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Means to the ends: The role of telomeres and telomere processing machinery in metastasis

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Abstract

Despite significant clinical advancements, cancer remains a leading cause of mortality throughout the world due largely to the process of metastasis and the dissemination of cancer cells from their primary tumor of origin to distant secondary sites. The clinical burden imposed by metastasis is further compounded by a paucity of information regarding the factors that mediate metastatic progression. Linear chromosomes are capped by structures known as telomeres, which dictate cellular lifespan in humans by shortening progressively during successive cell divisions. Although telomere shortening occurs in nearly all somatic cells, telomeres may be elongated *via* two seemingly disjoint pathways: (i) telomerase-mediated extension, and (ii) homologous recombination-based alternative lengthening of telomeres (ALT). Both telomerase and ALT are activated in various human cancers, with more recent evidence implicating both pathways as potential mediators of metastasis. Here we review the known roles of telomere homeostasis in metastasis and posit a mechanism whereby metastatic activity is determined by a dynamic fluctuation between ALT and telomerase, as opposed to the mere activation of a generic telomere elongation program. Additionally, the pleiotropic nature of the telomere processing machinery makes it an attractive therapeutic target for metastasis, and as such, we also explore the therapeutic implications of our proposed mechanism.

Keywords

Alternative lengthening of telomeres; DNA damage; DNA repair; Epithelial-mesenchymal transition; Metastasis; Signal Transduction; Telomeres; Telomere Homeostasis

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1. Introduction

When considered as a single disease, cancer is one of the leading causes of global mortality, with an estimated 14.9 million new cases and 8.2 million deaths attributable to cancer each year [1]. The incidence of many cancers is increasing in both developed and developing nations due in part to the prevalence of risk factors (*e.g.*, tobacco and obesity) in an expanding and increasingly aging population [2]. Metastasis, while comprising only a fraction of this growing clinical burden, is responsible for the overwhelming majority of cancer mortality. Indeed, although the rates of diagnosing metastatic disease are typically low in many cancers (<10–30 percent; [3–5]), approximately 90 percent of cancer-related deaths are attributable to metastasis [6]. The underlying lethality of metastasis reflects its molecular complexity, which has greatly limited the success of therapies targeting this process in both overt disease and adjuvant settings [7–9]. Thus, there remains a significant unmet need for novel therapeutic approaches to target metastasis.

Metastasis is most accurately thought of as a cascade of systemic and cellular events undertaken by a subset of cells within the primary tumor [10, 11]. Generally speaking, metastatic cells become liberated from well-vascularized, angiogenic primary tumors and undergo intravasation to gain access to the circulation, where they persist in the blood, lymph, or bone marrow. Upon reaching their target tissue, disseminated cells extravasate and initiate growth of pre-angiogenic “micrometastases” before fully colonizing the metastatic niche upon reinstatement of angiogenesis [10]. The classical view of metastasis as the terminal stage of cancer progression suggests that a subpopulation of primary tumor cells progressively acquire genetic alterations necessary for their dissemination and colonization, and that these cells remain rare until clonally expanded within secondary organs [12]. However, recent evidence indicates that the capacity of tumor cells to metastasize is present in the earliest stages of primary tumor development [13, 14] and that these variant cells are often genetically divergent from their primary tumor counterparts and from one another [15–18]. In many respects, metastases may be considered as discrete entities from their primary tumors of origin due in part to their acquisition of genomic alterations during dissemination and distant organ colonization, suggesting that distinct regulatory pathways are operant during metastasis *versus* those active in primary tumor development [19].

Telomeres have long been implicated in driving tumorigenesis, yet emerging evidence indicates that the established concept whereby telomeres and their homeostatic machinery serve solely as cellular “immortalizers” may be drastically oversimplified. Indeed, telomeres and telomeric proteins subserve diverse functions in many of the stages that define the metastatic cascade. Herein we examine the varying roles that telomeres play in driving the dissemination and interaction of cancer cells with the metastatic microenvironment. We also discuss the therapeutic potential of targeting telomeres as a novel means to alleviate metastatic disease.

