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Review

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

Nathaniel J. Robinson [a], William P. Schiemann [b,*]

[a] Department of Pathology, Case Western Reserve University, Cleveland, OH 44106, USA
[b] Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH 44106, USA

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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.

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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%; [3–5]), approximately 90% of cancer-related deaths are attributable to metastasis [6]. The underlying lethality of metastasis

* Corresponding author at: Case Comprehensive Cancer Center, Case Western Reserve University, Wolstein Research Building, Room 2131, 2103 Cornell Road, Cleveland, OH 44106, USA.
E-mail address: william.schiemann@case.edu (W.P. Schiemann).

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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 re-instatement 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, phosphatidylinositide 3-kinase (PI3K), AKT, and the mitogen-activated protein kinases

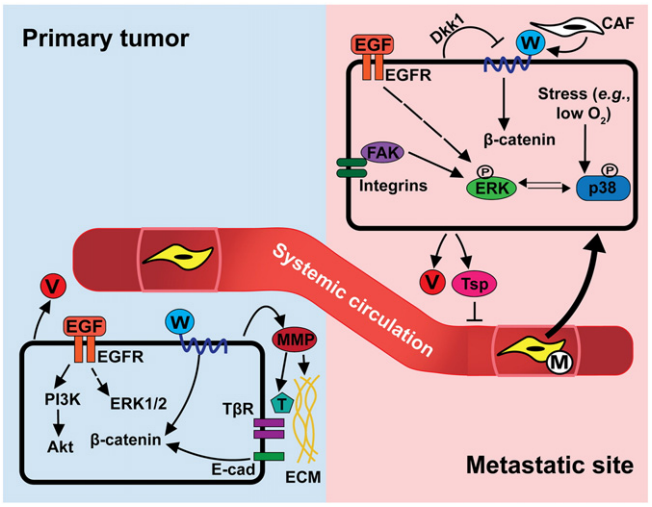

Fig. 1. Overview of the metastatic cascade. The ability of carcinoma cells to disseminate is dependent on the presence of a vascular supply and the cellular responses to multiple signaling inputs. Cancer cells secrete pro-angiogenic factors, such as vascular endothelial growth factor (VEGF; denoted by 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 (TβR) 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 is 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; and PI3K, phosphatidylinositide 3-kinase.

(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 β1 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