RESEARCH HIGHLIGHT

Telomerase exerts physiological and tumor promoting functions by stimulating ribosomal biogenesis

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The enzymatic activity of telomerase counters erosion of telomeric DNA that occurs as a biochemical consequence during replication. This primary function of telomerase resolves the ‘end-replication problem’ and prevents telomere dysfunction and replicative senescence ensuring cellular immortality. Most human cells lack telomerase activity but high telomerase activity can be detected in the vast majority of human cancers. Moreover, telomerase activity is a pre-requisite of sustained organ maintenance under physiological hyper-proliferative conditions such as liver regeneration and for the proliferation of activated B- and T- cells. Increasing evidence indicates at telomere length independent functions of telomerase, including DNA-damage repair, mitochondrial function and stem cell activity. Mechanisms of how telomerase can fulfill such diverse functions remain yet to be elucidated. Our recent findings showing that telomerase activates ribosomal biogenesis under oncogenic and regenerative conditions can shed a light to some of these questions.

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Telomerase has a pivotal function in maintaining chromosome integrity. The canonical function of telomerase reverse transcriptase is to assist the DNA replication machinery for complete synthesis of telomeric DNA to prevent loss of genetic material ^[1]. In humans, telomerase activity can be detected in embryonic tissues but is limited to the primitive stem cells and to some specialized cells with high proliferative capacity in adults ^[2-7]. In the absence of telomerase activity, telomere shortening is a biological barrier that limits proliferative capacity of checkpoint proficient human cells by the activation of cellular senescence ^[8-12]. In experimental models, depletion of telomerase results in telomere shortening, limiting self renewal capacity of stem cells, exhaustion of the stem cell pool and finally promoting premature aging whereas dysfunctional telomeres can cause age-related pathologies, depending on the functionality of the DNA-repair machinery ^[13-16]. Telomerase reactivation occurs during cellular

transformation to stabilize telomere function (in a subset of tumors, an alternative mechanism, the recombination based ALT pathway ensures telomere maintenance) ^[17-20]. Consequently, strong telomerase activity can be detected in most human tumors, indicating that the suppression of telomerase activity is a tumor protective mechanism ^[21, 22], see also ^[23, 24] for review. In line with this hypothesis, it was shown that telomerase activity is needed for tumorigenic conversion of primary human cells ^[25].

Accumulating evidence indicates that telomerase holoenzyme or its components TERT (the catalytic subunit of telomerase) and TERC (the telomerase RNA component) may also exert some fundamental biological functions independent of their role in telomere maintenance, including DNA-damage response, cell survival and apoptosis ^[26-32]. Importantly, studies from the Artandi laboratory indicated that TERT activates the proliferation of epidermal stem cells

in a transgenic mouse model independent of its the catalytic enzyme activity and in the absence of TERC^[33]. This function of TERT seems to involve the activation Wnt- β -catenin signaling pathway^[34, 35]. However, studies in two independent laboratories questioned these observations^[36, 37]. It remains to be shown whether the regulation of Wnt- β -catenin pathway by TERT has relevance under physiologic conditions. In a recent study, it was demonstrated that telomerase is involved in the regulation of the NF κ B signaling pathway^[38]. Of note, this study provides evidence that telomerase holoenzyme, rather than the TERT protein alone, is important for the NF κ B-dependent gene regulation.

Mutations in all three telomerase core components (TERT, TERC and DKC1) have substantial effects on cell proliferation in humans, resulting in stem cell exhaustion, aging phenotypes and cancer^[39-43]. Interestingly, mutations that impair telomere function were also found in NHP2 and NOP10, like DKC1, nucleolar factors that are associated with telomerase^[44-47]. Although most of these studies addressed the telomere length dependent function of telomerase components, it remains to be studied if specific mutations may impair non-telomeric functions of telomerase. The TERT subunit harbors a nucleolar localization signal^[48] and functional telomerase is assembled in the nucleolus during the G1/early S-phase and transported to the telomeres during the S-phase^[49-52]. Together, these data extensively document the localization of telomerase in nucleoli.