2. Metastasis at the cellular level

The metastatic cascade is defined by the following sequence of events: (*i*) primary tumor angiogenesis; (*ii*) cancer cell migration away from the primary tumor and intravasation into

the tumor vascular supply; *(iii)* cancer cell survival within the circulation; *(iv)* extravasation of circulating tumor cells at secondary organs; and *(v)* proliferation of disseminated tumor cells (DTCs) at these secondary sites [19]. Each of these stages is spatially and temporally regulated by a host of cancer cell-intrinsic and -extrinsic (microenvironmental) signaling inputs (Fig. 1). The initial dissemination of cancer cells is reliant upon the development of a tumor blood supply, a process known as angiogenesis. Neovascularization involves both the intussusception of the tumor into the surrounding vasculature and the recruitment of endothelial cells and other vascular precursors required to form new vessels [20]. This process is driven largely by the secretion of vascular endothelial growth factor (VEGF) and angiopoietin (Ang) family members by cancer or stromal cells, and by the endothelium of preexisting vessels [21, 22]. The spread of cancer cells is further restricted by a complex network of extracellular matrix (ECM) and proteoglycan-rich basement membrane. This network is readily remodeled by secreted matrix metalloproteinases (MMPs) in response to mechanical forces or chemical stimuli, including inflammatory cytokines and reactive oxygen species (ROS) [23]. In addition, MMPs have been implicated in regulating cell growth, thus disrupting the normal balance between proliferative and cytostatic signals. For instance, extracellular proteases release latent epidermal growth factor (EGF), which subsequently signals through its receptor (EGFR) and downstream effectors, phosphatidylinositol 3-kinase (PI3K), AKT, and the mitogen-activated protein kinases (MAPK) ERK1/2. Collectively, these signals coalesce to activate proliferative programs, as well as propagate MMP production [24, 25]. MMPs 2, 9, and 14 also proteolytically activate latent transforming growth factor- β (TGF- β), which in turn stimulates cancer cell invasion and epithelial-mesenchymal transition (EMT) in carcinomas [26–29]. EMT is a process whereby epithelial cells shed their native polarity and adhesive properties and adopt the migratory and invasive features of mesenchymal stem cells [29]. There is substantial evidence that EMT is essential in cancer cell dissemination, as EMT promotes invasion *via* alterations in the expression of cell adhesion proteins (*e.g.*, epithelial cadherin (E-cad) and β 1 integrin) [30–32]. Intravasation is mediated by many of the same factors that control angiogenesis and cell migration, including MMPs 1, 2, and 3, TGF- β , and VEGF and angiopoietin family members, as well as the EGF-related peptide, epiregulin (EREG) [33–35].

While persisting in isolation or as small clusters within the vasculature, circulating tumor cells (CTCs) must activate pro-survival programs, as well as escape immune detection [33]. AKT serves as the master regulator of CTC survival, doing so by suppressing apoptotic signals, and by blocking anoikis. These survival signals largely derive from inputs initiated by EGFR and VCAM-1 (vascular cell adhesion molecule 1) present on the surface of DTCs [36–38]. Interestingly, the survival of DTCs within secondary organs requires them to cooperate with cells in the newly colonized tissue, including stromal and resident immune cells [39]. Crosstalk between these malignant and normal cell types forms the so-called metastatic niche; however, not all cellular niches are permissive to DTC outgrowth. Indeed, some DTCs are maintained as single cells in a quiescent or senescent state within their niche, while others exist as small “micrometastases” whose growth is controlled by pro- and anti-angiogenic signals, and by the activation status of resident immune responses [40]. Collectively, these two phenomena define the stage of metastasis known as dormancy.

On a molecular level, the transition from dormancy and overt metastasis may reflect an intricate balance between the activation of proliferative and stress response pathways in DTCs, with proliferation being driven largely by ERK1/2 and stress responses by p38 MAPK [41]. The activation of ERK1/2 in dormant DTCs is mediated by EGFR and focal adhesion kinase (FAK), which functions synergistically with the Wnt and Notch pathways [42–44]. In contrast, p38 MAPK is responsive to microenvironmental stressors, such as oxidative stress and inflammation, and induces an endoplasmic reticulum stress response while simultaneously inhibiting ERK1/2 activation [40, 45–48]. Dormant states are also maintained by stromally-derived signals, such as bone morphogenetic proteins (BMPs), which diminish Wnt signaling in a p53-dependent manner [49, 50]. In addition to their role in metastatic dormancy, canonical Wnt-dependent and -independent activation of β -catenin fulfill pivotal functions along the entire metastatic cascade (Fig. 1; [51]). For instance, β -catenin is a critical mediator of both cell proliferation *via* Wnt, and of cell adhesion *via* E-cad and other cell adhesion molecules [52, 53]. Thus, the balance between proliferative and adhesive inputs that activate β -catenin influences cancer cell migration, intravasation, and invasion [54–56]. Furthermore, Wnt signaling stimulates EMT and regulates both cellular and immune-mediated micrometastatic dormancy [57, 58]. As we discuss in detail below, these links between Wnt/ β -catenin signaling and metastasis are particularly provocative because Wnt/ β -catenin are known to regulate the expression of multiple telomeric proteins [59–61].

