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Current Opinion in Cell Biology

Modes of cancer cell invasion and the role of the microenvironment

Andrew G Clark[1,2] and Danijela Matic Vignjevic[1,2]

Metastasis begins with the invasion of tumor cells into the stroma and migration toward the blood stream. Human pathology studies suggest that tumor cells invade collectively as strands, cords and clusters of cells into the stroma, which is dramatically reorganized during cancer progression. Cancer cells in intravital mouse models and *in vitro* display many 'modes' of migration, from single isolated cells with round or elongated phenotypes to loosely-/non-adherent 'streams' of cells or collective migration of cell strands and sheets. The tumor microenvironment, and in particular stroma organization, influences the mode and dynamics of invasion. Future studies will clarify how the combination of stromal network structure, tumor cell signaling and extracellular signaling cues influence cancer cell migration and metastasis.

Addresses
[1] Institut Curie, PSL Research University, 75005 Paris, France
[2] CNRS, UMR144, 75005 Paris, France

Corresponding author: Clark, Andrew G (andrew.clark@curie.fr)

Current Opinion in Cell Biology 2015, **36**:13–22

This review comes from a themed issue on **Cell adhesion and migration**

Edited by **Michael Sixt** and **Erez Raz**

http://dx.doi.org/10.1016/j.ceb.2015.06.004

Introduction

Metastasis is a hallmark of cancer and the leading cause of mortality among cancer patients [1]. Cancer, in its most virulent form, is thus not only a disease of uncontrolled cell growth, but also a disease of uncontrolled cell migration. The first step in metastasis is the migration of cancer cells away from the primary tumor, a process called *tumor invasion* (Figure 1). In solid epithelial tumors, or *carcinomas*, invading cells must first cross the basement membrane (BM). The BM is a natural barrier between the epithelium and the *stroma*, a network of extracellular matrix (ECM) populated by a number of other cell types that surrounds the tissue. Metastasizing cells migrate through the stroma to reach blood or lymph vessels, where they can be carried to other organs. In this review, we will focus on the migration of cancer cells through the stroma. The mechanisms of BM invasion have recently been reviewed [2].

We will first discuss general features of cancer cell migration through the stroma, with a focus on tumor cell morphology and migration mode. Next, we will summarize findings specifically from human pathology studies, *in vivo* studies employing intravital imaging techniques, and *in vitro* systems, highlighting tumor–stroma interactions. Finally, we draw attention to the similarities and differences in findings using these different systems and discuss outstanding questions in the field.

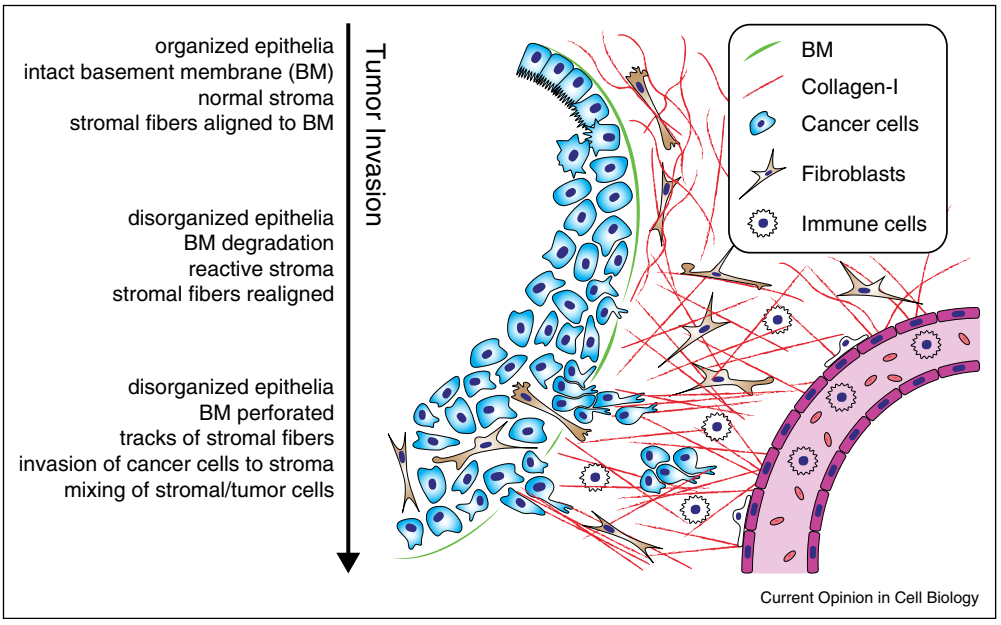
Modes of cancer cell migration

Both human pathology studies and intravital imaging studies in mouse systems have revealed a great diversity in the morphologies of invading cancer cells and the way these cells migrate. Cancer cells possess a unique ability to adapt to different environmental conditions, assuming different morphologies and migration characteristics in order to stay motile [3]. *In vivo*, motile tumor cells have been observed to migrate individually as single cells, as loosely-attached cell streams and as well-organized, adherent collectives. In human cancer pathology studies, cancer cells from epithelial tumors primarily invade collectively, while in intravital imaging studies, cancer cells display a wide range of different migration modes and morphologies (Box 1).

