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Abstract (49 words)

Matrix proteolysis mediated by MT1-MMP facilitates the invasive migration of tumor cells in dense tissues, which otherwise get trapped in the matrix because of limited nuclear deformability. A digest-on-demand response has been identified, which requires nucleus-microtubule linkage through the LINC complex and triggers MT1-MMP surface-exposure to facilitate nucleus movement.

Keywords

Invadopodia; MT1-MMP; microtubule; LINC complex; tumor invasion; centrosome

Text (1449 words)

Introduction

During their metastatic journey, tumor cells migrate through dense and complex 3D microenvironments within connective tissues. For carcinoma cells deriving from epithelial tumors, dissemination starts by breaching the 0.1-1 μm thick basement membrane (BM) made of laminins, cross-linked type IV collagen and proteoglycans that surround epithelial tissues. BM transmigration signals the transition from in situ to more aggressive infiltrating carcinomatous lesions; then invasive cells can disseminate, mostly in cohorts, through interstitial tissues consisting of bundles of type I collagen-rich fibrils interspaced with discontinuities. A salient feature of tumor cell invasion in confining environments is that it requires extensive nuclear deformation to squeeze the bulky and stiff nucleus through constricting pores within the tissue matrix [1]. Recent studies, which are detailed in Box 1, revealed that nuclear deformations during constricted migration can lead to nuclear envelope (NE) rupture with potential consequences for genome stability and tumor progression [2-5]. In addition, it has been shown that when the pore size of the matrix meshwork is below the deformability of nucleus, then cell movement stops [1]. It is also established that surface-exposed membrane-type (*i.e.* trans-membrane) matrix metalloproteinases (MT-MMPs) can enlarge the pores in the matrix [1, 6]. All together, these findings have raised important questions as to whether and how the matrix proteolysis machinery of cancer cells can scale with the level of confinement by the matrix and what is the influence of nuclear deformation and mechanosensing to this response and to the invasive potential of tumor cells.

Nucleus pulling and pushing schemes generate nuclear deformation during confined migration

Deformability and mechanical stability of the nucleus depends on nuclear lamina proteins lamin-A and B (LMNA, -B), which form a viscoelastic proteinaceous network underneath the inner nuclear membrane. Nuclear stiffness scales with LMNA levels: LMNA overexpression impedes transmigration by augmenting nuclear rigidity, while reducing LMNA levels increases invasion speed in 3D reconstituted matrix [1, 2, 7]. Thus, nuclear stiffness and limited nuclear deformability have been identified as restrictive factors that impede cell migration in confined environments [1, 2]. Interestingly, a gradient of LMNA expression decreasing from the core to the invasive front of tumor xenografts has been reported and LMNA-deficient tumors have a growth advantage [2, 5]. The downside is that nuclear resistance to mechanical insults drops with LMNA deficiency and repeated passages across constrictions can lead to cell death as a failure to repair consequent NE rupture and DNA damage [2-5] (see Box 1).

Nuclear deformation reflects the pulling and pushing schemes by tumor cells to move their nucleus across constrictions. As during cell migration in 2D, integrin-based adhesion to surrounding collagen fibers and actomyosin-based contractility produce traction forces that can propel the nucleus forward through constraining spaces ([8] and references herein). Additionally, pulling forces can result from dynein and kinesin molecular motors attached to the nuclear surface and moving the nucleus along the microtubule network [9]. Proteins responsible for nucleo-cytoskeleton attachment and motor association to the surface of the nucleus belong to the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex. This complex consists in SUN and nesprin proteins, which span the inner and outer nuclear

membrane, respectively, and interact through SUN domain and the carboxy-terminal Klarsicht, ANC-1, Syne Homology (KASH) domain of nesprins in the intermembrane space. Nesprin-2 was reported to be required for recruitment of LIS1, a regulatory protein of dynein motor function, to the cytoplasmic face of the nucleus in invasive MDA-MB-231 breast tumor cells [7]. In humans, LIS1 deficiency leads to lissencephaly (smooth brain) due to severe cortical neuron migration and positioning defects caused by impaired dynein-dependent nucleokinesis. It was recently found that LINC complex and LIS1 mediate nucleo-centrosome linkage and centrosome positioning ahead of the nucleus [7]. These observations suggest that Nesprin-2 and LIS1 contribute to pulling forces exerted on the nucleus by dynein moving along microtubules to support nucleus movement through confining environments generating nuclear deformation (Figure 1).