3. Telomeres: Dynamic structures with dynamic functions

Telomeres function as determinants of cellular age and replicative potential [62]. Consequently, aberrant telomere length imparts cells with replicative immortality, one of the hallmarks of cancer [63]. Indeed, abnormal telomere elongation is found in nearly all human cancers, with the vast majority of these exhibiting activation of the reverse transcriptase telomerase, while the remaining cases accomplish this task *via* the recombination-based Alternative Lengthening of Telomeres (ALT; [64–66]). In humans, somatic cells ordinarily undergo a finite number of cell divisions before entering senescence and ultimately undergoing apoptosis, thereby protecting them from accumulating pathologic genomic abnormalities [67]. In contrast, rapidly dividing cancer cells experience pronounced telomere attrition, which cells interpret as DNA double-strand breaks (DSBs). In the absence of functional mediators of the DNA damage response (DDR), such as p53, these cells bypass replicative senescence and enter a phase of widespread genomic instability known as crisis, which is characterized by the emergence of chromosome end-to-end fusions [68]. Reactivation of telomere maintenance programs occurs in a subset of cancer cells once their critical telomere length is reached. This endows these cells with expanded replicative potential and enables their transmission of abnormal chromosomal structures (*i.e.*, amplifications, deletions, translocations, inversions) that arise as a result of iterative chromosomal breakage-fusion-bridge (BFB) cycles [69–71]. Interestingly, recent evidence indicates that telomerase and other telomeric proteins directly modulate many of the key effectors of metastatic progression (*see below*).

Structurally, telomeres are nucleoprotein complexes, and as such, both DNA and protein elements are important regulators of telomere length and stability. In humans, telomeres are

composed of tandem (TTAGGG)_n repeats with an obligate 3' single-stranded overhang on the lagging strand [72]. The 3' overhang invades the telomeric DNA duplex, forming a duplex DNA loop (T-loop) with a terminal triplex structure (D-loop) that protects telomeres from recombination, fusion, and eliciting a DDR [73, 74]. T-loop formation is facilitated by the proteins Telomeric Repeat-binding Factor 1 (TRF1), Telomeric Repeat-binding Factor 2 (TRF2), and Protection of Telomeres 1 (POT1), which in turn are members of the larger shelterin complex that serves to protect telomeres from the DDR and coordinate telomere length homeostasis [75, 76]. TRF1 and TRF2 control T-loop formation by binding to telomeric DNA either as homodimers or oligomers that are capable of bridging distant intratelomeric loci [77, 78]. Other components of the shelterin complex include TRF1-Interacting Nuclear Protein 2 (TIN2), Repressor/Activator Protein 1 (RAP1), and TPP1. Once bound to double-stranded (TRF1 and TRF2) or single-stranded (POT1) telomeric repeat sequences, TRF1 and TRF2 assemble the remaining shelterin proteins to yield functional telomere protection units [76, 79].

Shelterin proteins perform diverse functions at telomeres, including shielding telomeres from degradation, recombination, and DDR activation, as well as controlling telomere length in conjunction with telomerase or ALT-associated factors [76]. Activation of DDRs occurs in response to telomere shortening, thereby reducing the degree of telomere protection by shelterin complexes [80]. Diminished protection of telomeres exposes telomeric double-stranded DNA (dsDNA) and single-stranded (ssDNA) 3' overhangs, which are sensed as DNA damage foci by the PI3K-related kinases Ataxia Telangiectasia Mutated (ATM; [81]) and Ataxia Telangiectasia and Rad3-related (ATR; [82]). ATM and ATR phosphorylate the cell cycle-specific checkpoint kinases, Chk1 and Chk2, whose activation blocks cell cycle progression and the transmission of damaged DNA [76]. The structural specificities of ATM and ATR are underscored by the fact that these proteins are impeded from activating DDRs at telomeres by TRF2 (*i.e.*, ATM) and POT1 (*i.e.*, ATR), whose DNA binding specificities mirror those of their corresponding kinase [83]. Chk1 and Chk2 further cooperate with ATM and ATR to activate p53, which inhibits cell cycle progression through induction of the cyclin-dependent kinase inhibitor p21 [84]. Conversely, the absence of functional p53 perpetuates telomere dysfunction and simultaneously prevents replicative senescence, thereby driving the evolution of cancer cells harboring significant chromosomal instability secondary to telomere crisis [85, 86].

