cellular physiological conditions to provide clear results. There is no direct molecular evidence to explain the role of telomerase in DNA repair, so a testable experimental model is needed to explain the telomere-independent role of telomerase in DNA repair.

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## REFERENCES

1. Olovnikov, A.M. A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. **J. Theor. Biol.** 41 (1973) 181-190.
2. Watson, J.D. Origin of concatemeric T7 DNA. **Nat. New Biol.** 239 (1972) 197-201.
3. Palm, W. and de Lange, T. How shelterin protects mammalian telomeres. **Annu. Rev. Genet.** 42 (2008) 301-334.
4. Liu, D., Safari, A., O'Connor, M., Chan, D.W., Laegerler, A., Qin, J. and Songyang, Z. POT1 interacts with POT1 and regulates its localization to telomeres. **Nat. Cell. Biol.** 6 (2004) 673-680.
5. Houghtaling, B.R., Cuttonaro, L., Chang, W. and Smith, S. A dynamic molecular link between the telomere length regulator TRF1 and the chromosome end protector TRF2. **Curr. Biol.** 14 (2004) 1621-1631.
6. Ye, J.Z., Hockemeyer, D., Krutchinsky, A.N., Loayza, D., Hooper, S., Chait, B.T. and De Lange, T. POT1-interacting protein PIP1: a telomere length regulator that recruits POT1 to the TIN2/TRF1 complex. **Genes Dev.** 18 (2004) 1649-1654.
7. De Lange, T. Shelterin: the protein complex that shapes and safeguards human telomeres. **Genes Dev.** 19 (2005) 2100-2110.
8. Henderson, E.R. and Blackburn, E.H. An overhanging 39 terminus is a conserved feature of telomeres. **Mol. Cell. Biol.** 9 (1989) 345-348.
9. Makarov, V.L., Hirose, Y. and Langmore J.P. Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for telomere shortening. **Cell** 88 (1997) 657-666.
10. McElligott, R. and Wellinger, R.J. The terminal DNA structure of mammalian chromosomes. **EMBO J.** 16 (1997) 3705-3714.
11. Woodring, E.W., Valerie, M.T., Kenneth, E.H., Stephen, D.L., and Jerry, W.S. Normal human chromosomes have long G-rich telomeric overhangs at one end. **Genes Dev.** 11 (1997) 2801-2809.
12. Greider, C.W. and Blackburn, E.H. Identification of a specific telomere terminal transferase enzyme with two kinds of primer specificity. **Cell** 51 (1985) 405-413.
13. Kim, N.W., Piatyszek, M.A., Prowse, K.R., Harley, C.B., West, M.D., Ho, P.L., Coviello, G.M., Wright, W.E., Weinrich, S.L. and Shay, J.W. Specific association of human telomerase activity with immortal cells and cancer. **Science** 266 (1994) 2011-2015.
14. Chang, J.T., Chen, Y.L., Yang, H.T., Chen, C.Y. and Cheng, A.J. Differential regulation of telomerase activity by six telomerase subunits. **Eur. J. Biochem.** 269 (2002) 3442-3450.
15. Greider, C.W. and Blackburn, E.H. A telomeric sequence in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis. **Nature** 337 (1989) 331-337.

16. Blasco, M.A., Funk, W., Villeponteau, B. and Greider, C.W. Functional characterization and developmental regulation of mouse telomerase RNA. **Science** 269 (1995) 1267-1270.
17. Feng, J., Funk, W.D., Wang, S.S., Weinrich, S.L., Avilion, A.A., Chiu, C.P., Adams R.R., Chang, E., Allsopp, R.C., Yu, J., Le, S., West, M.D., Harley, C.B., Andrews, W.H., Greider, C.W. and Villeponteau, B. The RNA component of human telomerase. **Science** 269 (1995) 1236-1241.
18. Chen, J.L. and Greider, C.W. Determinants in mammalian telomerase RNA that mediate enzyme processivity and cross-species incompatibility. **EMBO J.** 22 (2003) 304-314.
19. Lai, C.K., Miller, M.C. and Collins, K. Roles for RNA in telomerase nucleotide and repeat addition processivity. **Mol. Cell.** 11 (2003) 1673-1683.