Invadopodia mediate MT1-MMP-based matrix degradation by cancer cells

Membrane-anchored MT1-MMP (aka MMP14) is the sword arm of the collagenolytic program of carcinoma cells. Using the intraductal mammary gland xenograft model, it was reported that silencing of MT1-MMP impairs the ability of ductal carcinoma in situ tumor xenografts to progress into infiltrating lesions, providing validation for the prominence of MT1-MMP for BM transmigration by breast cancer cells in vivo [10]. MT1-MMP has also been implicated in invasive migration of mesenchymal cells through the fibrous interstitial type I collagen network, in the infiltration of vascular and lymphatic compartments and in extravasation during metastasis. Inhibition of MT1-MMP function can evoke protease-independent programs of cancer cells, which can switch to contractility-driven ameboid movement or use the nucleus as a piston to propel the cell ahead [6, 8]. Moreover, in support of a major role during cancer

dissemination, MT1- MMP is linked to malignancy of multiple tumor types including lung, gastric, colon, breast, and cervical carcinomas, gliomas, and melanomas. MT1-MMP is accumulated and spatially restricted to invadopodia that correspond to actin-based plasmalemmal subdomains, which combine membrane protrusive and matrix proteolytic functions to promote cancer cell invasion and metastasis [11]. Invadopodia form dynamically in association with constricting matrix fibers and can vary in shape and possibly in composition depending on extracellular matrix topology and components [12].

Nuclear Confinement triggers polarized MT1-MMP/invadopodia-based matrix degradation ahead of the nucleus

Contrasting with gel-like pseudopodial protrusions that can squeeze through narrow pores between matrix fibers, the nucleus has limited deformability (experimentally estimated to be ~10% of original nuclear cross section) [1]. When nuclear deformability limit is reached, cell migration physically stops as the nucleus becomes entrapped in the fibrous matrix network [1, 7]. It is well established that pericellular collagenolysis by matrix metalloproteinases (MMPs) can modulate restricting environmental conditions by widening ECM pores [1]. Recent studies demonstrated that MMP inhibition during confined migration of tumor cells in dense collagen environment leads to increased nuclear deformation and mechanical rupture of the NE [1, 3]. However, how mechanical input from the ECM microenvironment triggers the invadopodial response is unknown and of paramount importance in light of data showing that the biomechanical properties of the microenvironment have major impact on cancer progression [13].

Recent work in breast cancer cells revealed that surface exposure of MT1-MMP and pericellular collagenolysis are adaptive responses, which are switched off under low nucleus confinement, while decreased matrix pore size or increased nuclear stiffness trigger the collagenolytic program [7]. MT1-MMP is known to recycle from endolysosomal compartments to invadopodia in metastatic breast cancer cells [11]. It was found that MT1-MMP-storage endolysosomal compartments distribute ahead of the nucleus with a centrosome-centered polarization optimal to fuel MT1-MMP delivery to invadopodia forming at the nuclear anterior zone (Figure 1, inset 2) [7]. The polarization of MT1-MMP-secretory compartments and the assembly of functional invadopodia require integrity of nucleo-microtubule linkage depending on the LINC complex and LIS1 functionality (Figure 1, inset 1) [7]. Thus, a working model is that tension generated by dynein motor along the microtubule network through nuclear envelope and cell cortex anchoring, pulls the nucleus forward for movement; extra tension on trapped nucleus through constricting ECM fibrils triggers formation of proteolytically-active invadopodia and dissolution of the confining fibrils to open the way for nucleus migration during confined invasion of tumor cells (Figure 1).