Repairing DNA DSBs in mammalian cells is accomplished by one of two recombination-based mechanisms: (*i*) non-homologous end joining (NHEJ), or (*ii*) homologous recombination (HR). Telomere shortening, concurrent with the activation of the DDR and NHEJ or HR repair pathways, yields chromosome end-to-end fusions [68]. By shielding chromosome ends from being recognized as DSBs, shelterin proteins play an essential role in maintaining genomic integrity. ALT is also a HR-mediated process that is characterized by genomic instability in the presence of an intact DDR [87, 88]. Indeed, telomere elongation via ALT occurs by sister chromatid exchange (T-SCE), resulting in the appearance of heterogeneous telomeres within individual cells that are typically considerably longer than those found in terminally differentiated cells [89, 90]. Moreover, uncontrolled telomere recombination can induce HR within T-loops, resulting in the

production of extrachromosomal DNA circles (C-circles) that serve as markers of ALT activity [91]. As with generalized recombination-based DSB repair, ALT is mediated by the MRN complex (**M**RE11-**R**AD50-**N**BS1; [92]), which may be recruited to telomeres as part of the ALT-associated Promyelocytic leukemia (PML) Body (APB; [93]). PML is a functionally promiscuous protein that can assemble numerous multimeric protein complexes in response to both environmental and cell cycle-dependent cues [94]. The prevailing model suggests that critically short telomeres are recognized and spatially organized within APBs by PML, which also recruits MRN complexes to perform T-SCE [93].

By repressing DNA damage signaling and homology-directed repair at telomeres, shelterin proteins, particularly TRF1, TRF2, and POT1, exert regulatory control over ALT activity. Indeed, changes in expression [95–97] or modification state [98] of these proteins alter T-SCE and APB formation. Because the DDR is intact in cells performing ALT, these cells must bypass replicative senescence and apoptosis. Similar to their telomerase-positive counterparts, ALT-positive cells often carry mutations in *TP53*, the gene encoding p53 [99]. Thus, although DDR kinases (*i.e.*, ATM and ATR) can become activated in response to telomere shortening and uncapping, the absence of functional p53 prevents the acquisition of a senescent phenotype, resulting in ALT activation and cell proliferation [100]. p53 dependence provides a unifying mechanism for telomere maintenance *via* telomerase and ALT; however, the functional diversity of telomerase manifests important differences between these two pathways that may prove to be essential in driving human disease, including the acquisition of metastatic phenotypes.

4. Telomerase: More than a telomere machine

Telomerase is an RNA-dependent DNA polymerase that is composed of two moieties: (i) a RNA component (TR) that serves as a template for telomeric DNA replication, and (ii) a protein component (TERT) that is responsible for polymerase activity. The TERT protein contains four domains, and regions of the N- and C-terminal domains are sufficient to induce cell immortalization, even in the absence of catalytic activity [101]. Indeed, alternative splice variants of TERT lacking its catalytic domain continue to promote cell growth, independently of telomere length [102]. Moreover, Khattar *et al* [103] determined that TERT drives cancer cell proliferation in part by binding to and enhancing the chromatin localization of the RNA polymerase III subunit, RPC32, leading to elevated tRNA expression and increased protein synthesis in cancers of the skin, lung, and liver. These findings indicate that the phenotypic effects of telomerase may stem in part from non-telomeric functions of telomerase, which are termed “extratelomeric” and include its ability to regulate gene expression, modulate intracellular signaling, and transduce microenvironmental stimuli [104]. In the context of cancer, telomerase is not only sufficient to bestow cells with tumorigenic properties, but it is also uniquely poised to regulate multiple processes that have been implicated in metastatic progression (Fig. 2).

Investigations into the effects of TERT abundance on global patterns of gene expression have revealed a cohort of genes whose transcription appears to be directly controlled by TERT, most notably EGFR and VEGF [105, 106]. In addition to the role of VEGF in angiogenesis, EGF and VEGF signaling coalesce to promote cancer cell proliferation [107].

In vitro and *in vivo* studies have confirmed a link between TERT and growth factor signaling, such that genetic depletion of EGFR in TERT-overexpressing human mammary epithelial cells resulted in decreased cell proliferation [105]. Likewise, genetic and pharmacologic inhibition of telomere maintenance decreased VEGF expression and abrogated angiogenesis *in vivo* [108, 109]. Along these lines, TERT functions as a downstream effector of VEGF signaling [110], thus creating a positive feedback loop that can promote cancer cell intravasation and sustain tumor angiogenesis.

