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Modes of cancer cell invasion and the role of the microenvironment

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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.

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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.

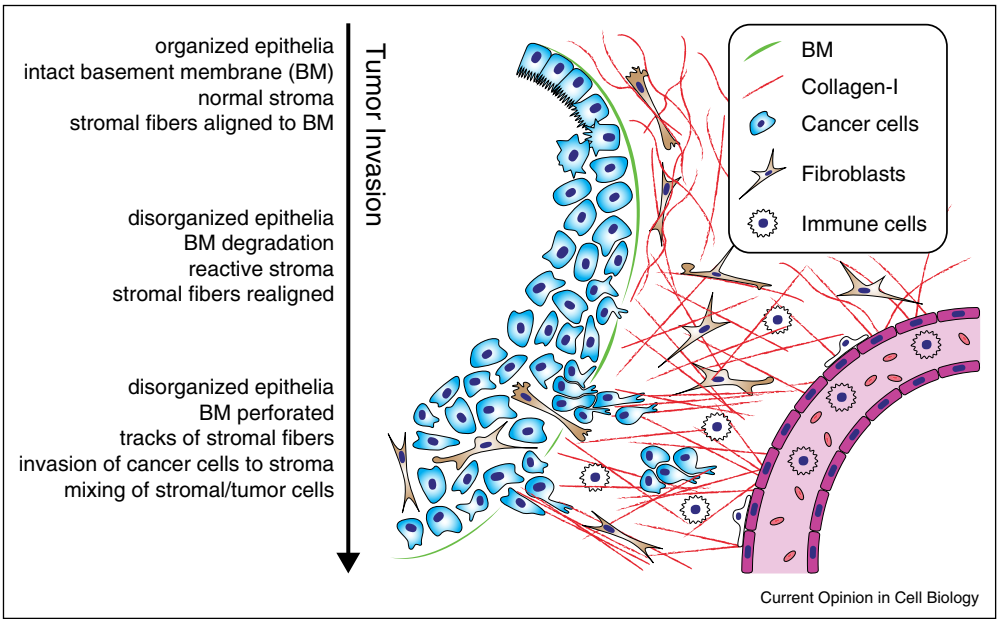
Modes of cancer cell migration

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

Box 1 Cancer cell migration modes and dynamics

Single-cell migration is characterized by a lack of cell-cell interactions during migration and low correlation in the migration pattern between a cell and its neighbors. Cells that migrate singly can display different phenotypes. In **amoeboid-like** migration, cells have a round cell-body phenotype and can differ dramatically in their protrusive activity. Amoeboid-like motility comes in several variants: 1) cells that rapidly change their morphology, have short thin protrusions, are devoid of blebs and move with high velocities (0.4–5 μm/min); 2) much slower cells with a blebbing morphology and chaotic movements; 3) cells with short cellular protrusions associated with proteolytic activity moving with low velocities (~0.1 μm/min). Other singly-migrating cells have a **mesenchymal** phenotype, which is characterized by an elongated ('spindle-shaped') cell body and longer protrusions. While the protrusions of such cells advance relatively rapidly (~0.4 μm/min, our unpublished data), in some cases, the cell rear stays immobile, resulting in relatively slow net translocation (~0.2 μm/min).

Multicellular streaming is characterized by loosely- or non-adherent cells that migrate along the same path. Cells in streams have typical speeds of 1-2 μm/min and significantly longer and straighter paths compared to isolated migratory cells and can display amoeboid-like or mesenchymal phenotypes [3].

Collective migration is characterized by groups of cells that retain cell-cell adhesions for long periods of time and display a high correlation in directionality between neighboring cells during migration. Cells move either as narrow linear strands lead by one leader cell or as broad, irregularly shaped sheets, which are multiple cells in diameter and lead by several leader cells [3,9,10•]. Collectively migrating cells can display mesenchymal or epithelial phenotypes, and the phenotypes may differ between 'leader' and 'follower' cells in some cases [11]. Collective migration is typically the slowest mode of cancer cell migration (0.01–0.05 μm/min), but faster collective migration (0.2-1 μm/min) has been observed during development *in vivo* (*e.g.*, [12–14]).

not currently clear if migration mode influences later metastasis. However, the same study demonstrated that such tumor cell clusters can more efficiently colonize secondary organs [27•].

Distant metastases typically recapitulate the epithelial/glandular morphology of the primary tumor, with epithelial-like phenotypes. This could suggest that metastases arise from tumor cells with preserved epithelial characteristics that migrate and colonize other organs as clusters. Alternatively, individually-invading cells that have undergone EMT could undergo a *Mesenchymal to Epithelial Transition* (MET), upon reaching a secondary organ [23,28,29]. Indeed, cells with mesenchymal phenotypes are enriched in CTC populations in some breast and colon cancer patients, and the presence of mesenchymal CTCs correlates with poor prognosis [30,31].