20. Chen, J.L., Blasco, M.A. and Greider, C.W. Secondary structure of vertebrate telomerase RNA. **Cell** 100 (2000) 503-514.
21. Seto, A.G., Umansky, K., Tzfati, Y., Zang, A.J., Blackburn, E.H. and Cech, T.R. A template-proximal RNA paired elements contributes to *saccharomyces cerevisiae* telomerase activity. **RNA** 9 (2003) 1323-1332.
22. Mitchell, J.R., Cheng, J. and Collins, K. A box H/ACA small nucleolar RNA-like domain at the human telomerase RNA 3' end. **Mol. Cell. Biol.** 19 (1999) 567-576.
23. Chen, J.L., Blasco, M.A. and Greider, C.W. Secondary structure of vertebrate telomerase RNA. **Cell** 100 (2000) 503-514.
24. Mitchell, J.R. and Collins, K. Human telomerase activation requires two independent interactions between telomerase RNA and telomerase reverse transcriptase. **Mol. Cell.** 6 (2000) 361-371.
25. Matera, A.G., Terns, R.M. and Terns, M.P. Non-coding RNA: lessons from the small nuclear and small nucleolar RNA. **Nat. Rev. Mol. Cell. Biol.** 8 (2007) 209-220.
26. Dragon, F., Pogacic, V. and Filipowicz, W. In vitro assembly of human H/ACA small nucleolar ribonucleoproteins reveals unique features of U17 and telomerase RNA. **Mol. Cell. Biol.** 20 (2000) 3037-3048.
27. Pogacic, V., Dragon, F. and Filipowicz, W. Human H/ACA small nucleolar ribonucleoproteins and telomerase share evolutionarily conserved proteins NHP2 and NOP10. **Mol. Cell. Biol.** 20 (2000) 9028-9040.
28. Zhu, Y., Tomlinson, R.L., Lukowiak, A.A., Terns, R.M. and Terns, M.P. Telomerase RNA accumulates in Cajal bodies in human cancer cells. **Mol. Biol. Cell.** 15 (2004) 81-90.
29. Cristofari, G., Adolf, E., Reichenbach, P., Sikora, K., Terns, R.M., Terns, M.P. and Lingner, J. Human telomerase RNA accumulation in Cajal bodies facilitates telomerase recruitment to telomeres and telomere elongation. **Mol. Cell.** 27 (2007) 882-889.
30. Zhang, Q., Kim, N.K. and Feigon, J. Architecture of human telomerase RNA. **Proc. Natl. Acad. Sci.** 108 (2011) 20325-20332.

31. Lendvay, T.S., Morris, D.K., Sah, J., Balasubramanian, B. and Lundblad, V. Senescence mutants of *Sachharomyces cerevisiae* with a defect in telomerase replication identify three additional EST genes. **Genetics** 144 (1996) 1399-1412.
32. Wick, M., Zubov, D. and Hagen, G. Genomic organization and promoter characterization of the gene encoding the human telomerase reverse transcriptase (hTERT). **Gene** 232 (1999) 97-106.
33. Lingner, J., Hughes, T.R., Shevchenko, A., Mann, M., Lundblad, V. and Cech, T.R. Reverse transcriptase motifs in the catalytic subunit of telomerase. **Science** 276 (1997) 561-567.
34. Nakamura, T.M., Morin, G.B., Chapman, K.B., Weinrich, S.L., Andrews, W.H., Lingner, J., Harley, C.B. and Cech, T.R. Telomerase catalytic subunit homologs from fission yeast and human. **Science** 277 (1997) 955-959.
35. Gillis, A.J., Schuller, A.P. and Skordalakes, E. Structure of the *Tribolium castaneum* telomerase catalytic subunit TERT. **Nature** 455 (2008) 633-637.
36. Mitchell, M., Gillis, A., Futahashi, M., Fujiwara, H. and Skordalakes, E. Structural basis for telomerase catalytic subunit TERT binding to RNA template and telomeric DNA. **Nature Struct. Mol. Biol.** 17 (2010) 513-518.
37. Bodnar, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C.P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S. and Wright, W.E. Extension of lifespan by introduction of telomerase in to normal human cells. **Science** 279 (1998) 349-352.
