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European Journal of Cell Biology 85 (2006) 213–218
European Journal of Cell Biology
www.elsevier.de/ejcb

Invadopodia and podosomes in tumor invasion

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Abstract

Cell migration through the extracellular matrix (ECM) is necessary for cancer cells to invade adjacent tissues and metastasize to an organ distant from primary tumors. Highly invasive carcinoma cells form ECM-degrading membrane protrusions called invadopodia. Tumor-associated macrophages have been shown to promote the migratory phenotypes of carcinoma cells, and macrophages are known to form podosomes, similar structures to invadopodia. However, the role of invadopodia and podosomes in vivo remains to be determined. In this paper, we propose a model for possible functions and interactions of invadopodia and podosomes in tumor invasion, based on observations that macrophage podosomes degrade ECM and that podosome formation is regulated by colony-stimulating factor-1 signaling.
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Keywords: Invadopodia; Podosomes; Carcinoma; Macrophage; WASP; N-WASP; Invasion; Metastasis; EGF; CSF-1

Introduction

Metastasis, dissemination of malignant tumors to a distant organ, is the major cause of cancer mortality. To metastasize, carcinoma cells must dissociate from primary tumors, invade underlying tumor stroma, and intravasate into blood or lymphatic vessels (Chambers et al., 2002). These processes require migration of cancer cells in association with local remodeling of the extracellular matrix (ECM) in the primary tumor, particularly, that of basement membranes associated with epithelial glands, and blood and lymphatic vessels (Condeelis and Segall, 2003; Friedl and Wolf, 2003; Wiseman and Werb, 2002; Yamaguchi et al., 2005b). Highly invasive cancer cells cultured on physiological substrates form specialized membrane protrusions rich in actin filaments, called invadopodia, that have the capacity to degrade underlying matrix (Buccione et al., 2004). Invadopodia are thought to be necessary for carcinoma cell invasion through the local remodeling of ECM structures in the path of the invading cell.

Carcinoma cell migration in primary tumors is affected by the tumor microenvironment, including chemoattractants/growth factors, ECM proteins, and resident stromal cells (Mueller and Fusenig, 2004). Tumor-associated macrophages (TAMs) have been shown to promote cancer progression and metastasis (Pollard, 2004), and a paracrine interaction between tumor cells and macrophages has been identified in vivo that is required for invasion and intravasation (Wyckoff et al., 2004). Macrophages are known to form ventral membrane protrusions and invaginations called

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0171-9335/$ - see front matter Published by Elsevier GmbH.
doi:10.1016/j.ejcb.2005.10.004

podosomes that are similar to invadopodia in molecular composition (Calle et al., 2004; Linder and Aepfelbacher, 2003; Linder and Kopp, 2005). Podosomes are thought to function as dynamic adhesion structures required for cell migration and are also proposed to have a role in ECM remodeling.

Recently invadopodia and podosomes have attracted the interest of many researchers as model systems for studying the regulation of the dynamics of the actin cytoskeleton at the cell membrane. However, little is known about the physiological functions of invadopodia and podosomes in vivo. Here, we describe some molecular mechanisms regulating formation of invadopodia and the possible functions of invadopodia in vivo. We also show that macrophage podosomes have a matrix degradation activity and that colony-stimulating factor-1 (CSF-1), a growth factor and chemoattractant for macrophages, regulates the formation and organization of macrophage podosomes. These observations have allowed us to propose a model for the functions and interactions of these specialized protrusive structures, invadopodia and podosomes, in tumor invasion.

Materials and methods

Cell culture and stimulation

Day 5 bone marrow-derived macrophages from C57Bl/6 mice were prepared as previously described (Stanley, 1997) and cultured in supplemented α-MEM (Life Technologies) containing 15% FBS (Life Technologies) and 120 ng/ml human recombinant CSF-1 (a gift of Chiron Corp., Emeryville, CA). Human peripheral blood monocyte-derived macrophages were prepared as follows: the mononuclear fraction was enriched from peripheral blood using the Ficoll-Hypaque (Sigma) gradient centrifugation method and adherent cells were selected, followed by 7 d culture in RPMI medium with 15% FBS and 120 ng/ml human recombinant CSF-1. BAC1.25F5 cells were cultured as described previously (Chitu et al., 2005). For the CSF-1 stimulation assay, bone marrow-derived macrophages cultured on fibronectin/gelatin-coated coverslips were starved of CSF-1 for 18 h and then stimulated with CSF-1 (120 ng/ml) for 30 min.

ECM degradation assay

Fluorescently labeled fibronectin- and gelatin-coated coverslips were prepared as described previously (Chen et al., 1994; Yamaguchi et al., 2005a). Cells were cultured on the coverslips overnight and then fixed and stained with rhodamine phalloidin (Molecular Probes). Images were taken with a confocal laser scanning microscope (model radiance 2000, Bio-Rad Laboratories) or with an Olympus microscope equipped with a cooled CCD camera.-

Results and discussion

Invadopodia in carcinoma cell invasion and intravasation

Invadopodia contain a variety of proteins involved in actin cytoskeleton regulation, cell-matrix adhesion, membrane remodeling, and matrix degradation (Buccione et al., 2004). However, the molecular mechanisms that govern the formation and dynamics of invadopodia are not well understood. We have previously reported that epidermal growth factor (EGF), a key chemoattractant for carcinoma cells involved in the invasion and metastasis of breast tumors (Wang et al., 2004; Wyckoff et al., 2004), and EGF receptor signaling are necessary for invadopodium formation by mammary adenocarcinoma cells (Yamaguchi et al., 2005a). Similarly, N-WASP, a ubiquitously expressed member of the Wiskott-Aldrich syndrome protein (WASP) family, is essential for invadopodium formation (Yamaguchi et al., 2005a). N-WASP has been demonstrated to be active at the base of invadopodia using fluorescent resonance energy transfer (FRET) imaging with an N-WASP biosensor (Lorenz et al., 2004). N-WASP induces the rapid actin polymerization required for membrane protrusion by activating actin nucleation activity of the Arp2/3 complex in response to various extracellular stimuli including EGF. N-WASP is activated directly by several signaling/adaptor molecules, including Cdc42, Nck1, and WIP (Miki and Takenawa, 2003; Stradal et al., 2004). Indeed, these N-WASP-activating proteins are also necessary for invadopodium formation, suggesting that EGF receptor activation stimulates the formation of invadopodia via the N-WASP/WIP/Arp2/3 complex signaling pathway that is mediated by Cdc42 and/or Nck-1 (Yamaguchi et al., 2005a).

However, the function of invadopodia in vivo has not been established. Given that EGF is a key chemoattractant for carcinoma invasion, intravasation, and metastasis, EGF-mediated invadopodium formation is likely to contribute to these processes (Condeelis et al., 2005). Intravital imaging of GFP-labeled primary mammary tumors by multiphoton microscopy showed that carcinoma cells in the process of intravasation extend invadopodium-like protrusions that penetrate blood vessel walls into the blood space (Condeelis and Segall, 2003; Yamaguchi et al., 2005b). This observation indicates that carcinoma cells breach the basement membrane surrounding blood vessel walls through the action of invadopodia. Moreover, degradation of ECM in the basement membrane and stromal tissue surrounding primary tumors is necessary for carcinoma cells to