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Review

Feedback regulation of telomerase reverse transcriptase: New insight into the evolving field of telomerase in cancer

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ABSTRACT

Telomerase reverse transcriptase (TERT) is the catalytic component of telomerase, especially the rate-limiting determinant of telomerase activity. So far, TERT has been reported to be over-expressed in more than 90% of cancers, thereby playing a critical role in sustained proliferation and survival potentials of various cancer cells. Over the past decade, a comprehensive network of transcription factors has been shown to be involved in the regulation of TERT. Furthermore, accumulating evidence has suggested that TERT could modulate the expression of numerous genes involved in diverse group of cellular processes, including cell cycle regulation and cellular signaling. Therefore, it indicates that TERT is both an effector and a regulator in carcinoma. However, the mechanisms of the interaction between TERT and its target genes are still not fully understood. Thus, it is necessary to consolidate and summarize recent developments of the cross-talk between TERT and related genes in cancer cells or other cells with cancer cell characteristics, and elucidate these relevant mechanisms. In this review, we focus on various signaling pathways and genes that participate in the feedback regulation of TERT and the underlying feedback loop mechanism of TERT, further providing new insights into non-telomeric functions of telomerase and potentially to be used as a novel therapeutic target for cancer.

Contents

Abbreviations: TERT, telomerase reverse transcriptase; RNP, ribonucleoprotein; TR, telomerase RNA; CSCs, cancer stem cells; TEN, telomerase essential N-terminal; TRB, TERT RNA-binding; RT, reverse transcriptase; CTE, C-terminal extension; DAT, dissociates activities of telomerase; pRb, phosphorylation of the retinoblastoma protein; RNAi, RNA interference; SCLC, small cell lung cancer; TRAP, telomeric repeat amplification protocol; TRF, terminal restriction fragment; APC, adenomatous polyposis coli; GSK3β, glycogen synthase kinase 3β; Frz, frizzled; LRP5/6, low-density lipoprotein receptor-related protein 5/6; Tcf-Lef, T-cell factor-lymphoid enhancer-binding factor; ES, embryonic stem; BRG1, Brahma-related gene 1; SMARCA4, SWI/SNF-related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 4; Klf, Kruppel-like factor 4; EMT, epithelial-mesenchymal transition; IκBα, inhibitor of NF-κB; IKK, inhibitor of nuclear factor kappa-B kinase; IL-6, interleukin-6; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α; PI3K, PtdIns3 kinase; RTKs, receptor tyrosine kinases; VEGF, vascular endothelial growth factor; ATL, adult T-cell leukemia; HIF1-α, hypoxia inducible factor 1α; COX-2, cyclooxygenase-2; PTEN, phosphatase and tensin homologue deleted from chromosome 10; TGF-β, transforming growth factor-β; Jab1, c-Jun activation domain-binding protein-1; MMP-9, matrix metalloproteinase-9; ECM, extracellular matrix; FAK, focal adhesion kinase; bHLHZip, basic helix–loop helix–leucine-zipper.

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1. Introduction

The ends of linear eukaryotic chromosomes are formed by a special heterochromatic structure, known as the telomere, which protects them from degradation and therefore plays a pivotal role in ensuring chromosome stability and integrity [1,2]. Most somatic cells have a finite proliferative capacity, largely caused by the inability of DNA polymerase to replicate the distal ends of chromosomes, finally leading to a continuously shortening of telomere after each cell division. However, immortal tumor cells overcome this barrier mainly by increasing telomerase transcription, a specialized reverse transcriptase which can extend telomeric ends [3]. So far, telomerase activation is observed in over 90% of human cancers, while most normal tissues and cells contain inactivated telomerase [4]. It has been known that the catalytic core of telomerase is a ribonucleoprotein (RNP) composing of at least two components, the telomerase reverse transcriptase catalytic subunit (TERT) and the telomerase RNA (TR), which act as a template for the synthesis of TTAGGG repeats by TERT [5,6].

It has long been recognized that TERT was a highly attractive target for the development of cancer therapeutics [7,8]. Recent major advances in the rapidly evolving field of cancer stem cells (CSCs) have opened novel insights and exciting opportunities for telomerase-targeted cancer therapies [9,10]. Similar to normal stem cells, CSCs also have the ability to self-renew as well as undergo differentiation to give rise to the diverse phenotypes of cancer cells. Thus, although CSCs are maintained at low numbers in most tumors, the treatment of human cancer with TERT inhibition may shift the population of stem cell from a maintenance mode to a depletion mode, eventually resulting in loss of the putative stem cell population. In a word, TERT is a universal therapeutic target for the CSCs as well as the bulk of the tumor [11–13].