38. Cayuela, M.L., Flores, J.M. and Blasco, M.A. The telomerase RNA component Terc is required for the tumour-promoting effects of Tert overexpression. **EMBO Rep.** 6 (2005) 268-274.
39. Canela, A., Martin-Caballero, J., Flores, J.M. and Blasco, M.A. Constitutive expression of tert in thymocytes leads to increased incidence and dissemination of T-cell lymphoma in Lck-Tert mice. **Mol. Cell. Biol.** 24 (2004) 4275-4293.
40. Engelhardt, M., Drullinsky, P., Guillem, J. and Moore, M.A. Telomerase and telomere length in the development and progression of premalignant lesions to colorectal cancer. **Clin. Cancer Res.** 3 (1997) 1931-1941.
41. Hara, E., Tsurui, H., Shinozaki, A., Nakada, S. and Oda, K. Cooperative of antisense-Rb and antisense-p53 oligomers on the extension of lifespan in human diploid fibroblasts, TIG-1 **Biochem. Biophys. Res. Commun.** 179 (1991) 528-534.
42. Counter, C.M., Avilion, A.A., LeFeuvre, C.E., Stewart, N.G., Greider, C.W., Harley, C.B. and Bacchetti, S. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. **EMBO J.** 11 (1992) 1921-1929.
43. Shay, J.W. and Wright, W.E. Quantitaion of the frequency of immortalization of normal human diploid fibroblasts by sv 40 large T-antigen. **Exp. Cell Res.** 184 (1989) 109-118.

44. Zhu, J., Wang, H., Bishop, J.M. and Blackburn, E.H. Telomerase extends the lifespan of virus-transformed human cells without net telomere lengthening. **Proc. Natl. Acad. Sci. USA** 96 (1999) 3723-3728.
45. Ding, D., Zhou, J., Wang, M. and Cong, Y. Implications of telomere-independent activities of telomerase reverse transcriptase in human cancer. **FEBS J.** 280 (2013) 3205-3211.
46. Kim, M., Xu, L. and Blackburn, E.H. Catalytically active human telomerase mutants with allele-specific biological properties. **Exp. Cell. Res.** 288 (2003) 277-287.
47. Kenkichi, M., Possemato, R., Wong, J.M., Currier, J.L., Tothova, Z., Manola, J.B., Ganesan, S., Lansdorp, P.M., Collins, K. and Hahn, W.C. The telomerase reverse transcriptase regulates chromatin state and DNA damage responses. **Proc. Natl. Acad. Sci. USA** 102 (2005) 8222-8227.
48. Stampfer, M.R., Garbe, J., Levine, G., Lichtsteiner, S., Vasserot, A.P. and Yaswen, P. Expression of the telomerase catalytic subunit, hTERT, induces resistance to transforming growth factor beta growth inhibition in p16INK4A (-) human mammary epithelial cells. **Proc. Natl. Acad. Sci. USA** 98 (2001) 4498-4503.
49. Lindvall, C., Hou, M., Komurasaki, T., Zheng, C., Henriksson, M., Sedivy, J.M., Bjorkholm, M., Teh, B.T., Nordenskjold, M. and Xu, D. Molecular characterization of human telomerase reverse transcriptase-immortalized human fibroblasts by gene expression profiling: activation of the epiregulin gene. **Cancer Res.** 63 (2003) 1743-1747.
50. Choi, J., Southworth, L.K., Sarin, K.Y., Venteicher, A.S., Ma, W., Chang, W., Cheung, P., Jun, S., Artandi, M.K., Shah, N., Kim, S.K. and Artandi, S.E. TERT promotes epithelial proliferation through transcriptional control of a Myc- and Wnt related developmental program. **PLoS Genet.** 4 (2008) e10.
51. VanMater, D., Kolligs, F.T., Dlugosz, A.A. and Fearon, E.R. Transient activation of beta-catenin signaling in cutaneous keratinocytes sufficient to trigger the active growth phase of the hair cycle in mice. **Genes Dev.** 17 (2003) 1219-1224.
