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Review

Invadopodia: The leading force

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ABSTRACT

Metastatic spread of cancer cells is the leading cause of mortality from cancer. Metastatic cancer cells must penetrate through several barriers to escape the primary tumor and gain entry into the bloodstream in order to spread to other tissues. It is believed that invasive cancer cells penetrate these barriers by forming specialized F-actin rich protrusions called invadopodia that localize matrix degrading activity to cell-substrate contact points. Invadopodia gain their protrusive ability by combining the physical force generated by actin polymerization with the chemical activity of matrix degradation. Accumulating data over the past few years have shed light on the molecular mechanisms as well as kinase signaling pathways that regulate the complex process of actin polymerization in invadopodia. Here we review some of these mechanisms, the signaling pathways that regulate this process, as well as the in vivo relevance of invadopodial structures. Understanding the mechanisms that govern invadopodia formation and function is an essential step in the prevention of cancer invasion and metastasis.

Introduction

Metastasis, the dissemination of cancer cells from the primary tumor and their spread to distant sites of the body, is the leading cause of mortality in breast cancer patients. The primary tumor arises from a host of different genetic causes, making it difficult to target treatments in many individuals. However, metastasis is a common phenomenon in all tumors, and therefore an important process to target for therapeutic intervention. Metastatic cancer cells leave the primary tumor and invade through the basement membrane, a network of extracellular matrix (ECM) proteins that support the overlying epithelium. Once they have escaped the tumor, cancer cells must also degrade the vascular sub-endothelial basement membrane to gain entry into the bloodstream. Invasive cancer cells penetrate these barriers by forming invadopodia, F-actin rich protrusions that localize matrix-degrading activity to cell-substrate contact points and represent sites in which cell signaling, proteolytic, adhesive, cytoskeletal, and membrane-trafficking pathways physically converge (Weaver, 2006, 2008). As such, understanding the mechanisms governing the formation and function of invadopodia will provide insights into the biology, regulation, and potential therapeutic approaches to cancer metastasis.

Invadopodia versus podosomes: definition, similarities, and differences

Invadopodia of cancer cells are reminiscent of podosomes, another cytoskeletal structure used by normal, highly motile cells. Podosomes and invadopodia are specialized F-actin rich protrusions that form on the ventral membrane of the cell and have close contact with the extracellular matrix. They can degrade the ECM by modulating the focused release and activation of proteases, such as matrix metalloproteases (MMPs), that promotes the ability of cells to cross tissue barriers (Buccione et al., 2009; Gimona et al., 2008; Linder, 2009; Linder et al., 2011; Stylli et al., 2009; Weaver, 2006). Podosomes were initially discovered in the early 1980s as adhesive structures with degrading ability in Rous sarcoma virus transformed fibroblasts (Chen et al., 1985; David-Pfeuty and Singer, 1980; Tarone et al., 1985) and later on in normal cells such as osteoclasts (Zambonin-Zallone et al., 1989). Similar structures with matrix degrading ability were described several years later in human cancer cell lines by Chen (1989) and were termed invadopodia ("invasive feet") to emphasize their protrusive nature (Fig. 1).

Podosomes and invadopodia share numerous similarities in organization, composition and function. Understanding the similarities and differences between these structures is currently a subject of much research and controversy. Both appear in culture as punctate F-actin rich structures that contain actin regulatory

Abbreviations: ECM, extracellular matrix; MMP, matrix metalloproteinase; EMT, epithelial to mesenchymal transition; NPF, nucleation promoting factor; FAK, focal adhesion kinase.