This model raises several questions. One question is how migrating cells negotiating changing environments, recognize nucleus-constricting fibers and degrade them, while ECM fibrils involved in integrin-based adhesion and cell movement at the cell front are spared from degradation? One possible mechanism for segregation of specialized degradation and adhesion contact zones is that distinct collagenic receptors selectively trigger assembly of peripheral focal adhesions to mediate traction force generation, and invadopodia, ahead of the nucleus. While beta1 and -3 integrin receptors are classically involved in cell-collagen adhesion,

147 receptors mediating invadopodia formation in association with collagen fibers remain
148 poorly defined and need to be identified. Another conundrum is how mechanical
149 constrains on the nucleus can trigger invadopodia formation and MT1-MMP delivery.
150 As tumor cells have constantly to adapt to changes in the matrix environment, these
151 responses have to happen on a fast time-scale, too fast for genetic regulation.
152 Several studies revealed the ability of the nucleus to sense and respond to forces
153 supporting the emerging concept of nuclear mechanotransduction [14]. Nuclear
154 mechanosignaling effectors have been recently identified including the nuclear
155 membrane protein emerin, which can contribute to nucleo-centrosome linkage and
156 mediates the response to tensional force applied to the nucleus in relation with LINC
157 complex and LMNA function [14]. Therefore, a possible mechanism to be further
158 explored is that nuclear tension and mechanosignaling can control the recycling
159 machinery ensuring MT1-MMP delivery to invadopodia. These observations also
160 highlight the possibility to target the machinery linking nuclear tension with MT1-MMP
161 surface delivery as a new therapeutic road to target cancer metastasis.
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BOX 1: Mechanical stress on the nucleus in cancer (200 words max/206 words)

Cells are submitted to compressive forces during physiological and disease conditions. Tumor growth can generate compressive forces due to overgrowth and confinement by the tissue environment. In addition, cancer cells are submitted to elastic deformations of the cell body and bulky nucleus as they invade across interstitial spaces during metastatic spread [6]. Recent studies reported that extensive nuclear deformation could result in local NE rupture, which can be rapidly repaired [3, 4]. Leakage of nuclear DNA repair factors as well as transient exposure of nuclear DNA to cytoplasmic nucleases such as three-prime repair exonuclease (TREX)1 can lead to DNA damage and double-strand breaks (DSB) as indicated by appearance of foci of DNA damage repair marker γ-H2AX [3, 4, 15]. Sensitivity of tumor cells to mechanical nuclear stress somehow scales with LMNA levels. Genomic analysis of cancer cell lines following repeated cycles of migration through small microfabricated rigid pores revealed that NE rupture can lead to genomic alterations and chromosomal copy-number changes [15]. All together, repetitive nuclear rupture could contribute to cancer progression by favoring genomic instability and chromosomal rearrangements, an idea that remains to be experimentally tested.

Legend Figure 1: Model of invadopodia-, MT1-MMP-based matrix digest-on-demand response triggered upon nucleus confinement during cancer invasion

Confined migration of tumor cells through dense 3D collagen network results in nucleus confinement by constricting collagen fibrils. Nucleus-microtubule/centrosome linkage and nucleus pulling is mediated by LINC complex interacting with dynein-Lis1 molecular motor (see inset 1). Cortical anchoring of microtubules is required for centrosome and MT1-MMP-positive endosome positioning and for targeted delivery of MT1-MMP to invadopodia. Nucleus movement is facilitated by localized invadopodia-based pericellular proteolysis of confining fibrils ahead of the nucleus (right inset). Left inset, scheme of nucleus-cytoskeletal linkage through LINC complex components nesprin and SUN in association with lamins. Lis1 in complex with dynein associates to the NE depending on Nesprin-2 and is involved in nucleus-microtubule linkage and nucleus pulling (adapted from [7]). Inset 2, model of polarized surface-delivery of MT1-MMP from recycling endolysosomes (adapted from [11]).

Figure

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