The most well-characterized transcriptional effects of TERT revolve around the Wnt/ β -catenin pathway. Indeed, when localized to the promoters of Wnt-dependent genes, TERT physically interacts with SMARCA4, a SWI/SNF-related chromatin remodeling protein that induces the expression of MMP-7, VEGF, and the mitogen-responsive transcription factor c-Myc [59, 111]. In turn, TERT expression is reciprocally controlled by β -catenin [112]. In addition, telomerase activity is greatly enhanced by phosphorylation of TERT by AKT [113], as is its nuclear localization [114]. Thus, AKT may influence both the canonical and extratelomeric functions of telomerase. Indeed, upon gaining entry into the nucleus, TERT may bind directly to nuclear factor- κ B (NF- κ B), thereby enhancing the expression of NF- κ B-dependent targets, particularly MMPs [115, 116]. NF- κ B-dependent MMP expression likely contributes to changes in adhesive properties and increased invasiveness seen in cancer cells treated with telomerase template antagonists [117, 118]. Additionally, NF- κ B acts synergistically with TGF- β to promote EMT and cancer cell dissemination [119, 120], while TERT enhances EMT stimulated by TGF- β by interacting directly with ZEB1, which suppresses E-cad expression [121, 122]. Taken together, these findings demonstrate that TERT functions at the center of an intricate transcriptional network that controls multiple regulators of metastasis.

TERT plays a crucial part in mediating the interactions of DTCs with their surrounding microenvironments. For instance, telomerase activity is increased in response to inflammatory cytokines (*e.g.*, interleukin-6), which also facilitate tumorigenesis, invasion, and NF- κ B-dependent activation of tumor-associated macrophages [115, 123]. Of note, TERT expression has been shown to be suppressed by BMPs, which are prevalent at metastatic sites and negatively regulate DTC outgrowth. Thus, downregulation of TERT *via* BMP signaling may serve as a mechanism for establishing the dormant niche [124, 125]. Mitochondrial function is also reliant on both expression and subcellular localization of TERT, whose association with mitochondria protects cells from oxidative stress [126]. As such, TERT activation may serve to stimulate mitochondrial biogenesis concomitant with increased glucose uptake and utilization [127, 128]. This holds functional significance, as reactive oxygen species (ROS) within primary tumor microenvironments can induce DNA mutations that instill cancer cells with a growth advantage [129], while systemic ROS production serves as a defense against cancer cell dissemination [130]. Thus, one mechanism underlying telomerase-driven metastasis may be a spatiotemporally regulated response to oxidative stress.

As telomere protectors, shelterin proteins dictate the accessibility of telomerase to its substrate. Predictably, each shelterin protein functions as a negative regulator of telomerase-mediated telomere extension [76]. POT1 and TPP1 play specific and well-studied roles in

this process. For instance, POT1 and telomerase both bind to telomeric ssDNA and are, in effect, competitive inhibitors of one another [131]. TPP1 is primarily responsible for recruiting and activating telomerase at shortened telomeres [132]. As such, defects in either of these proteins may preclude telomerase-mediated telomere elongation and shift the cellular TERT reserve toward performing extratelomeric functions. As a separate consideration, some shelterin components function within the same extratelomeric regulatory networks as TERT. Indeed, TRF2 expression is regulated by Wnt signaling [61] and excess TRF2 promotes angiogenesis by binding directly to the promoter of the platelet-derived growth factor receptor- β (PDGFR β ; [133]). Moreover, TIN2 is localized to telomeres *via* its interaction with TPP1 and abolishing this interaction targets TIN2 to mitochondria where it regulates energy metabolism and oxidative stress response [134]. Hence, telomeres may be considered cellular control centers for metastasis, doing so by integrating information regarding replicative potential with programs that guide each step of the metastatic cascade.

5. Telomere homeostasis: Determinant or consequence of metastatic progression?

Associations between cancer and mutations in various telomeric proteins are continually being discovered (Table 1). However, the extent to which these aberrations specifically influence metastatic progression remains unclear. To date, many studies that have examined the association between cancer progression and telomere homeostasis have employed descriptive readouts of telomere dynamics, primarily differences in either telomere length or telomerase expression or activity that are then correlated with tumor and/or metastatic progression [64, 66, 135]. In adopting this approach, these studies failed to address two important questions. First, does the identity of the maintenance program (*i.e.*, telomerase *versus* ALT) influence the natural history of disease progression? Second, at what stage(s) of cancer progression do telomere maintenance mechanisms (TMMs) become activated? We postulate that the selection of TMMs in cancer cells represents a critical determinant of their metastatic capability, such that subsets of TERT-positive cancer cells become more prone to disseminate from primary tumor sites and form overt metastases in distant organs (Fig. 3). Thus, consideration of TMMs may permit more precise diagnostic evaluations of high-risk patients and catalyze the development of novel therapies capable of alleviating these pathways.