52. Reya, T. and Clevers, H. Wnt signaling in stem cells and cancer. **Nature** 434 (2005) 843-850.
53. Wege, H., Heim, D., Lütgehetmann, M., Dierlamm, J., Lohse, A.W. and Brümmendorf, T.H. Forced activation of  $\beta$ -catenin signaling supports the transformation of hTERT-immortalized human fetal hepatocytes. **Mol. Cancer Res.** 9 (2011) 1222-1231.
54. Chiodi, I. and Mondello, C. Telomere-independent function of telomerase in nuclei, cytoplasm, and mitochondria. **Front. Oncol.** 2 (2012) 1-6.
55. Rao, T.P. and Ku'hl, M. An updated overview on Wnt signaling pathways: a prelude for more. **Circ. Res.** 106 (2010) 1798-1806.
56. Komiya, Y. and Habas, R. Wnt signal transduction pathways. **Organogenesis** 4 (2008) 68-75.

57. Barker, N., Hurlstone, A., Musisi, H., Miles, A., Bienz, M. and Clevers, H. The chromatin remodeling factor Brg-1 interacts with beta-catenin to promote target gene activation. **EMBO J.** 20 (2001) 4935-4943.
58. Surendran, K. and Simon, T.C. CNP gene expression is activate by Wnt signaling and correlates with *Wnt4* expression during renal injury. **Am. J. Physiol. Renal Physiol.** 284 (2003) F653-F662.
59. Jho, E., ZhanDomon, C., Joo, C.K., Freund, J.N. and Costantini, F. Wnt/  $\beta$  -catenin/Tcf signaling induces the transcription of Axin2 a negative regulator of the signaling pathway. **Mol. Cell. Biol.** 22 (2002) 1172-1183.
60. Katoh, M and Katoh, M. Integrative genomic analyses of *WNT11*: Transcriptional mechanisms based on canonical WNT signals and GATA transcription factors. **Int. J. Mol. Med.** 24 (2009) 247-251.
61. Park, J.I., Venteicher, A.S., Hong, J.Y., Choi, J., Jun, S., Shkreli, M., Chang, W., Meng, Z., Cheung, P., Ji, H., McLaughlin, M., Veenstra, T.D., Nusse, R., McCrea, P.D. and Artandi S.E. Telomerase modulates Wnt signaling by association with target gene chromatin. **Nature** 460 (2009) 66-72.
62. Shkreli, M., Sarin, K.Y., Pech, M.F., Papeta, N., Chang, W., Brockman, S.A., Cheung, P., Lee, E., Kuhner, F., Olson, J.L., Kuo, C.J., Gharavi, A.G., D, Agati, V.D. and Artandi, S.E. Reversible cell-cycle entry in adult kidney podocytes through regulated control of telomerase and Wnt signaling. **Nat. Med.** 18 (2012) 111-119.
63. Hoffmeyer, K., Raggioli, A., Rudloff, S., Anton, R., Hierholzer, A., Valle, I.D., Hein, K., Vogt, R. and Kemler, R. Wnt/beta-catenin signaling regulates telomerase in stem cells and cancer cells. **Science** 336 (2012) 1549-1554.
64. Zhang, Y., Toh, L.L., Lau, P. and Wang, P. Telomerase reverse transcriptase (TERT) is an ovel target of Wnt/ $\beta$ -catenin pathway in human cancer. **J. Biol. Chem.** 287 (2012) 32494-32511.
65. Maida, Y. and Masutomi, K. RNA-dependent RNA polymerases in RNA silencing. **Biol. Chem.** 392 (2011) 299-304.
66. Maida, Y., Yasukawa, M., Furuuchi, M., Lassmann, T., Possemato, R., Okamoto, N., Kasim, V., Hayashizaki, Y., Hahn, W.C. and Masutomi, K. An RNA- dependent RNA polymerase formed by TERT and the RMRP RNA. **Nature** 461 (2009) 230-235.
67. Ridanpaa, M., Eenennaam, H., Pelin, K., Chadwick, R., Johnson, C., Yuan, B., Venrooij, W., Pruijn, G., Salmela, R., Rockas, S., Kitie, O.M., Kaitila, I. and Chapelle, A. Mutations in the RNA component of RNase MRP cause a pleiotropic human disease, cartilage-hair hypoplasia. **Cell** 104 (2001) 195-203.