Apart from the telomere elongation, many biological functions of TERT have been shown to be associated with tumorigenesis and tumor progression. During the past decades, a large body of evidence has accumulated concerning the non-telomeric functions of TERT, independently of telomere maintenance. These functions included regulation of gene expression [14–16] and modulation of cellular signaling [17,18] or cell cycle regulation [19], protection of mitochondria and mtDNA [20,21], inhibition of apoptosis [22–24] and modulation of DNA damage response [25]. After the discovery of TERT, it took almost twenty years for scientists to realize that TERT is not a final effector. High throughput analyses of gene expression showed that TERT could modulate the expression of about 300 genes, including genes involved in cellular processes ranging from cell cycle regulation to cellular signaling and cell proliferation [26]. Thus, the functions and importance of TERT were evidently underestimated. The biological significance of these findings implicated novel molecular mechanisms of TERT functions in many essential cellular processes by targeting genes and pathways besides its well defined function in telomere biology for cancer.

Meanwhile, when research of the structure and function of TERT had begun, attempts to regulate the expression of TERT were initiated soon. Nevertheless, TERT expression is subject to multiple factors which may be crucial regulators of TERT in cancer cells or other cells with cancer cell characteristics [27–30]. Further discoveries have led to characterization of various genes and pathways that participate in the negative or positive feedback regulation of TERT. However, the mechanisms of the interaction between TERT and its target genes are still not fully elucidated. Deciphering the network of TERT interacting factors is necessary to better understand the non-telomic function of telomerase in tumorigenesis. In this review, we highlight some pathways and genes which existed in the underlying feedback loop of TERT regulation, further potential to be used as a novel therapeutic target for telomerase-based anti-cancer therapy.

2. The structure and function of hTERT

hTERT is encoded by a single copy gene, mapped to the short arm of human chromosome 5 (5p15,33), more than 2 Mb away from the telomere [31]. The gene consists of 15 introns and 16 exons spanning about 40 kb [32,33]. Sequence analysis revealed that the hTERT promoter lacked both TATA and CAAT boxes, but contained many binding sites for several transcription factors, including the suppressor genes p53 and p21, and the oncogenes Sp1 and c-Myc [34,35]. The core region of hTERT promoter extends from 330 bp upstream of the translational start site to 37 bp of the second exon [32]. Furthermore, this regulatory region as well as upstream sequences interplays with various regulators of hTERT *via* an abundance of transcriptional binding sites [33].

In addition, the protein encoded by hTERT consists of 1132 amino acid residues. Current models define four distinct domains of hTERT protein based on various functions [36]. These are: telomerase essential N-terminal (TEN) domain; TERT RNA-binding (TRB) domain; reverse transcriptase (RT) domain; and TERT C-terminal extension (CTE) domain. These regions are all responsible for the telomere lengthening function of telomerase. The TEN domain is indispensable for appropriate action of telomerase at the telomere, as mutations in the dissociated activities of telomerase (DAT) region abolish telomere lengthening *in vivo* but not the catalytic activity. The TRB domain contains several conserved RNA binding sequences, including the telomerase-specific T motif which is required for binding of TERC. The RT domain contains five conserved RT motifs that are responsible for telomerase activity. The CTE domain participates in numerous protein–protein interactions and regulates enzyme localization and processivity. Together, these domains contain seven functional motifs and one telomerase-specific T motif. It has been demonstrated that these motifs in human telomerase are the vital determinant of enzymatic activity.

Transfection of hTERT into normal telomerase-negative human cells could protect the cells from senescence, thereby extending the replicative life span [37]. Furthermore, introduction of hTERT into U2OS cells, a hTERT-negative malignant cell line, enabled telomerase activity and further promoted their invasive and metastatic potential [38]. Meanwhile, hTERT-transduced cells had a significantly increased expression of cell proliferation-related signaling pathways and genes. Conversely, the inhibition of hTERT expression remarkably abrogated the cell viability in the short term [39].

3. The feedback regulation of TERT *via* cellular signaling

3.1. pRb/E2F pathway

Cell proliferation is regulated by cell cycle. Before cell mitosis and division, the cell is required to pass through several checkpoints, including the pivotal G_1/S restriction point governed by the successive phosphorylation of the retinoblastoma protein (pRb) [40,41]. This phosphorylation is controlled by the interaction between CDKs and cyclin D or E. Upon mitogenic stimulation, cyclin D activates its associated CDK4

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