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Mini Review

Invadosomes in their natural habitat

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ABSTRACT

Podosomes and invadopodia (collectively known as invadosomes) are small, F-actin-rich protrusions that are located at points of cell-ECM contacts and endow cells with invasive capabilities. So far, they have been identified in human or murine immune (myelomonocytic), vascular and cancer cells. The overarching reason for studying invadosomes is their connection to human disease. For example, macrophages and osteoclasts lacking Wiskott–Aldrich syndrome protein (WASp) are not able to form podosomes, and this leads to altered macrophage chemotaxis and defective bone resorption by osteoclasts. In contrast, the ability of cancer cells to form invadopodia is associated with high invasive and metastatic potentials. While invadosome composition, dynamics and signaling cascades leading to their assembly can be followed easily in *in vitro* assays, studying their contribution to pathophysiological processes *in situ* remains challenging. A number of recent papers have started to address this issue and describe invadosomes *in situ* in mouse models of cancer, cardiovascular disease and angiogenesis. In addition, *in vivo* invadosome homologs have been reported in developmental model systems such as *C. elegans*, *zebrafish* and sea squirt. Comparative analyses among different invasion mechanisms as they happen in their natural habitats, *i.e.*, *in situ*, may provide an outline of the invadosome evolutionary history, and guide our understanding of the roles of the invasion process in pathophysiology *versus* development.

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; cAMP, 3, 5-cyclic adenosine monophosphate; Ab, antibody; AC, anchor cell; Arp2/3, complex containing actin regulators 2 and 3; BM, basement membrane; CAM, chicken chorioallantoic membrane; Cdc42, Cell division control protein 42 homolog, small protein of RhoGTPase family; DIC, differential interference contrast; EC, endothelial cells; ECM, extracellular matrix; EMT, Epithelial-mesenchymal transition; GPCR, G-protein-coupled receptors; hpf, hours post-fertilization; HUVEC, human umbilical vascular endothelial cells; KD, knock down; KO, knock out; LSEC, liver sinusoidal endothelial cells; (N)-WASp, (Neural)-Wiskott-Aldrich syndrome protein; MMP, matrix metalloproteases; miR, microRNAs; myoB, myosin family protein; PDGFR, platelet-derived growth factor receptor; ROS, reactive oxygen species; Rac, small signaling G proteins, subfamily of Rho-GTPases; RTK, receptor tyrosine kinases; TGFβ, transforming growth factor; Tks5, Src substrate otherwise known as Fish; TNFα, tumor necrosis factor; UNC-6, netrin receptor; VSMC, vascular smooth muscle cells; Src, short for sarcoma, a non-receptor tyrosine kinase; PI3K, phosphatidylinositol 3-kinase; PIP2/PIP3, phosphatidylinositol bi/triphosphate; PKC, protein kinase C; p53, a tumor suppressor protein; PdBU, Phorbol (ester) 12,13-dibutyrate; PMA, phorbol myristate acetate.

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Introduction

Podosomes and invadopodia, collectively known as invadosomes, are specialized microdomains of the plasma membrane. They are defined by their morphology, structure and function as small F-actin-rich protrusions located at points of cell-extracellular matrix (ECM) contact that have (ECM)-degrading capability (Linder et al., 2011). Invadosomes commonly precede invasion; cell movement through obstacles into a new type of environment. Invasion occurs during both physiological and pathological processes, including different stages of embryonic and tissue development, inflammation, wound-healing and cancer metastasis. So far, invadosomes have been studied *in vitro* and reported in vascular cells, myelomonocytic cells (osteoclasts, monocytes), cancer cells and fibroblasts which are either transformed with oncogenic viruses or associated with cancer cells (cancer-associated fibroblasts) (Goicoechea et al., 2014).

Historically, the structures were discovered in chicken embryo fibroblasts transformed with the Rous Sarcoma Virus oncogene v-Src (David-Pfeuty and Singer, 1980) and named rosettes. In subsequent studies, the term podosome was preferred for describing these cell-ECM contacts containing individual actin-rich cores (Tarone et al., 1985), while a podosome rosette referred to self-organized groups of podosomes (Destaing et al., 2003). Finally, the term invadopod/invadopodium was introduced when a third team discovered that these structures were not only mediating adhesion but also invasion through their capability to degrade the ECM (Chen, 1989). When invadosomes were detected in other cell types, it became clear that invadosome architecture depends both on the cell type considered and on the experimental setting used for their observation. For a while, nomenclature became a major issue that complicated the interpretation and comparison of published data. Different classifications arose, including use of the term podosome when the structure extends upwards from the ventral cell surface into the cytoplasm on stiff substrata whereas long filopodia-like membrane extensions that penetrate into the ECM were referred to as invadopodia. Currently, a consensus seems to have emerged: invadosomes are referred to as podosomes when they are found in vascular and myelomonocytic cells and as invadopodia when they are found in cancer cells. The term invadosome is used when no distinction is being made between podosomes and invadopodia and also includes structures found in Src-transformed fibroblasts (which present mixed features of podosomes and invadopodia).

