Review

Breaking away: matrix remodeling from the leading edge

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Substantial progress has been made in recent years toward understanding the molecular mechanisms by which tumor cells, and the supporting stroma, degrade confining matrix during migration. Significant attention has been focused on understanding the biology of several dynamic and distinct, but remarkably related, cell structures that include lamellipodia, focal adhesions (FAs), filopodia, podosomes, and invadopodia. How these invasive organelles assemble and function is a topic of intense study. Most exciting has been the recent progress made by combining advanced microscope technologies with a wide variety of different 3D matrices, tissue explants, or even living model organisms. From these approaches, it has become increasingly evident that the conventional definitions of these invasive structures may be less clear than was previously thought.

Adhere and degrade: invasive dissemination as a key component of the metastatic process

It is well documented that many malignant tumors are characterized by modest encapsulation that permits aggressive dissemination from the site of origin into peripheral stroma, vessels, and, subsequently, other organs. In addition to known defects in genomic stability, cell cycle check points, and contact inhibition, many aggressive tumors possess the capacity actively to degrade and remodel the surrounding stroma by the combined processes of matrix metalloprotease (MMP) secretion [1–3] and chemotactic migration [4]. This invasive process is supported not only by the primary neoplastic lesion, but by a complex stroma that includes cancer associated fibroblasts (CAFs), macrophages, and endothelial cells that contribute distinct motogenic cytokines and matrix components. This complicated mix forms what is commonly referred to as the tumor microenvironment and provides a supportive milieu for tumor cell migration either individually or in a 'collective' fashion (Figure 1a) [1].

Lamellipodia, FAs, podosomes, filopodia, and invadopodia are known to share many components that are mixed and matched on a central core of branched or bundled actin filaments. This core usually can sustain protrusive deformation of a plasma membrane domain that may exhibit some enrichment in specific phosphoinositides. A large variety of different actin scaffolding proteins – including WASP, N-WASP, VASP, and actin bundling/remodeling proteins such as cortactin, gelsolin, and many others – can attach to this central scaffold. In turn, this network provides a platform for integrin binding and recruitment of small Rho GTPases, myosins, Src kinases, dynamin, and many other components (for helpful tables and illustrations that compare the content and characteristics of these structures see [5–7]). As lamellipodia, filopodia, FAs, and the *bona fide* matrix-degrading structures invadopodia and podosomes are similar in composition, it is likely that these structures interact, exchange, interconvert, and even coalesce at the leading edge of migrating tumor cells and/or accompanying fibroblasts (Figure 2). This is not a comprehensive review of the literature on invasive migration, which has been done by others [5,6,8]. Instead, a focus on recent observations implicating the advancing lamellipodium as a multipurpose, degradative, and contractile structure or 'invadosome' is provided.

Due to structural and functional similarities, the terms podosome and invadopodium have been used interchangeably in the literature. More recently, the field has arrived at some clarity in the use of these descriptors. Although perhaps identical in structural content, podosomes are now generally viewed as degradative organelles of more differentiated cell types that can include invading macrophages, vascular smooth muscle cells, bone remodeling osteoclasts, and others. By contrast, invadopodia could be viewed as renegade aberrations of neoplastic transformation in which oncogenic activation leads to inappropriate mobilization of the actin cytoskeleton and associated proteins. Indeed, normal epithelial cells of ductule-based organs such the liver, breast, or pancreas would seem to have little obvious need for these invasive structures. More likely, these are assembled from pre-existing components upon transformation as cells lose their characteristic polarized organization and contacts with adjacent cells are compromised.

If most healthy differentiated epithelial cells do not normally degrade the surrounding matrix, how are invadopodia formed on neoplastic transformation? Although many cultured tumor cell lines do not form *bona fide* invadopodia, those that do could utilize FAs or equivalent structures as nucleating precursors. As detailed in a recent review [6], these structures share a substantial, nearly complete set of components. In fact, it can be challenging to distinguish the two structures biochemically or by cell staining. There is strong evidence, however, for FAs functioning as precursors for invadopodia formation [9]. In this study, an accumulation of phosphoinositide ($PtdIns(3,4)P_2$) at FAs was key to

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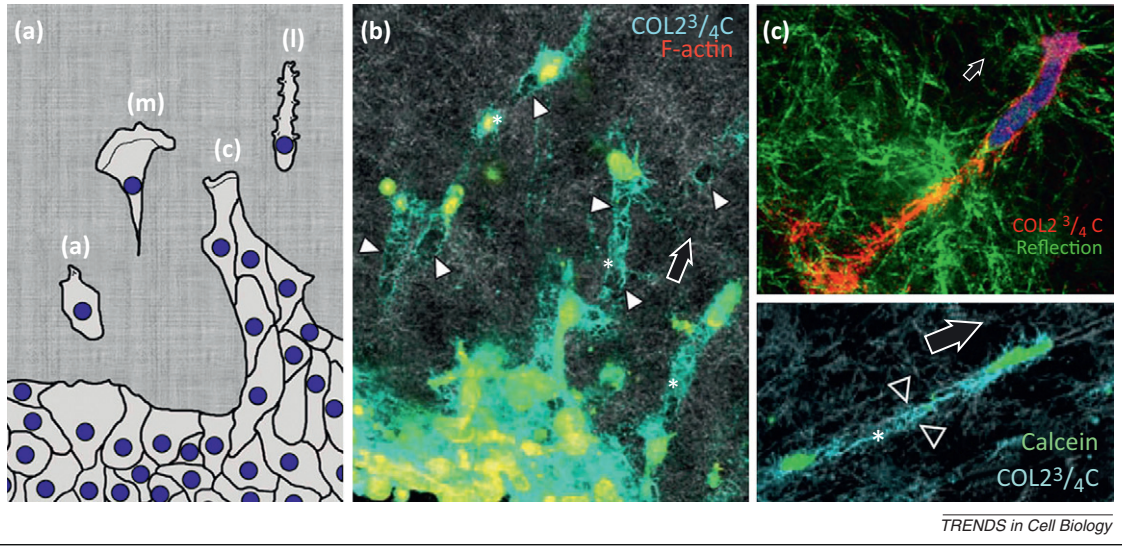

