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Review

Alternative mechanisms of telomere lengthening: Permissive mutations, DNA repair proteins and tumorigenic progression

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ABSTRACT

Telomeres protect chromosome termini to maintain genomic stability and regulate cellular lifespan. Maintenance of telomere length is required for neoplastic cells after the acquisition of mutations that deregulate cell cycle control and increase cellular proliferation, and can occur through expression of the enzyme telomerase or in a telomerase-independent manner termed alternative lengthening of telomeres (ALT). The precise mechanisms that govern the activation of ALT or telomerase in tumor cells are unknown, although cellular origin may favor one or the other mechanisms. ALT pathways are incompletely understood to date; however, recent publications have increasingly broadened our understanding of how ALT is activated, how it proceeds, and how it influences tumor growth. Specific mutational events influence ALT activation, as mutations in genes that suppress recombination and/or alterations in the regulation of telomerase expression are associated with ALT. Once engaged, ALT uses DNA repair proteins to maintain telomeres in the absence of telomerase; experiments that manipulate the expression of specific proteins in cells using ALT are illuminating some of its mechanisms. Furthermore, ALT may influence tumor growth, as experimental and clinical data suggest that telomerase expression may favor tumor progression. This review summarizes recent findings in mammalian cells and models, as well as clinical data, that identify the genetic mutations permissive to ALT, the DNA repair proteins involved in ALT mechanisms and the importance of telomere maintenance mechanisms for tumor progression. A comprehensive understanding of the mechanisms that permit tumor cell immortalization will be important for identifying novel therapeutic targets in cancer.

Contents

Abbreviations: ALT, alternative lengthening of telomeres; APB, ALT-associated PML body; ECTR, extrachromosomal telomere repeat; GBM, glioblastoma multiforme; MEN1, multiple endocrine neoplasia type 1; MRN, MRE11/RAD50/NBS1; PanNET, pancreatic neuroendocrine tumor; TIF, telomere dysfunction-induced focus; TRAP, telomere repeat amplification protocol.

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1. Introduction

Telomeres are DNA-protein structures that protect chromosome ends and are composed of $(TTAGGG)_n$ sequence repeats in vertebrates [1]. Telomeres protect DNA by concealing the chromosome end in a looped structure resembling a replication D-loop. The 3′ single-stranded telomere overhang folds back and invades a double-stranded telomere region to create a T-loop [2]. This structure is maintained by a number of telomere-binding proteins, including telomere repeat binding factor 1 (TRF1), telomere repeat binding factor 2 (TRF2), and protection of telomeres 1 (POT1). These proteins, along with adrenocortical dysplasia protein homolog known as TPP1, TRF1-interacting nuclear protein 2 (TIN2), and TRF2-interacting telomeric protein 1 (RAP1), form the shelterin complex [3]. Shelterin functions as a telomere cap that distinguishes the chromosome end from a DNA break, protecting chromosomal integrity.

Telomeres prevent the eventual loss of coding DNA due to the end replication problem, a replicative limitation that yields progressive telomere shortening with each round of cell division [4]. Progressive telomere shortening accompanies organismal aging [4–6], while rapidly dividing cells actively maintain their telomeres. For example, the high proliferation rate of neoplastic cells necessitates the maintenance of telomere length to facilitate immortalization and tumorigenesis. Telomere maintenance is thus not only critical for genomic stability, but also represents a key step in tumor cell immortalization.

In human cells, two telomere maintenance mechanisms are known: expression of telomerase or activation of telomerase-independent pathways termed alternative lengthening of telomeres (ALT). Telomerase is an enzyme expressed during development that catalyzes the addition of telomere repeats to chromosome ends. The enzyme is primarily composed of a reverse transcriptase catalytic subunit (TERT) and an RNA template (TERC). Post-neonatal human somatic cells repress telomerase expression [7,8], although telomerase continues to be expressed in proliferative cells such as germ cells and stem cells. Most neoplastic cells de-repress telomerase expression to support immortalization and tumor formation [7,9], although some neoplastic cells use telomere recombination or ALT [10] for the addition of telomere repeats without telomerase activity. High levels of telomere recombination characterize ALT cells [11]; both inter-telomeric [12] and intra-telomeric [13] recombination have been observed in immortalized human ALT cell lines, demonstrating that telomeres can use unique templates in their maintenance. ALT cells contain both linear and circular extrachromosomal telomeric repeats (ECTR) [14] that may represent other telomeric templates for recombination.