During tumor progression, the stroma, which surrounds the epithelial tissues, also undergoes profound changes. The stroma is comprised primarily of a collagen-I network. In normal stroma, collagen fibers typically appear 'curly' and anisotropic [32]. During early cancer progression, the amount of collagen in the stroma increases, and collagen fibers appear straighter and are aligned parallel to the tumor border [33••]. Similar patterns have been found using a murine breast cancer model [34]. In invasive tumors, collagen fibers become bundled and are oriented perpendicularly to the BM, providing 'tracks' for cancer cells to migrate away from the primary tumor (Figure 1). This particular organization of collagen is correlated with poor patient survival [33••].

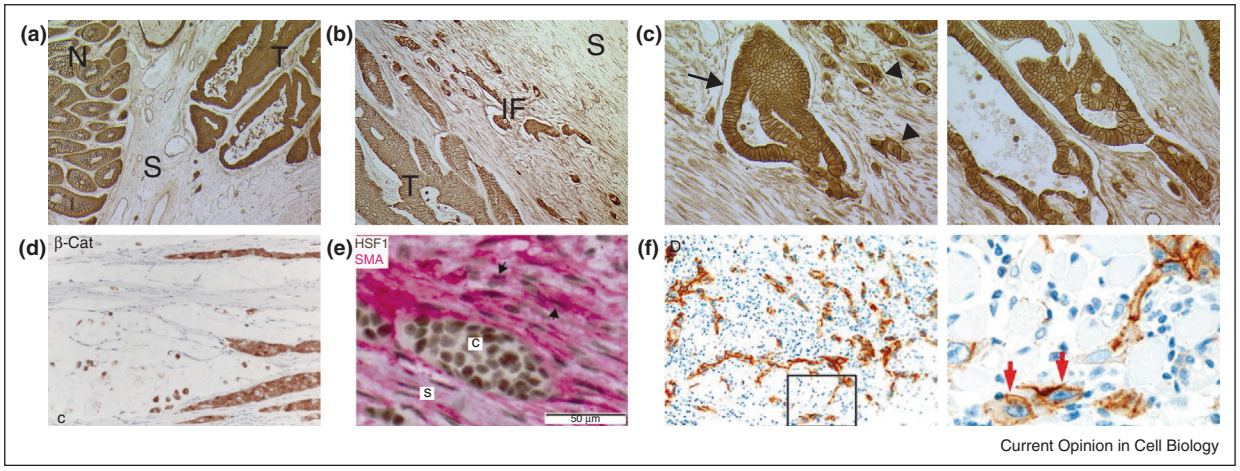
The reorganization of the stromal network is primarily mediated by stromal cells, most prominently fibroblasts. Cancer-associated fibroblasts (CAFs) reorganize the stroma by secreting ECM and enzymes that covalently cross-link collagens fibers and by physically pulling on the collagen network [32,35,36]. As a result, the stromal network becomes stiffer. In breast cancers, neoplastic tissue can be two- to ten-fold stiffer than normal tissue. This increase in tissue stiffness is thought to primarily reflect changes in the stroma [37–39], while tumor cells themselves appear to become softer [40]. Breast tumors are more likely to develop in regions of high tissue density [41], and tissue density itself is a predictive risk factor in the development of breast cancer [42–44]. The dramatic reorganization of the stroma in invasive cancers is likely to contribute to changes in the migratory properties of tumor cells that lead to later metastasis [32]. Although many details of stromal evolution during tumor progression are yet to be determined, an increase in network stiffness and reorganization into thicker bundles appears to correlate with an increased risk for metastasis.

Intravital imaging of cancer cell invasion

With the introduction of multiphoton-based intravital imaging about 15 years ago, it became possible to observe cancer cell behavior during tumor invasion *in vivo* [45,46]. In intravital cancer cell migration experiments, tumors are usually generated by subcutaneously or orthotopicaly xenografting cancer cells that express fluorescent proteins. Alternatively, cancer cell migration can be studied in genetically modified animals that develop tumors spontaneously. Fluorescently-labeled dextran can be intravenously injected to highlight the vasculature, and stromal collagen fibers can be visualized using second harmonic generation (SHG). However, it is not currently possible to visualize changes in the stromal network during tumor progression, due to the relatively slow timescale of this process and challenges in long-term imaging in intravital studies. New tissue preparation methods may allow for the observation of the co-evolution of stromal structure and tumor progression on longer timescales. For excellent reviews on current intravital imaging techniques, refer to [9,47,48].