We hypothesized that the nucleolar localisation of telomerase may indicate a function beyond its telomere elongating activity. In nucleoli, transcription of ribosomal DNA is executed by the activity of RNA polymerase I (Pol I) that can account for up to 70% of the whole transcription in a growing cell. As ribosomal biogenesis is vital for growth and proliferation^[53] and telomerase stimulates cellular proliferation, we asked if telomerase exerts this effect through stimulating rDNA transcription.

Our analyses revealed a significant association of TERT/telomerase with rDNA promoter and gene-internal sites in ChIP experiments^[54]. Further on we used ChIP/re-ChIP and co-immunoprecipitation experiments to demonstrate a close interaction of RNA polymerase I and telomerase. These initial proof-of-principle experiments were performed with endogenous and ectopic expression of TERT in 293 cells, a transformed human cell line that forms tumors in nude mice. Altogether, these first results proved that TERT binds to the rDNA promoter and physically interacts with Pol I. Importantly, overexpression of TERT in normal human fibroblasts activated robust telomerase activity but did not result in rDNA binding of telomerase, indicating a

specific activation of these interactions under certain conditions. To assess the physiological relevance of these interactions we investigated TERT binding in resting and regenerating mouse liver -after partial hepatectomy- in mice and by transformation of human fibroblasts by the previously described protocol by the Weinberg group^[25]. These data clearly demonstrated the association of telomerase with rDNA and Pol I under hyper-proliferative conditions, in the regenerating liver and in transformed cells. These findings were further confirmed by ChIP experiments with mouse and human hepatocellular carcinoma specimens. Of note, strong telomerase activity is detectable in resting mouse liver and TERT-immortalized, non-transformed human primary cells but no impact on rDNA transcription or Pol I association was observed in these samples. Interestingly, telomere sequences in the ChIP precipitates of telomerase could only be detected in tumors, whereas no telomeric localization of telomerase was detectable in normal mouse liver that is also positive for telomerase. These findings indicate that telomere elongation and rDNA transcription by telomerase are co-regulated events. Transfection experiments with a rDNA reporter plasmid revealed that TERT strongly stimulates Pol I transcription in an oncogenic context and that this stimulation requires the presence of TERC, as the depletion of TERC or transfection of TERT in TERC negative cells severely decreased Pol I activity and impaired binding of telomerase to the rDNA. Together, these results provide strong evidence that the telomerase holoenzyme stimulates rDNA transcription in an oncogene dependent manner. Mechanistic studies with in vitro transcription assays where partially purified telomerase was admixed to cell free reporter assay revealed that telomerase enhances initiation complex formation by elevating the amount of Pol I at the rDNA promoter. During the transcription cycle TERT remains bound at the promoter and does not seem to migrate with the Pol I enzyme. The cellular consequences of the absence of telomerase were addressed by shRNA-mediated knock-down or dominant-negative inhibition of telomerase showing that these conditions reduce the ribosomal content and retard growth of tumor cells in a manner comparable to the inhibition of Pol I transcription itself thus indicating that the growth promoting properties of telomerase^[55] might be at least partially mediated by its influence on ribosomal biogenesis.

Together, our results reveal a novel physiological function of telomerase in regulation of ribosomal biogenesis in response to hyper-proliferative signals and support the idea that tumor cells abuse telomerase to stimulate ribosomal transcription and to enhance protein synthesis capacity of the cell for unrestrained growth. It is an exciting idea to target the nucleolar function of telomerase in cancer cells for tumor therapy without interfering with its telomere maintenance

capacity in telomerase positive normal cells. One important question in this context is how telomerase negative cancer cells (ALT cells) manage to boost ribosomal biogenesis. Another interesting question that needs to be addressed is whether this novel function of telomerase is of physiological relevance in the stem cells, the major telomerase positive cell population in human tissues. Interestingly, telomerase mutations (e.g. dyskeratosis congenita) are followed by bone marrow failure, a symptom that is a hallmark of ribosomopathies (Diamond-Blackfan Anemia) ^[56]. One fascinating area of future research is therefore to explore potential telomere length independent contributions of telomerase to the pathomechanism of dyskeratosis congenita.

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