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Invited Mini Review

Telomerase reverse transcriptase in the regulation of gene expression

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Telomerase plays a pivotal role in the pathology of aging and cancer by maintaining genome integrity, controlling cell proliferation, and regulating tissue homeostasis. Telomerase is essentially composed of an RNA component, Telomerase RNA or *TERC*, which serves as a template for telomeric DNA synthesis, and a catalytic subunit, telomerase reverse transcriptase (TERT). The canonical function of TERT is the synthesis of telomeric DNA repeats, and the maintenance of telomere length. However, accumulating evidence indicates that TERT may also have some fundamental functions that are independent of its enzymatic activity. Among these telomere-independent activities of hTERT, the role of hTERT in gene transcription has been investigated in detail. Transcriptional regulation is a fundamental process in biological systems. Several studies have shown a direct involvement of hTERT in gene transcription. This mini-review will focus on the role of hTERT in gene transcription regulation, and discuss its possible mechanisms. [BMB Reports 2014; 47(1): 8-14]

INTRODUCTION

Telomeres are located at the ends of eukaryotic chromosomes, and consist of repeat GC sequences that are bound by multiple telomeric proteins, which help guarantee chromosomal integrity (1). Experimental data from genetically modified mice and human premature aging diseases clearly indicate that intact telomere function is crucial for cell proliferation and survival; whereas, dysfunctional telomeres can lead to either cancer or aging pathologies, depending on the integrity of the cellular stress response pathways. Human telomerase is essentially composed of RNA subunits (*hTR*), together with a reverse transcriptase catalytic subunit (hTERT), whose main function is to maintain telomere length, allowing cells to subvert the Hayflick limit (2). Telomerase activity is repressed during embryonic differentiation, but remains active in the germ line, and in stem cells and activated lymphocytes. Telomerase is reactivated in 90% of all cancers (3), suggesting that the activation of telomerase is a critical step in human carcinogenicity. Telomerase has also been linked to aging, and aging-associated pathologies. Dysfunctional telomeres may result in tissue atrophy, deficient tissue regeneration, or stem cell depletion (4). In humans, loss-of-function mutations in either *hTERT* or *hTR* are associated with diseases, such as aplastic anemia, pulmonary fibrosis, and dyskeratosis congenita (5).

In addition to the role of telomerase in bypassing the replicative senescence of normal human cells, the upregulation of telomerase activity in cancer cells promotes cell proliferation, invasion, and resistance to apoptosis. Increasing evidence indicates that hTERT also has non-telomeric functions that are independent of its conventional function in telomere synthesis. It has been demonstrated that telomerase directly regulates the expression of specific genes belonging to the NF-κB signaling pathway or the Wnt/β-catenin pathway (6-9), participates in DNA damage repair, and promotes cell survival under oxidative stress or endoplasmic reticulum stress conditions (10). Additionally, it has been shown that hTERT protects developing neurons from DNA damage-induced cell death (11). TERT also regulates mitochondrial function and cell metabolism (12-15). Among the telomere-independent activities of hTERT, the role of hTERT in gene transcription has been investigated in detail. Several studies have shown a direct involvement of hTERT in gene transcription.

EVIDENCE THAT TERT REGULATES GENE EXPRESSION

An increasing number of studies have indicated that telomerase has a regulatory role in gene expression. An early study showed that ectopic expression of hTERT in human mammary epithelial cells resulted in the upregulation of five growth promoting genes, and the downregulation of seven growth inhibitory genes (16). Expression profiling analysis of the human foreskin fibroblasts BJ cells and BJ cells transfected with an hTERT expression construct identified 172 differentially expressed genes, and further demonstrated that epiregulin, a potent growth factor, was highly expressed in the hTERT-BJ cells, and that the inhibition of epiregulin expression triggered a senescence program in the hTERT-BJ cells (17). It has also been re-

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ported that hTERT regulates Mac-2BP, VEGF, and cyclin D1 expression at the transcriptional levels (18, 19). Additionally, global expression profiling experiments in bovine adrenocortical cells with ectopically expressed hTERT showed that a total of 284 genes that were associated with cell cycle regulation, metabolism, differentiation and apoptosis were altered by hTERT overexpression (20).