ALT cells are characterized by the ability to maintain telomeres in the absence of telomerase and by highly heterogeneous [10,15,16] telomere lengths [17,18]. The majority of ALT cells contain ALT-associated promyelocytic (PML) bodies (APBs) that differ from PML bodies in other cell types by the inclusion of telomeric DNA and proteins [19]. ALT cells often display spontaneous DNA damage at the telomere, localized to foci called telomere-dysfunction-induced foci (TIF), and sometimes overexpress mitochondrial regulators due to prevailing mitochondrial dysfunction [20]. Although a definitive enzymatic assay for ALT recombination has not been developed, ALT is characterized by heterogeneous telomere lengths, APBs, telomere recombination and/or ECTR.

The majority of human tumors express telomerase. However, the likelihood of one or the other telomere maintenance mechanism varies with specific tumor types—cancers that are mesenchymal in origin more frequently activate ALT, while carcinomas, epithelial in origin, more frequently express telomerase [21,22]. Osteosarcomas have the highest incidence of ALT, with 59% of cases exhibiting ALT characteristics. Many, but not all, soft tissue sarcomas display ALT characteristics (27% overall), along with gastric carcinoma (38%), low-grade (grade 1–3) astrocytoma (37%), and diffuse malignant pleural mesothelioma (17%). The variation of ALT frequency associated with tumor type suggests that cell-specific differences favor one telomere maintenance mechanism over another. For example, mesenchymal stem cells express little to no detectable telomerase [23] and may predispose cells from this lineage to use ALT due to a continued chromatin-mediated repression of telomerase expression [24]. Although cell type-specific differences may exist, the question of how cells activate one mechanism over the other remains unresolved.

Experimental evidence from cell culture and mouse models demonstrates that following the primary genomic alterations that confer an increased proliferative capacity and a high tolerance for subsequent genomic alterations, neoplastic cells activate a telomere maintenance mechanism. Primary human adrenocortical cells transformed with *H-RAS* and *SV40* form tumors following implantation in immunodeficient mice, although subsequent activation of telomerase is required to prevent crisis or the cellular senescence induced by telomere shortening [25]. In a hepatocellular carcinoma mouse model, genomic instability in the absence of telomerase expression favors tumor initiation, although these tumors display a limited ability for progression [26]. Telomere dysfunction in $Terc^{-/-}$, $p53^{+/-}$ or $Terc^{-/-}$, $p53^{-/-}$ cells is associated with a loss of cell cycle checkpoint control in mouse embryonic fibroblasts (MEFs) [27] and the promotion of epithelial tumors in mice [28]. These tumors exhibit greater genomic instability than tumors arising without telomere dysfunction [29], suggesting that telomere dysfunction may permit tumor initiation and promote the acquisition of additional mutations that drive tumor progression. Furthermore, histologically dysplastic but non-invasive regions of human skin, breast and colon cancers demonstrate colocalization of DNA damage markers with telomeres, although telomere dysfunction is absent in the adjacent malignant or invasive tumor [30]. These data support a model in which early mutations in neoplastic cells enhance proliferative capacity, genomic instability and telomere shortening, creating a bottleneck for the activation of a telomere maintenance mechanism to promote tumor cell immortalization (Fig. 1). Activation of telomere maintenance represents a key target for limiting tumorigenesis.

2. How is ALT activated?

Specific genetic alterations in tumors can be associated with a higher likelihood for telomerase- or ALT-associated mechanisms of telomere maintenance (Fig. 2). The regulation of genes encoding telomerase components has been extensively studied and is governed by complex interactions of transcription factors, signaling pathways [31] and epigenetic regulation [32]. These data suggest that mutations affecting telomerase regulation may facilitate ALT activation. Additionally, mutations associated with ALT activation are found in genes that suppress recombination, also facilitating recombination-mediated telomere maintenance. These genes include *p53, ATRX, DAXX* and *H3F3A*, the latter three encoding proteins that affect chromatin remodeling at the telomere.

2.1. p53 mutation

Alteration or loss of function of the tumor suppressor p53 is a common event in many tumors and tumor-derived cell lines regardless of the telomere maintenance mechanism employed. *In vitro* and *in vivo* studies suggest, however, that impaired p53 may be associated with ALT activation. p53 is a DNA binding and signaling protein that alters transcription of target genes, controls