While most studies agree that the vast majority of cancer cells *in vivo* are immobile over periods of several hours, a wide range of different migration modes and phenotypes have been described for motile cancer cells (Table 1, Figure 3). In general, grafted cells that display amoeboid-like morphology, such as A375 and B16F2 melanoma

Figure 2



Tumor invasion in human epithelial cancers. **(a–c)** Colon tissue section from colon cancer patient stained with a β-catenin antibody (brown). **(a)** Section highlighting the difference in epithelial organization between normal glandular tissue (N) and a tumorous gland (T) separated by stroma (S). **(b)** Colon cancer tissue section showing the edge of a tumorous gland (T), the invasive front (IF) and more distal stroma (S), which has not yet been invaded. **(c)** Examples of invasion phenotypes from colon cancer tissue sections showing invasive gland structure (*left, arrow*), small tumor buds that have separated from body of the tumor (*left, arrowheads*) and a cord of connected invasive cells (*right*). **(d)** Finger-like strands of invading cancer cells in a singlet ring-type colon carcinoma, with β-catenin staining (brown). Modified from [83]. **(e)** Tissue section from breast cancer patient showing a group of invasive cancer cells (C) surrounded by stroma (S). Cells are stained for the transcriptional regulator heat shock factor 1 (HSF1; brown) and smooth muscle actin (SMA; pink). Modified from [84]. **(f)** Tissue section from an oral squamous cell carcinoma displaying thin cords and strands of tumor cells as well as smaller tumor buds at the invasive front. Cells are stained for the nerve growth factor receptor $p75^{NTR}$ (brown) and counterstained with hematoxylin (blue). *Right*: high-magnification zoom of outlined region in *left* image. Red arrows indicate invasive cells expressing $p75^{NTR}$. Modified from [85].

cells, tend to migrate as single cells or as streams. Cells with predominantly mesenchymal phenotypes, like MTLn3 or HT-1080, are more prone to switching between single-cell and collective migration modes. Intravital imaging has been essential to building an understanding of tumor cell invasion dynamics and how the native tumor environment can impact invasion and metastasis.

Intravital imaging studies suggest that the stromal collagen network can significantly impact the mode and direction of cancer cell migration. In breast cancer models, while areas with low collagen density contain mostly non-motile cells, motile cells can be found in the proximity of large collagen bundles [55•]. In fast-migrating cancer cells, both with amoeboid-like and mesenchymal phenotypes, cells orient their protrusions parallel to collagen bundles, using the bundles as 'highways' for efficient, directional migration [9,59,64]. Loosely connected streams of migrating cells and collectively migrating cell strands also align parallel to collagen fibers [9,61]. It has recently been suggested that breast tumor cell behavior (slow-moving and high ECM degradation *vs.* fast-moving and invasive) is influenced by a combination of collagen structure, presence of macrophages and proximity to blood vessels [57].

The microenvironments of nearby tissues can also influence tumor cell migration. For example, melanoma cells in the dermis tend to migrate as fast, directional and collective strands along linear tracks of muscle fibers and nerves. In contrast, cells migrating in fatty connective tissue migrate slowly as a broad, poorly organized multicellular group lead by several leader cells, from which single cells occasionally detach [10•]. Similarly for fibrosarcoma cells implanted in the deep dermis, loosely organized fat tissue favors single-cell migration, while muscle fibers and lymph vessels promote collective migration of multicellular strands [61]. These studies suggest that more organized/aligned environments may promote more collective migration.

The presence of nearby blood vessels can also influence migration, though it is unclear if this is due to differences in organization of the stromal network or signaling. Cancer cells are typically more motile in the proximity of blood vessels; however, there is no correlation between the occurrence of single-cell migration *vs.* streaming and blood vessel density [54,58,65]. The presence of macrophages, which are usually found near blood vessels, positively correlates with cancer cell motility [62]. It is hypothesized that macrophages help cancer cells to invade the stroma and to intravasate into blood vessels [62,66,67].