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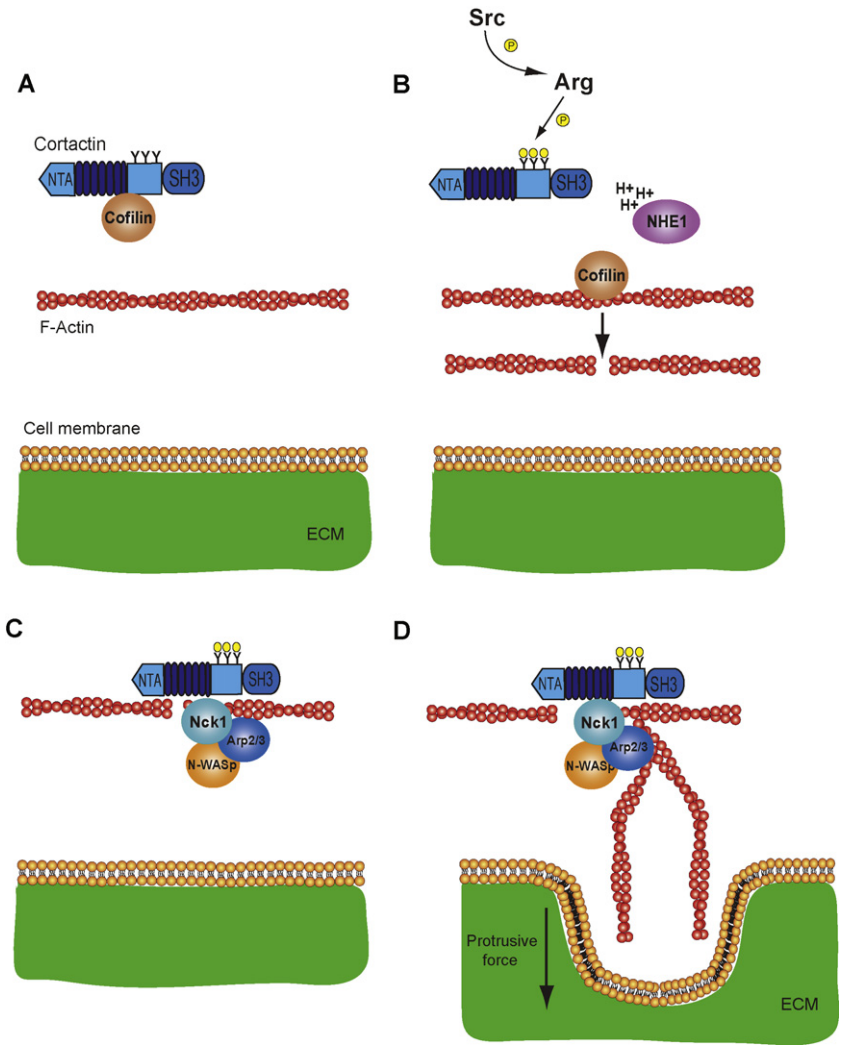

Fig. 1. Cortactin phosphorylation regulates actin polymerization in invadopodia. (A) In unstimulated cells, cofilin is bound to cortactin in an electrostatic interaction. (B) Following stimulation (i.e. EGF) cortactin is phosphorylated by a Src-Arg cascade on its three major tyrosines (Tyr 421, 466, 482). Phosphorylated cortactin recruits NHE1 which increases local pH at invadopodia. As a result, cofilin is released from cortactin and can now severe actin to generate free barbed ends. (C) Phosphorylated cortactin recruits the Nck1-N-WASp-Arp 2/3 complex, which induces actin polymerization that produces the physical force that pushes the invadopod membrane into the matrix and enables invasion of the cell (D).

proteins (Arp2/3, cortactin, WASp or N-WASp), focal adhesion proteins (paxillin, talin), and signaling proteins such as protein kinases, adaptor proteins and integrins. ECM degradation takes place in both structures and co-localizes with sites of active actin polymerization (Buccione et al., 2004, 2009; Gimona and Buccione, 2006; Linder, 2007; Linder and Aepfelbacher, 2003; Weaver, 2006).

Podosomes and invadopodia have several differences that distinguish them from each other. The first and most significant difference that defines these two structures is the type of cell in which they are found: Podosomes usually form in normal, non-pathological highly motile cell types such as monocytes, macrophages, dendritic cells and osteoclasts, as well as in endothelial cells, smooth muscle cells and transformed fibroblasts, while invadopodia are mostly found in cancer cells including metastatic mammary carcinoma cells, melanoma cells, and aggressive head and neck squamous carcinoma cells. A second difference is in their size and abundance. Podosomes are relatively small, approximately 1 μm in diameter, 0.4 μm in height, and are present in large numbers (20–100). In contrast, invadopodia are larger, 8 μm diameter and 5 μm height, and are less abundant (1–10 per cell). Podosomes and invadopodia differ markedly in their dynamics: Podosomes are highly dynamic and have a lifetime of several minutes, while invadopodia are considered more stable and can persist for over one hour. As a consequence, the pattern of degradation that is resulted by invadopodia activity is deeper and more focused compared to the shallow, widespread degradation by podosomes (Gimona et al., 2008; Linder, 2007, 2009). Much effort has been focused towards defining specific markers that can distinguish between podosomes and invadopodia. Among these, vinculin has been suggested as a podosome marker (Gimona et al., 2008) while Nck1 has been shown to be invadopodium-specific (Oser et al., 2011)

Invasive structures: not only cancer

There is a striking similarity between the processes of cancer metastasis and embryonic development. This similarity results by a common process called the epithelial-to-mesenchymal transition (EMT), in which cells change their gene expression program to enable them to become motile and invasive. Growing experimental evidence link the formation of migratory membrane protrusions such as podosomes or invadopodia to the process of EMT and cancer metastasis (Eckert et al., 2011; Sung et al., 2009). Indeed, protrusive cytoskeletal structures that resemble podosomes and invadopodia have been documented in several embryonic processes, such