It should be noted that while TERT- and ALT-positive cancer cells both readily respond to metastasis-promoting signals (*e.g.*, TGF- β , Wnt, etc.), TERT uniquely functions in binding ZEB1 and amplifying EMT programs stimulated by TGF- β [121, 122]. TERT also interacts with NF- κ B to enhance the production of MMPs, thereby promoting the liberation of carcinoma cells from the primary tumor en route to the systemic circulation [115, 116]. These events are complemented by the formation of nascent blood vessels in response to TERT-driven VEGF production and secretion within the TME [106, 108, 109] (Fig. 3). Interestingly, although ALT-positive cells are incapable of activating the aforementioned ancillary pathways, they nevertheless remain competent in responding to TERT-independent microenvironmental cues that support carcinoma dissemination. Within the metastatic niche,

TERT-positive DTCs to undergo self-renewal *via* the continued secretion of VEGF and resultant activation of a VEGFR2→ERK1/2 signaling axis. A potential consequence of these events manifests in the reactivation of proliferative programs in previously dormant micrometastases, as reflected by high ERK1/2:p38 MAPK activation ratios [41]. Conversely, microenvironmental stressors, such as the production of ROS by resident macrophages, can inversely skew the activation ratio between ERK1/2:p38 MAPK by stimulating p38 MAPK activity, thereby enhancing metastatic latency [40, 45–48]. Protection from oxidative damage is imparted by mitochondrial translocation of TERT [126], and by activation of the Wnt/β-catenin driven survival programs. We posit that in the absence of competing signals that arise from TERT that ALT-positive DTCs may possess elevated susceptibility to cytostatic signaling, particularly those stimulated by BMPs that (i) antagonize Wnt/β-catenin signaling, (ii) repress TERT expression, and (iii) promote DTC dormancy [49, 50, 124, 125]. Moreover, diminished TERT function may render ALT-positive DTCs more susceptible to DNA damage induced by oxidative stress, and to the acquisition of dormant phenotypes *via* ROS-mediated p38 MAPK activation (Fig. 3). Importantly, our model of telomere homeostasis during metastatic progression does not limit individual cancers, or even single cancer cells, to a singular mode of telomere maintenance. Indeed, we envision a scenario wherein DTCs experience a dynamic fluctuation between TERT- and ALT-based signaling, particularly in response to various selective pressures encountered during dissemination, and potentially in response to various therapeutic regimens. Likewise, it remains plausible that ALT-positive cells may express TERT in the absence of TR, thus devoting TERT entirely to the performance of extratelomeric functions (Fig. 3).

There is a dearth of mechanistic data available regarding the link between ALT activation and cancer progression beyond the notion that TMMs drive cellular immortalization. Specifically, the factors (both cell-intrinsic and microenvironmental) that preferentially activate ALT *versus* telomerase remain largely uncharacterized. Clinical observations have revealed that ALT-driven primary tumors tend to be of mesenchymal origin, although carcinomas of the lung, liver, kidney, esophagus, breast, and brain are also observed [99, 136, 137]. Importantly, evidence of ALT is present in only a subset of these cancers, and the presence of ALT carries prognostic value, specifically with respect to disease progression and patient survival [99, 138–140]. In particular, ALT generally predicts for a more indolent clinical course [138, 139]; however, ALT coupled with lost expression of either Daxx (death domain-associated protein) or ATRX (alpha thalassemia/mental retardation X-linked) is strongly associated with shorter disease-free and disease-specific survival, thereby implicating ALT in mediating metastatic progression [140]. Taken together, these findings clearly implicate ALT in mediating metastatic progression. Likewise, tumors that exhibit telomerase activity have been associated with both reduced survival and increased lymph node metastasis [141, 142]. While the mechanisms that underlie these observations remain to be elucidated, there is mounting evidence to suggest that TMM selection substantially influences the rate and severity of cancer progression.

TMMs are classically considered to be static properties of specific cancers, such that tumors typically adopt a single TMM during malignant transformation and maintain this program indefinitely. However, recent studies offer two alternatives to this model. First, ALT may serve as an adaptive mechanism of chemoresistance in some cancers treated with anti-

telomerase agents [143]. Second, matched primary tumors and metastases may rely on different TMMs [144]. Collectively, these findings suggest that telomere elongation may not simply be a necessary condition for tumorigenesis, but instead may be a spatiotemporally regulated balance between telomerase and ALT that gives rise to genetically distinct cancer cells that possess differential metastatic capabilities [145, 146]. Further studies are needed to determine whether telomerase and ALT activation may occur throughout the metastatic cascade, or whether cells utilizing either telomere maintenance program defines distinct populations within primary tumors that become enriched or depleted in metastatic lesions through clonal selection.