However, the detailed mechanisms by which telomerase participates in gene expression are largely unknown, and several hypotheses have been proposed. One theory suggests that telomerase may be involved in epigenetic modifications or the modulation of chromatin structure, which indirectly affect gene expression. In support of this hypothesis, it has been observed that overexpression of hTERT upregulated and stabilized DNA 5-methylcytosine transferase I in normal human fibroblasts (21), and the suppression of hTERT expression altered the overall configuration of chromatin (22). Another possible mechanism is that telomerase may interact with transcription factors or chromatin modifying factors that directly regulate certain gene transcription programs. In this regard, it has been shown that hTERT interacts with NF-κB p65 and β-catenin, and mediates the transcriptional regulation of target gene expression.

TERT AND THE NF-κB PATHWAY

It has been well established that telomerase activation is a prerequisite for tumorigenesis and malignant transformation (23, 24). However, the mechanisms by which hTERT is employed in cancer progression remain unclear. It was recently demonstrated that TERT regulates NF-κB-dependent gene transcription, independent of its telomerase enzymatic activity. The NF-κB signaling pathway is a master regulator of cellular and developmental events, and the key transcription factor p65/p50 dimer regulates the expression of a large number of genes, in response to physiological and pathological stimuli and stress, which play important roles in the canonical activation of the NF-κB signaling pathway (10, 25, 26). Both TERT and NF-κB are activated in cancerous cells. Studies have suggested that NF-κB activates the expression of TERT, by binding to the promoter of TERT (27), and p65 was shown to modulate hTERT translocation from the cytoplasm to the nucleus in MM.1S cells, and promote the expression of hTERT in HepG2 cells (28, 29). These results indicated that hyperactivation of NF-κB could promote tumorigenesis, by activating the expression of TERT. However, Ghosh *et al*. recently suggested that hTERT directly regulates the expression of the NF-κB-dependent genes by binding to p65, and localizing to a subset of NF-κB promoters, such as *IL-6*, *TNF-α* and *IL-8*. They observed that si-TERT had a more pronounced effect on the expression of *TNF-α* and *IL-6*, compared to *MCP1* and *IκBα*. They further found that TERT selectively occupied the *IL-6*, *TNF-α* and *IL-8* promoters; however, TERT binding to the MCP1 promoter was weak, and binding was almost absent at the *IκBα* promoter.

Chip sequencing analysis indicated that TERT is required for optimal p65 binding to a small proportion of NF-κB dependent target genes, following TNF-α stimulation. It appears that the TERT-mediated gene expression is dependent on the context of the NF-κB binding site in the promoter. They further found that, in addition to the NF-κB binding site, there was a T_2G_3 sequence within the *IL6A*, *IL8A* and *TNF-α* promoters that may function as a putative hTERT binding site (30). These results indicate that TERT-mediated NF-κB dependent gene transcription may rely on the presence of the T_2G_3 sequence near the NF-κB binding site in their promoters. However, this hypothesis needs more experimental support. Importantly, we recently showed that TERT regulates MMP family gene expression via NF-κB-dependent transcription, in a manner independent of its telomerase activity. We observed that both TERT and TERT K626A regulated the expression of NF-κB target genes, such as *IL-6*, *IL-8*, *MMP1* and *IκBα*, in U2OS and HeLa cells (7). Given that both telomerase and NF-κB are hyperactivated in an equally large number of cancers, the functional interaction of telomerase and NF-κB might be a key missing molecular link that mediates the effects of reactivated telomerase in cancer cells. These data could explain the upregulation of inflammation-related gene expression in patients with metabolic syndromes (31), and the regulation of immune cell functions by telomerase activity (32). Taken together, these findings indicated that the feed-forward loop between NF-κB and telomerase not only drives multiple hallmarks of cancer, but also is an early event that promotes cancer progression.

