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Review article

Regulation of invadopodia by mechanical signaling

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ABSTRACT

Mechanical rigidity in the tumor microenvironment is associated with a high risk of tumor formation and aggressiveness. Adhesion-based signaling driven by a rigid microenvironment is thought to facilitate invasion and migration of cancer cells away from primary tumors. Proteolytic degradation of extracellular matrix (ECM) is a key component of this process and is mediated by subcellular actin-rich structures known as invadopodia. Both ECM rigidity and cellular traction stresses promote invadopodia formation and activity, suggesting a role for these structures in mechanosensing. The presence and activity of mechanosensitive adhesive and signaling components at invadopodia further indicates the potential for these structures to utilize myosin-dependent forces to probe and remodel their ECM environments. Here, we provide a brief review of the role of adhesion-based mechanical signaling in controlling invadopodia and invasive cancer behavior.

Contents

1. Introduction

Cells sense the biomechanical properties of the ECM through interactions facilitated by matrix adhesions [1]. Intracellular adhesion proteins link ECM receptors to downstream force-sensing pathways, including non-muscle myosin II (NM II)-dependent contractility of adhesion-associated actin [2] and conformational changes of mechanosensitive proteins [3]. Changes in mechanical

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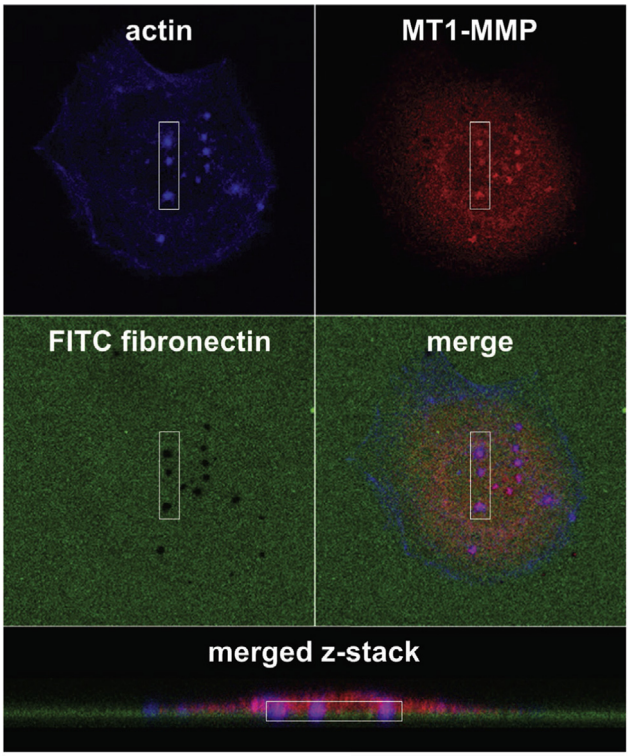

Fig. 1. Invadopodia are actin-rich proteolytic protrusions that are often identified through colocalization of markers with ECM degradation. The *in vitro* invadopodia assay typically consists of invasive cancer cells cultured on fluoroscently-labeled ECM, in this case FITC-fibronectin-coated crosslinked gelatin. After 6–48 h, the cells are fixed and stained for molecular markers of invadopodia including actin filaments, cortactin, Arp2/3 complex, Tks5, and/or MT1-MMP [44,45,76,99,108,109,119]. In this case, invadopodia are identified by colocalization (purple) of actin filaments (blue) and MT1-MMP (red) using confocal microscopy imaging. Mature invadopodia are further recognized by colocalization of invadopodia markers with areas of ECM degradation (black holes in the green FITC-labeled fibronectin). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

signaling pathways can alter cellular phenotypes and contribute to a number of diseases, including deafness, cardiac hypertrophy, and muscular dystrophy [4]. In breast cancer, increased ECM rigidity during tumorigenesis has been shown to drive a malignant phenotype through biomechanical adhesion signaling [5–8], including enhanced invasion and metastasis [8–11]. ECM rigidity changes in breast cancer are thought to occur as a result of a number of factors, including tumor cell packing, ECM deposition and crosslinking, and higher fluid pressures [12]. These factors are common features of many types of cancers [7,12–14], and several other tumor types have also been quantitatively shown to have greater mechanical properties than neighboring normal tissues [15–17]. Recent studies have shown that mechanical factors alter the invasive properties of diverse cancer cell types in vitro [18–21] suggesting common rigidity-dependent regulatory pathways.