6. Future directions: Telomere-directed therapy for metastasis

The deadly nature of metastatic disease necessitates the development of therapies that specifically target essential pathways operant during dissemination. Current efforts aimed at anti-telomerase therapy have adopted several approaches, including direct enzyme inhibition, telomere destabilization, anti-telomerase immunotherapy, and telomerase-driven suicide gene therapy [147]. Enzyme inhibitors include both small molecules and RNA template antagonists, both of which have demonstrated robust antitumor activity (*i.e.*, against primary and metastatic tumors) in preclinical models of breast and lung cancer [148, 149]. Clinical studies indicate potential telomere length-dependent therapeutic benefit with these agents, although results to date have been inconsistent [150]. Telomere destabilization can be accomplished by incorporating mutant-template TR into catalytically active TERT, resulting in nucleotide misincorporation into telomeric repeat sequences. The effects of telomere destabilization on cancer cells have only begun to be investigated in preclinical settings [151], and methods for clinically implementing such therapies may be difficult to devise. Immunotherapy consists of injection of either a recombinant TERT peptide (*i.e.*, vaccination) or dendritic cells exhibiting constitutive TERT expression. Each of these approaches have been assessed in clinical trials, and while an immunologic response may be achieved [152, 153], this response does not appear to confer a survival advantage [154]. Lastly, gene therapy seeks to parlay telomerase overexpression in cancer cells in order to drive expression of a cytotoxic or oncolytic gene product under the control of the *TERT* promoter [155]. Unfortunately, gene therapy as a means to target cells with aberrant telomerase activity remains in its infancy and has not been rigorously validated in either preclinical investigations or clinical trials.

ALT has recently emerged as a novel therapeutic target in cancer, as some malignancies rely on ALT as their primary TMM, while others rely upon telomerase or ALT in a context-dependent manner [136, 144]. Moreover, pathways that are preferentially active during ALT may serve as effective drug targets in these cancers [156, 157]. As the relative contributions of each TMM to metastatic progression become clearer, anti-telomerase and anti-ALT therapies may emerge as viable strategies to target metastatic disease (Fig. 3). Theoretically, administering TERT inhibitors should provide clinical benefit to patients whose metastatic tumors are TERT-positive, particularly since progression through the metastatic cascade is reliant upon pathways and effectors regulated by TERT (Fig. 2). Unfortunately, existing TERT inhibitors only target the enzymatic activity of this polymerase, resulting in modest clinical efficacy due in part to the (i) extratelomeric functions of TERT, and (ii) potential

compensatory activation of ALT machinery in TERT-inhibited cells. Moreover, we submit that future ALT-directed therapies should aim to maintain DTCs in a perpetual quiescent/senescent state, doing so either by delivering “ALT activators,” or by providing agents capable of shifting telomere regulatory networks towards ALT (*e.g.*, BMP agonists). Along these lines, combining anti-TERT and anti-ALT agents holds tremendous promise to eradicate metastatic disease, thereby providing a two-pronged approach to simultaneously target quiescent (ALT-positive) and proliferating (TERT-positive) DTCs, and to prevent chemotherapy-induced compensatory transitions between these mechanisms. Moving forward, significant attention should be devoted to (*i*) exploring improved therapies that target telomerase and ALT, including specific modulation of telomerase extratelomeric functions; (*ii*) propelling these drugs into clinical trials; and (*iii*) developing methods for longitudinal assessment of patient TMM status in both primary tumors and metastases. These advances will shed light on the dynamic interplay between telomerase and ALT in cancer progression and, potentially, improve the survival of patients afflicted with one of the world’s most lethal diseases.

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Fig. 1.

Overview of the metastatic cascade. The ability of carcinoma cells to disseminate is dependent on the presence of a vascular supply and the cellular responses to multiple signaling inputs. Cancer cells secrete pro-angiogenic factors, such as vascular endothelial growth factor (VEGF; denoted by V), that facilitate tumor invasion of existing vasculature and recruit endothelial cells for neovascularization. These cells similarly secrete matrix metalloproteinases (MMPs), which remodel the surrounding extracellular matrix (ECM). MMPs also cleave and activate latent signaling molecules, including transforming growth factor- β (TGF- β ; denoted by T). TGF- β binds and activates its receptors (T β R) to promote cancer cell migration, intravasation, and epithelial-mesenchymal transition (EMT). ECM proteins and secreted growth factors, including epidermal growth factor (EGF) and Wnt (denoted by W), activate convergent signaling pathways that further stimulate cancer cell growth and invasiveness and impart these cells with mesenchymal properties. Once in the circulation, disseminated tumor cells (DTCs) persist in isolation, in small clusters, or in association with macrophages (denoted M) until they reach distant organs that are amenable to DTC outgrowth. Metastatic outgrowth is determined in part by the relative activities of the protein kinases ERK1/2 and p38 MAPK. ERK1/2 are activated downstream of EGF receptor (EGFR) and integrin stimulation, while p38 MAPK is activated in response to environmental stressors, such as hypoxia. In addition, DTCs secrete pro- (VEGF) and anti-angiogenic (thrombospondin-1; Tsp) factors that control vascular supply and tumor growth. DTC growth at metastatic sites is further influenced by cells of the surrounding tissues, including cancer-associated fibroblasts (CAFs) that release Wnt and other factors into the microenvironmental milieu. In response, DTCs negatively regulate Wnt signaling in an autocrine manner. Dkk1, Dickkopf-related protein 1; E-cad, epithelial cadherin; FAK, focal adhesion kinase; PI3K, phosphatidylinositol 3-kinase.