Additionally, we previously reported that hTERT could activate the transcription of vascular endothelial growth factor (VEGF), independent of telomerase activity in HeLa cells (33). However, we performed a mammalian one-hybrid assay in multiple cell lines, and we found that hTERT has no direct transcriptional activity. The mechanism by which TERT regulates *VEGF* may be mediated by its interaction with other transcription factors, such as p65 and Sp1. Liu *et al*. suggested that TERT promotes the transcription of epithelial mesenchymal transition (EMT)-related genes, such as *vimentin* and *snail1*, independent of telomerase activity in gastric cancer (34). They further found that hTERT interacted with β-catenin, and occupied the promoters of β-catenin target genes, such as *vimentin*. Given that the MMP family, EMT, and angiogenesis-related genes, such as *VEGF*, are important for cell invasion, transformation, and angiogenesis, respectively, these studies may thus explain how hTERT also exhibits non-telomeric functions in tumor progression.

TERT AND THE WNT/β-CATENIN PATHWAY

Canonical Wnt/β-catenin signaling regulates many biological processes, including cell fate decision, axis formation and organ development, and mutations in the Wnt pathway resulting in tumorigenesis (35). The Wnt signaling pathway is composed

of extracellular protein ligands, receptors on the cell membrane, the cell signal transduction system in the cytoplasm, and gene transcription regulation elements within the nucleus. The transcriptional activator β-catenin is a key effector of this pathway. When Wnt signaling is turned off, the tumor suppressor adenomatous polyposis coli (APC), Axin, and β-catenin form protein complexes in the cytoplasm, leading to the phosphorylation of β-catenin by CKI and GSK3β, and its degradation by the 26S proteasome. When Wnt signaling is turned on, the APC-Axin-GSK3β complex is destabilized through Dsh, and β-catenin is no longer degraded, leading to its accumulation in the cytoplasm. Stabilized β-catenin enters the nucleus, and activates the transcription of downstream target genes, in concert with the TCF/LEF family of transcription factors (36). The Wnt pathway regulates the self-renewal of stem cells and/or progenitor cells in a variety of tissues and organs, and dysfunction of the Wnt pathway has been implicated in a number of cancers (37). Telomerase activity is repressed during embryonic differentiation, but remains active in the germ line. The Wnt pathway and telomerase have both been implicated in embryonic development and tissue homeostasis, suggesting that they have a functional link and/or regulation. Studies showed that TERT can directly promote the cell cycle entry of quiescent epidermal stem cells (38, 39), suggesting that TERT has its own activity in the stem cell, independent of its telomerase activity. However, the mechanism by which TERT regulates stem cell behavior is not understood.

In 2008, Artandi's group first studied the link between the Wnt pathway and TERT. They investigated the genome-wide transcriptional response to acute changes in TERT or $TERT^{ci}$ (TERT mutant lacking RT function) expression in mammalian skin, an ideal tissue for progenitor cell biology research. They observed that TERT induced the anagen phase of the hair cycle, promoted hair growth, activated hair follicle stem cells, and caused hyperproliferation in the basal layer of interfollicular skin, independent of telomerase activity. They further discovered that acute withdrawal of TERT resulted in a substantial change in the expression of genes related to epithelial development, adhesion and signaling transduction. Gene Set Enrichment Analysis (GSEA) revealed a significant correlation between TERT, and two pathways, Wnt and Myc. Activated TERT upregulated Wnt, Shh and BMP pathway related genes, such as *Bambi*, *Bmp8a*, *Ccnd2*, *Lef1*, *Nkd2*, *Smad*, *Wnt5a* and *Wnt11*. MSigDB (a database detailing which genes harbor conserved elements for each specific regulatory motif) analysis further suggested that E-boxes, which are recognized by Myc/Max heterodimers, and TCF/TEF binding sites, which bind β-catenin, were significantly enriched in the TERT-activated and TERT-repressed genes (9). Artandi's group further revealed a novel role of telomerase in the regulation of the Wnt/β-catenin signaling pathway. TERT acts as a cofactor in the β-catenin transcription complex; in this complex, TERT interacts with BRG1, a chromatin remodeling factor, to regulate the Wnt/β-catenin signaling pathway (8). It has been reported that TERT, BRG1 and NS/GNL3L (GTP-binding protein nucleostemin) form a complex that regulates the cancer cell state (40). Overexpression of TERT in normal human fibroblasts activated the expression of DNMT1 (DNA methyltransferase I), through the STAT3 transcription factor (41). Studies showed that EGFR acts as a transducer of the signal between TERT and STAT3 (42). Because elevated levels of NS/GNL3L increased the phosphorylation of STAT3 (40), it is possible that TERT indirectly regulates STAT3 through NS/GNL3L. Additionally, Xu's lab found that TERT indirectly interacts with β-catenin, and occupies the promoters of β-catenin target genes, to promote the epithelial mesenchymal transition in cancer cells (34). However, Liu *et al*. failed to observe a physical interaction between hTERT and BRG1 in human cells. They demonstrated that hTERT could directly interact with β-catenin, and prevent its nuclear retention and degradation (34). Additionally, Greider's lab suggested that there were indistinguishable phenotypes between the mice deficient in mTERT and mTR, and no apparent Wnt pathway defects were observed in the $mTERT^{-/-}$ mice (43). Ghosh *et al*. failed to verify the physical association of hTERT and BRG1, following stimulation with TNF-α (30).