Figure 1. Breaking away: synergistic movement by tumor and stromal cells leaving a destructive path. **(a)** Cartoon illustrating distinct classes of cellular movement from the site of the primary tumor. These include mesenchymal (m), lobopodial (l), amoeboid (a), and collective (c) cell motility. These cellular movements exhibit different morphologies and requirements for ROCK, Rho, myosin, and other components while supporting tumor dissemination and matrix remodeling. Modified with permission from [1]. **(b–d)** HT1080 cells were fixed while migrating through a collagen lattice *in vitro*. Blue (b,d) or red (c) represents paths of degraded collagen using a specific antibody (COL2^3/$_4$C); other markers indicate the cell body. Arrows indicate direction of migration; arrowheads point to degraded collagen trails. Republished with permission from [16] and [2].

initiating Src-induced formation of invadopodia in NIH3T3 cells (Figure 3a). These authors proposed a stepwise model for invadopodium formation that utilizes pre-existing FA sites through activation of Src and FA kinases (FAKs) to stimulate the formation of PtdIns(3,4)P_2 that, in turn, recruits N-WASP, dynamin, cortactin, and the invadopodium-specific adaptors Tks4/5.

In addition to acting as nucleation sites for invadopodium formation, it is now believed that FAs can degrade matrix directly. This concept was first introduced following observations that exogenously expressed membrane type 1 (MT1)-MMP was recruited to FAK-positive adhesive sites at the leading edge of migrating cells [10,11]. This recruitment not only supported matrix degradation at FA sites, but appeared to alter integrin clustering and thereby promote turnover of cell adhesions to facilitate motility. Because HeLa cells express low levels of endogenous MT1-MMP, many of these observations used HeLa cells over-expressing exogenous MT1-MMP or a human fibrosarcoma cell line (HT1080) that expresses high levels of endogenous MT1. Because of these elevated protease levels, one could predict that recruitment to FAs occurs on saturation of the invadopodium machinery based on the similarity of these structures. These findings did, however, draw a functional comparison between FAs and invadopodia.

Very recently our group has made several observations that implicate FAs as *bona fide* matrix-degrading organelles [12]. On examination of several different human pancreatic adenocarcinoma cell lines (Panc-1, BxPC3, Panc 04.03), as well as human fibroblasts and HT-1080 cells, we observed marked degradation of extracellular matrix at FA sites (Figure 3b). Because of the similarity between FAs and invadopodia, multiple criteria were utilized to discriminate between these structures in the cells examined. In general, FAs have an oblong shape, reside near the cell periphery, provide initiation sites for actin stress fibers,

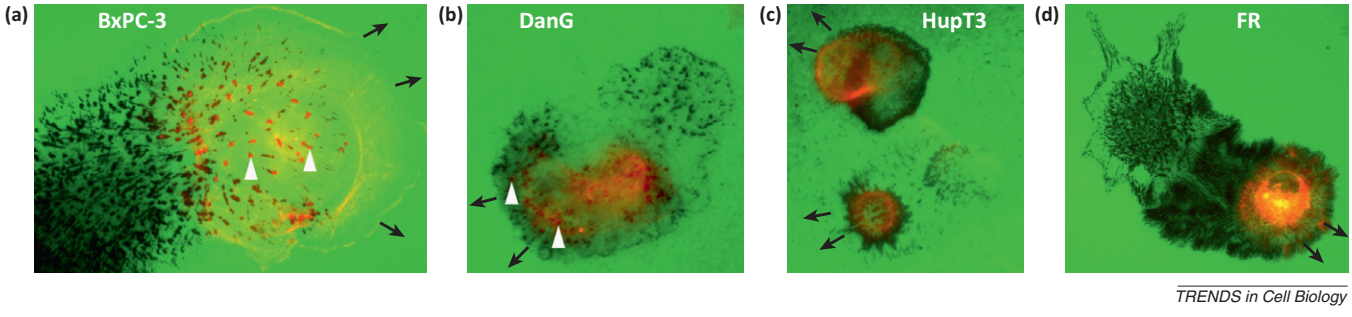

Figure 2. Migratory degradation by tumor and stromal cells in 2D culture. Images of cultured cells moving on top of a fluorescent gelatin matrix. **(a–c)** Human pancreatic tumor cells, and **(d)** rat fibroblast (FR) transfected to express membrane type 1 matrix metalloproteinase (MT1-MMP). Cells were plated for 3–15 hours before fixation and staining for actin (a–c) or zyxin (d). Most striking are the numerous black voids that are left by the degradative action of actin-rich invadopodia (arrowheads), focal adhesions, and other structures. A trail of degradation that occurred before fixation reveals the migratory path made by each cell. The black arrows are situated at the advancing lamellipodium of each cell and indicate the direction of movement. The white arrowheads point to sites of matrix degradation that correspond to actin structures likely to be invadopodia.