Invadosomes on 2D surfaces appear as punctate dynamic protrusions formed at the points of cell-ECM contact, initiated by growth factors or hypoxia (Diaz et al., 2013), highly enriched with filamentous actin (F-actin) and oriented perpendicularly to the substratum (Murphy and Courtneidge, 2011). Actin-regulatory proteins (*e.g.*, Arp2/3, WASp/N-WASp, cortactin, dynamin, gelsolin and cofilin) are consistently found in invadosomes in close association with F-actin, together with integrins (*e.g.*, β1, β2, β3) (Beaty et al., 2013; Calle et al., 2006), adhesion molecules (*e.g.*, talin), lipids (*e.g.*, PI(3,4)P2), GTPases (*e.g.*, Cdc42) and non-receptor tyrosine kinases (*e.g.*, Src). The scaffolding protein and Src substrate Tks5 also colocalizes with F-actin and constitutes a reliable invadosome marker (Murphy and Courtneidge, 2011). A key feature of invadosomes, which is in contrast to other cell adhesion devices (focal adhesions) or other protrusions related to motility (*e.g.*, pseudopodia, lamellipodia), is their ECM-degrading capability enabled by resident metalloproteases (MMPs) which provide *invasive* capabilities. Such a distinction is not absolute as one study reports on low levels of ECM degradation by focal adhesions (Wang and McNiven, 2012) whereas another one describes physical displacement of the ECM as a key element enabling invasion (Hagedorn et al., 2013). In addition to the role in invasion, dynamic behavior of the organelle has now been associated with a mechanosensing function in some cell types (Collin et al., 2008; Labernardie et al., 2010; Van den Dries et al., 2013). Invadosomes are therefore expected to contribute to a wide range of biological processes. As mentioned, the structures may differ in their size and appearance, abundance, dynamics and subcellular localization and arrangement of structural components, depending on the cell type considered (for more on differences between invadopodia and podosomes, see (Murphy and Courtneidge, 2011)). Thus, the architecture, properties and subcellular distribution of invadosomes may reflect, at least in part, the cellular processes in which they are involved.

One of the main results of invadosome research is the connection of both podosomes and invadopodia with human diseases, which revealed their integral role in biological processes. Invadopodia are commonly studied in cell lines derived from solid cancers. The evidence for their existence in human tumors came from primary cells isolated from head and neck, bladder and brain tumors (Clark et al., 2007; Sutoh et al., 2010; Stylli et al., 2008). Cancer cells with invadopodia have high invasive potential *in vitro* and metastatic potential in mouse transplants (Coopman et al., 1998). Podosomes are extensively studied in cells of the myelomonocytic lineage such as macrophages, immature dendritic cells (iDCs) and osteoclasts. Pioneering studies performed by Linder and colleagues reported that macrophages from patients expressing truncated forms of WASp completely lack podosomes (Linder et al., 1999). Macrophages and iDCs devoid of podosomes show impaired chemotaxis, and WASp null osteoclasts exhibit abnormal patterns of bone resorption both *in vitro* on bone slices and *in vivo* (Calle et al., 2004). Other cell types, such as endothelial cells (ECs) or smooth muscle cells (VSMCs) in the vascular system, also have the ability to form podosomes. In these cells, podosomes are not present in quiescent cells but arise in response to certain cytokines, providing a conceptual framework to explore the role of podosomes in the pathogenesis of some vascular diseases.

Podosomes and invadopodia have been extensively studied in *in vitro* models and on two-dimensional (2D) surfaces. Their similarities and differences, as well as their relationship to focal adhesions, lamellipodia and ruffles have been reviewed in detail (Murphy and Courtneidge, 2011; Hoshino et al., 2013) (Table 1). Invadosome composition as well as the extracellular cues and intracellular signaling cascades leading to their assembly, have been extensively studied in such models. However, most cells evolve within three-dimensional (3D) contexts inside living organisms, surrounded by other cells and diverse ECM components, raising the question to what extent observations made on single cells on planar surfaces apply to *in vivo* situations. Such thinking is strengthened by reports on differences between cell migration in 2D and 3D conditions (Meyer et al., 2012; Baker and Chec, 2012) and motility *in vivo* (Patsialou et al., 2009). For these issues, which are intrinsically of wide interest to all biologists, there may be light at the end of the tunnel. Taking one step at a time, a number of approaches for studying invadosomes have been developed in 3D *in vitro* (Table 1), and show major differences in invadosome morphology in 3D environments as compared to 2D surfaces (Wiesner et al., 2014). Analysis of invadosomes has now been reported for *in situ* tissue explants, tissue sections and in *in vivo* models. Concerted efforts aim at tracking signs of their presence in optimized *in situ* settings to support their relevance and subsequent association with human diseases. Moreover, as invasion programs also take place during embryogenesis, invadosomes and related protrusions may be essential to developmental processes. These studies open the way to address the role of these structures in pathophysiological processes as well as their participation in developmental programs, leading toward establishment of the causative link between invadosomes and invasion.

Herein, we describe the studies where evidence for the occurrence of invadosomes or their homologs has been provided *in situ* or *in vivo*. These include mouse models of cardiovascular disease,

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