Recently, Blackburn's lab investigated the effects of TERT on the Wnt pathway in MCF7 and HeLa cells (44). They failed to verify the physical association of hTERT and BRG1 or β-catenin. One possible explanation for this discrepancy may be that two mechanisms are employed by hTERT and mTERT, respectively, to regulate the Wnt/β-catenin pathway (34). Many studies showing a role for TERT in the regulation of gene expression have been based on the upregulation or downregulation of TERT in a transient fashion. However, whether the telomere-independent functions of TERT on gene regulation, or the Wnt signaling pathway, are physiologically relevant, remain to be seen. It is possible that the impact of TERT on the regulation of the Wnt/β-catenin signaling pathway may become biologically relevant, under particular physiopathological conditions (45). It has been found that TERT not only activated the Wnt/β-catenin pathway, but β-catenin could also directly regulate the transcription of TERT, by interacting with KLF4 in the NTera2 and SW480 carcinoma cell lines (46). Together, these studies suggest that hTERT could influence stem cell functions in two ways: TERT could activate the transcription of β-catenin-dependent genes, or Wnt/β-catenin could regulate the transcription of TERT, which results in telomere maintenance.

TERT AND RMRP IN THE REGULATION OF GENE EXPRESSION

Many reports have shown that TERT plays a direct role in the regulation of gene expression. Recently, Maida *et al*. suggested that TERT may regulate gene expression in a telomere-independent manner at the posttranscription level (47). It has been suggested that hTERT could interact with the RNA com-

ponent of the mitochondrial RNA-processing endoribonuclease (RMRP), to form a ribonucleoprotein complex that has RNA-dependent RNA polymerase activity. Using RMRP as a template, the TERT-RMRP complex could produce dsRNAs, which are processed into 22 nucleotide siRNAs that specifically silence the expression of RMRP, in a Dicer-dependent manner. RMRP is a 267 nucleotide non-coding RNA that is highly expressed in many human and murine tissues. RMRP mutations are associated with Cartilage Hair Hypoplasia (CHH) (48, 49). Rosenbluh *et al*. were unable to obtain viable homozygous RMRP null mice, and they claimed that RMRP is essential for early embryonic development (50). It has also been suggested that RMRP RNA binds to the mitochondria, and modifies the RNase MRP complex at posttranscriptional level (49). Recently, Sharma *et al*. suggested the mitochondrial hTERT could bind different mitochondrial RNAs, and acts in a telomere-independent manner (51). It is possible that there are RNAs other than RMRP that associate with TERT, to regulate gene expression in the mitochondria. A growing body of research suggests that TERT has important effects on mitochondrial function in a telomere-independent manner (14, 52-55); however, the mechanism by which TERT protects mitochondrial function is not completely understood. Whether TERT-RMRP or TERT-RNAs (non-RMRP) are required for mitochondrial function under physiological or pathological conditions remains to be elucidated.