Proteolytic degradation of ECM promotes cancer cell invasion by allowing migration through dense cross-linked tissues such as the basement membranes that surround carcinomas and underlie blood vessels [22]. In addition, proteolytic remodeling of stromal collagen may allow collective migration of cancer cells through tissues [23]. In order to degrade ECM, cancer cells form actin-rich adhesive protrusions called invadopodia (Fig. 1) [24]. Invadopodia are cellular hotspots for secretion of matrix-degrading proteinases [25–27]; thus, formation of invadopodia greatly accelerates matrix remodeling. The ability of cancer cells to form invadopodia correlates well with their *in vitro* and *in vivo* invasive behavior [28–35]. In addition, upregulation in tumors of key invadopodia molecules, such as the matrix metalloproteinase MT1-MMP, and the actin assembly protein cortactin, are associated with poor patient prognosis [36,37]. Similar structures called podosomes are formed in a variety of other cell types that need to remodel tissue or cross tissue barriers, including osteoclasts, endothelial cells, and macrophages [38].

In addition to invadopodia and podosomes, invadopodia-independent proteolytic degradation mechanisms have been described in normal and cancer-associated fibroblasts (CAFs) [39,40]. Matrix degradation by fibroblasts at focal adhesions was regulated by signaling mechanisms that also control invadopodia (e.g. Src, FAK, p130Cas) [40]. However, invadopodia-independent plasma membrane sites were identified that do not depend on the critical invadopodia regulators Cdc42 or Src [39]. These data suggest some flexibility in the mechanisms controlling proteinase expression on the plasma membrane. In contrast, pancreatic CAFs expressing high levels of palladin have been shown to enhance invasion and metastasis of tumor cells through invadopodia-dependent ECM degradation [41]. While invadopodia appear to be the dominant mechanism used by invasive cancer cells to degrade ECM, further investigation is required to elucidate the role and regulation of proteolytic structures in tumor-associated stromal fibroblasts.

2. Invadopodia formation and structure

Invadopodia are formed in response to signaling events that lead to dynamic branched actin assembly at membrane sites [25,32,42]. Shortly thereafter, proteinases are secreted and promote ECM degradation. MT1-MMP has been the most studied proteinase in invadopodia and is essential for degradation of *in vitro* crosslinked gelatin substrates [43–45] (Fig. 1). However, many proteinases are secreted at invadopodia and could collaborate to promote degradation of ECM in tissues. These proteinases include MT1-MMP, MMP-2, MMP-9, seprase, cathepsin B, ADAM12, and uPAR [27,28,44,46–50]. Some of these proteinases are also likely to activate latent ECM- and cell-associated growth factors [51–56].

By electron microscopy (EM), invadopodia are long, slender protrusions that are typically 50 nm in diameter and ~0.5–2 μm in length [30,32,57,58]. While dynamic branched actin is found at the cortex and is an essential part of the formation process, the resemblance to filopodia by EM suggests that the actin found within the invadopodial protrusion is likely to be unbranched. Indeed, key filopodia proteins including fascin, Myosin X, mDia1, and fimbrin have been shown to be essential for invadopodia stabilization and elongation [32,59,60]. Thus, both the branched and unbranched actin nucleation machineries collaborate to form stable, active invadopodial protrusions.

Many signaling proteins localize to and regulate invadopodia formation and stability, including tyrosine kinases such as Src, EGFR, and Arg, adhesion proteins such as integrins, focal adhesion kinase (FAK), p130Cas, and integrin-linked kinase (ILK), and scaffold proteins such as Tks5 [24,25,61]. Many of these molecules also control podosome and focal adhesion formation and activity [62] (reviewed elsewhere in this issue). Src kinase is a particularly important regulator, as exemplified by the spontaneous formation of invadopodia-like structures in cells engineered to exogenously express constitutively active Src [63–65]. Given their similarities, invadopodia, podosomes, and Src-induced invadopodia-like structures are often referred to collectively as invadosomes [66].

3. ECM rigidity and cellular contractility control invadopodia formation and activity

One of the first indications that invadopodia might be involved in mechanical signaling came from our work demonstrating that

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