68. Mukherjee, S., Firpo, E.J., Wang, Y. and Roberts, J.M. Separation of telomerase functions by reverse genetics. **Proc. Natl. Acad. Sci. USA** 108 (2011) E1363-E1371.
69. Santos, J.H., Meyer, J.N., Skorvaga, M., Annab, L.A. and VanHouten, B. Mitochondrial hTERT exacerbates free-radical- mediated mtDNA damage. **Aging Cell** 3 (2004) 399-411.

70. Santos, J.H., Meyer, J.N. and Van Houten, B. Mitochondrial localization of telomerase as a determinant for hydrogen peroxide- induced mitochondrial DNA damage and apoptosis. **Hum. Mol. Genet.** 15 (2006) 1757-1768.
71. Haendeler, J., Hoffmann, J., Rahman, S., Zeiher, A.M. and Dimmeler, S. Regulation of telomerase activity and anti-apoptotic function by protein-protein interaction and phosphorylation. **FEBS Lett.** 536 (2003) 180-186.
72. Sharma, N.K., Reyes, A., Green, P., Caron, M.J., Bonini, M.G., Gordon, D.M., Holt, I.J. and Santos, J.H. Human telomerase acts as a hTR-independent reverse transcriptase in mitochondria. **Nucleic Acids Res.** 40 (2012) 712-725.
73. Haendeler, J., Hoffmann, J., Diehl, J.F., Vasa, M., Spyridopoulos, I., Zeiher, A.M. and Dimmeler, S. Antioxidants inhibit nuclear export of telomerase reverse transcriptase and delay replicative senescence of endothelial cells. **Circ. Res.** 94 (2004) 768-775.
74. Zhang, P., Chan, S.L., Fu, W., Mendoza, M. and Mattson, M.P. TERT suppresses apoptosis at a premitochondrial step by a mechanism requiring reverse transcriptase activity and 14-3-3 protein-binding ability. **FASEB J.** 17 (2003) 767-769.
75. Massard, C., Zermati, Y., Pauleau, A.L., Larochette, N., Metivier, D., Sabatier, L., Kroemer, G. and Soria, J.C. hTERT: a novel endogenous inhibitor of the mitochondrial cell death pathway. **Oncogene** 25 (2006) 4505-4514.
76. Lee, J., Sung, Y.H., Cheong, C., Choi, Y.S., Jeon, H.K., Sun, W., Hahn, W.C., Ishikawa, F., and Lee, H.W. TERT promotes cellular and organismal survival independently of telomerase activity. **Oncogene** 27 (2008) 3754-3760.
77. Ray, S., Karamysheva, Z., Wang, L., Shippen, D.E. and Price, C.M. Interactions between telomerase and primase physically link the telomere and chromosome replication machinery. **Mol. Cell. Biol.** 22 (2002) 5859-5868.
78. Sharma, G.G., Gupta, A., Wang, H., Scherthan, H., Dhar, S., Gandhi, V., Iliakis, G., Shay, J.W., Young, C.S. and Pandita, T.K. hTERT associates with human telomeres and enhances genomic stability and DNA repair. **Oncogene** 22 (2003) 131-146.
79. Sharma, G.G., Hwang, K.K., Pandita, R.K., Gupta, A., Dhar, S., Parenteau, J., Agarwal, M., Worman, H.J. and Wellinger R.J. Human heterochromatin protein 1 isoforms HP1 (Hsalph) and HP1 (Hsbeta) interfere with hTERT-telomere interactions and correlate with changes in cell growth and response to ionizing radiation. **Mol. Cell. Biol.** 23 (2003) 8363-8376.
80. Shin, K.H., Kang, M.K., Dicterow, E., Kameta, A., Baluda, M.A. and Park, N.H. Introduction of human telomerase reverse transcriptase to normal human fibroblasts enhances DNA repair capacity. **Clin. Cancer Res.** 10 (2004) 2551-2560.
81. Smith, L.L., Collier, H.A. and Roberts, J.M. Telomerase modulates expression of growth-controlling genes and enhances cell proliferation. **Nat. Cell. Biol.** 5 (2003) 474-479.