TERC IN THE REGULATION OF GENE EXPRESSION

Kashani-Sabet's group showed that stable suppression of *mTERC* in the murine melanoma B16 cell line resulted in the significant downregulation of 138 genes, 8 of which are involved in the glycolytic pathway. Interestingly, changes in the expression of glycolytic enzymes indicated a functional link between telomerase and cancer metabolism (56). Cancer cells frequently experience endoplasmic reticulum stress, such as glucose deprivation conditions; therefore, the association of hTERT expression with the glycolytic pathway may contribute to the resistance of cancer cells to regulation by the tumor microenvironment. Blackburn's group demonstrated that targeting *hTR* in the human colon cancer HCT116 cell line resulted in the suppression of specific genes that have been implicated in angiogenesis and metastasis (57). It seems that the telomerase template RNA (*TERC*) can also participate in the telomerase-mediated NF-κB transcription. The expression of some NF-κB target genes is decreased in the *TERC* knockout mice, similar to the *TERT* knockout mice (30). The concrete mechanism by which telomerase participates in the regulation of NF-κB target genes requires further investigation.

CONCLUDING REMARKS

The main function of TERT is to maintain the telomere. However, accumulating evidence indicates that TERT may also have some functions that are independent of its telomerase activity. A number of studies suggest that telomerase plays a role in the regulation of gene expression in a telomere-independent manner (Fig. 1). Telomerase/TERT may act as a transcription modulator through its interaction with transcription factors, such as p65, β-catenin or BRG1; and may regulate certain gene transcription programs, as described for the NF-κB signaling pathway, and the Wnt/β-catenin pathway. TERT may be able to form different complexes in different cell contexts, to participate in the regulation of gene expression in certain pathways. Additionally, the function of TERC in the regulation of gene expression is not clear. It appears that TERT can form complexes with or without *TERC*. It will be interesting to learn whether TERC is required for the TERT-mediated gene transcription regulation. However, changes in telomerase/TERT levels, in response to certain physiological or pathological conditions, may also regulate telomere length or state in a transient fashion, which subsequently affect the telomeric

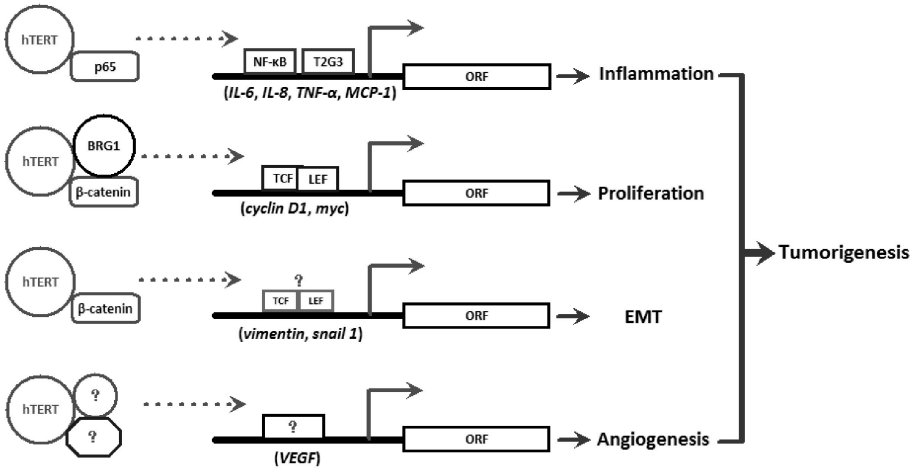

Fig. 1. hTERT in regulation of gene expression.

or sub-telomeric chromatin, and in turn, can regulate gene transcription. Whether telomerase/TERT directly participates in gene transcription is currently under debate, and results from the different research groups are controversial. Future work should include detailed biochemical studies and model systems to confirm the existing data, and to understand the biological significance.

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