In vitro studies have identified several intrinsic factors regulating migration mode and morphology. In cancer cells migrating individually, increased contractility, under control of the Rho-pathway, favors amoeboid-like migration, while lower contractility (and/or increased adhesion) favors more mesenchymal phenotypes [4–7]. Increased cell-cell interactions *via* cadherins and cell-ECM binding *via* integrins can promote collective migration in cancer cells (reviewed in [3]). It is not currently well understood how cell morphology affects a cell's ability or tendency to migrate individually or collectively. However, cells with amoeboid-like morphologies tend to migrate individually or in streams, while epithelial cells migrate collectively. Cells with mesenchymal morphologies can most readily switch between single-cell, streaming and collective migration modes. For example, in hepatocyte growth factor-treated MDCK cells, which have a mesenchymal phenotype, upregulation of N-cadherin activity can promote a switch from individual to collective cell migration [8].

In addition to intrinsic factors, the microenvironment plays a significant role in determining cancer cell migration mode and morphology. In the remainder of the

Figure 1



Summary of tumor progression and invasion. In tumors of epithelial origin, or *carcinomas*, hypertrophic cell growth causes the epithelial layer to become many cell-layers thick. At more advanced stages, carcinoma cells often lose apical-basal polarity and apical cilia and may appear disorganized, due to reduction in cell-cell contacts and cytoskeletal reorganization (see also Figure 2a; [28]). At this 'carcinoma *in situ*' stage, the cancer cells are still encapsulated by the BM. Due to cross-talk between tumor cells and stromal cells, the stroma becomes reactive. Reactive stroma is characterized by an increased presence of immune cells and fibroblasts, which can help to deposit ECM and reorganize the stromal network (mostly made of collagen-I). Stromal network fibers are initially loosely organized and appear 'curly' and later increase in density and stiffness. At late stages, collagen bundles form 'tracks' perpendicular to the BM. In invasive tumors, cancer cells perforate the BM or migrate through regions of dysfunctional BM deposition, allowing the tumor cells to invade the stroma and migrate toward the blood stream (reviewed in [2]). Stromal cells can also enter the tumor, leading to a mixing of cell types and further disorganization of the tissue.

review, we will describe different migration modes that have been observed in human pathology studies, intravital imaging studies and *in vitro* experiments and focus on the role of the microenvironment in determining migration mode.

The pathology of tumor invasion in humans

In human epithelial cancers such as colorectal and breast cancers, invasive cells are typically observed to migrate collectively [15,16,17••]. Invasive carcinomas often display a disorganized glandular structure (Figure 2a). From these neoplastic glands, strands and cords of tumor cells project into the stroma at the *invasive front* (Figure 2b–f; [18–20]). Scattered clusters of ~5 cells (*tumor buds*) have also been observed (Figure 2b,c,f; [21,22]). This suggests that invading tumor cells *in vivo* typically preserve cell-cell contacts, leading to collective migration of groups of cancer cells.

Invading cells often display characteristic Epithelial to Mesenchymal Transition (EMT) markers, such as down-regulation of E-cadherin and upregulation of Vimentin, and lose some epithelial characteristics, such as apical-basal polarity [23]. Despite these changes, in human cancers, invading cells usually do not have a typically spindle-shaped mesenchymal phenotype. This has fueled a debate over the role of EMT in human cancer progression [15,20]. However, in pathological examinations, it may be difficult to distinguish stromal cells from individual tumor cells with spindle-shaped phenotypes, potentially leading to the rarity of observed mesenchymal tumor cells in human cancers. A recent study using 3D reconstructions of serial tissue slices found that invading tumor cells invade almost exclusively in a collective manner. Cells in invading buds only rarely display changes in morphology to spindle-shaped (mesenchymal) or rounded phenotypes, while exhibiting some changes in EMT markers [17••].

Recent studies have uncovered that clusters of circulating tumor cells (CTCs), also called tumor microemboli, are present in the circulation of patients with invasive melanoma, lung cancer and renal cell carcinoma [24–26]. It is possible that the presence of microemboli in the circulation is due to intravasation of small groups of collectively-migrating tumor cells. A recent study using a mouse breast cancer model suggests that tumor cell clusters in the circulation may indeed arise from the entry of groups of tumor cells into vessels, rather than aggregation of cancer cells following intravasation [27•]. It is