Fig. 2.

Extratelomeric functions of TERT coupled to cancer cell dissemination and tumor formation. **(Left Panel)** Schematic representation of the metastatic cascade, encompassing (A) primary tumor formation, (B) endothelial cell (EC) recruitment and angiogenesis, (C) cancer cell migration and invasion into surrounding tissue, (D) cancer cell intravasation and survival within the system circulation, and (E) extravasation and colonization of distant organs either as single cells/micrometastases or (F) overt metastatic lesions. **(Right Panel)** Depicts the pathways regulated by telomerase TERT (denoted by T) that influence specific stages of the metastatic cascade. Canonical TERT activity is responsible for amplifying telomeric repeat DNA, an event that is essential for malignant transformation, and for tumor growth and development (A). In addition to its role in telomere elongation, TERT also controls gene expression by acting as a transcription factor. In doing so, TERT targets the expression of vascular endothelial growth factor (VEGF), as well as genes governed by the Wnt:β-catenin axis (Wnt denoted by W; β-catenin by β). VEGF orchestrates multiple stages of the metastatic cascade, particularly tumor angiogenesis (B), intravasation (D), and proliferation of disseminated cells at distant sites (F). Wnt signaling promotes EMT-mediated cancer cell migration (C) and intravasation (D). Additionally, Wnt signaling regulates the proliferation and outgrowth of DTCs in part by governing various features of the metastatic niche (F). Activation of Wnt-responsive genes, which includes VEGF, by TERT is dependent on its association with the chromatin remodeler SMARCA4 (SM4). In turn, TERT expression is reciprocally regulated by β-catenin-dependent transcription. TERT function and localization are controlled by both stimulatory (e.g., AKT) and inhibitory (e.g., bone morphogenetic proteins, BMPs) signals present in the metastatic microenvironment. AKT is an important regulator of cancer cell dissemination and survival in circulation (D), doing so by preventing the death of CTCs. In contrast, BMPs inhibit DTC outgrowth and maintain these cells as subclinical micrometastases (E). Under conditions of oxidative stress, TERT may translocate to mitochondria and regulate the expression of mitochondrial genes (Mitochondrion, left arrow) related to glucose metabolism and energy production; it also mediates an antioxidant response to reactive oxygen species (ROS; Mitochondrion, right bar). ROS are ordinarily present as a defense mechanism against disseminated tumor cells both in the circulation (D), and in the metastatic niche (F). Thus, expression of TERT in these cell may provide them with a selective advantage in colonizing metastatic sites.

Fig. 3. Hypothetical model of TMM selection and therapeutic targeting during metastasis. The top row (*blue box*) depicts the dissemination of a telomerase-positive cancer cell from the primary tumor site to a distant organ, while the bottom row (*pink box*) depicts these same events in an ALT-positive cell. *See Section 5. "Telomere homeostasis: Determinant or consequence of metastatic progression?"* for details.

Table 1

Mutations in telomeric proteins are associated with various cancers and cancer predisposition syndromes.

Protein	Cancer(s)	Reference(s)
Shelterin		
TRF1/TRF2	Gastric	[158]
POT1	Leukemia (CLL)	[159]
	Melanoma	[160]
	Glioma	[161]
TPP1	DC	[162]
	Melanoma	[163]
TIN2	DC	[164]
RAP1	Melanoma	[163]
Telomere elongation		
TERT	Glioma	[165, 166]
	Bladder	[165, 167]
	Thyroid	[165, 168]
	Melanoma	[165, 169]
	Breast/Ovarian	[170]
TERC	MDS	[171]
MRE11	Colorectal	[172]
	Breast	[173]
NBS1	Prostate	[174]
	Leukemia (ALL)	[175]
RAD50	Breast	[176]

Abbreviations: ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; DC, dyskeratosis congenita; MDS, myelodysplastic syndrome.