In spontaneous murine colorectal tumors, distinct invasive regions have been identified. In one region, thick and

Table 1

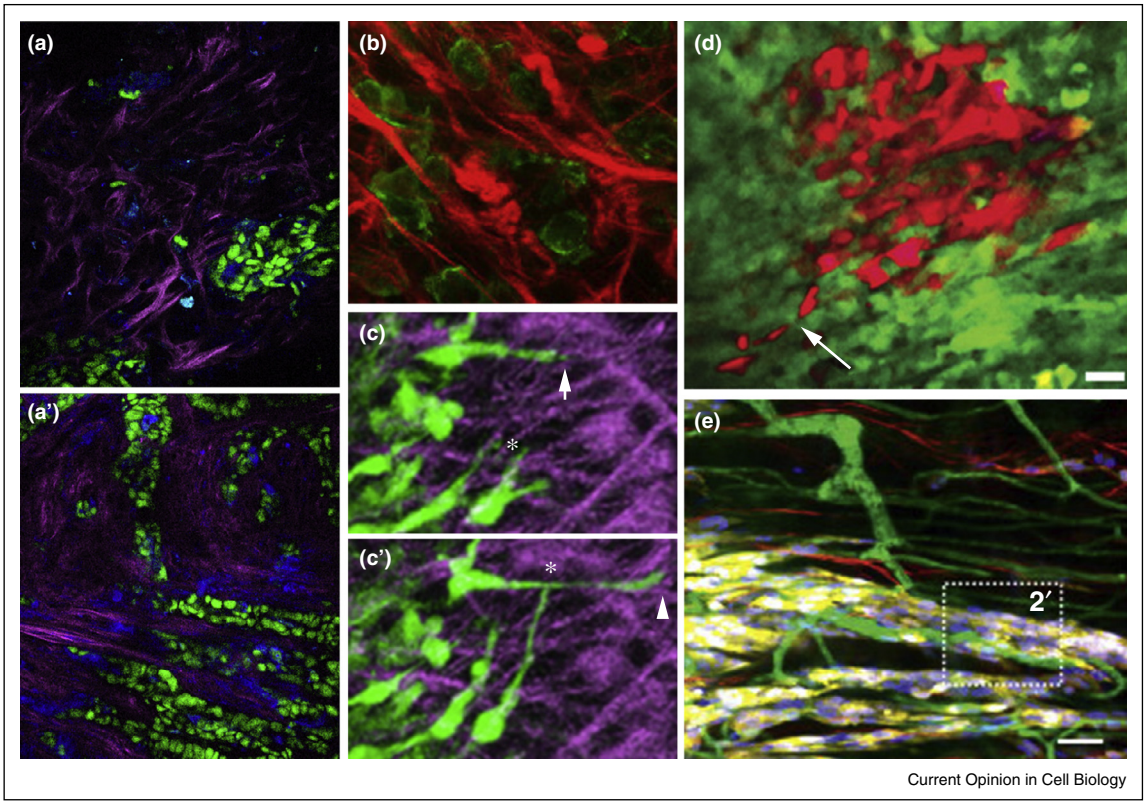
Migration modes and dynamics observed by intravital imaging.

Cell type	Fraction of motile cells	Migration mode	Speed	Reference
A375M2 human melanoma		70% amoeboid-like (single-cell; with and without blebs) 30% mesenchymal	0.5–10 μm/min amoeboid-like: ~3 μm/min mesenchymal: ~1 μm/min	[6]
A375 human melanoma	1–5%	55% amoeboid-like (single cell; without blebs) 10% amoeboid-like (single cell; with blebs) 35% mesenchymal	1–5 μm/min	[49,50]
B16F2 mouse melanoma	6.6% (0–22% per field of view)	56% single-cell (amoeboid-like) 44% multicellular streaming	0.4–6.7 μm/min amoeboid: ~1 μm/min streaming: ~2 μm/min	[51]
B16F2 mouse melanoma	Most immobile	Single-cell (amoeboid-like, without blebs)	0.2–5 μm/min	[52]
B16F10 mouse melanoma		Collective (strands 3-5 cell diameters)	~0.25 μm/min	[10•]
MTLn3 rat breast cancer	1.6% mobile	Single-cell		[53]
MTLn3 rat breast cancer	10–20 cells per field	30% single-cell 70% multicellular streaming	Single-cell: ~0.77 μm/min streaming: ~2.1 μm/min	[54]
MTLn3E rat breast cancer	1–5% (0–15% per field of view)	30% single-cell 55% multicellular streaming 15% collective	Single-cell: ~2.5 μm/min streaming: ~1 μm/min	[55•,56]
MDA-MB-231 human breast cancer	15%	Single-cell (without blebs or with invasive protrusions) multicellular streaming	Single-cell (without blebs): 0.4–4.2 μm/min single-cell (invasive protrusions): 0.03–0.25 μm/min	[57]
MDA-MB-231 human breast cancer	~5% (93% of fields have ≥1 motile cell)	44% single-cell 56% multicellular streaming	Single-cell: ~0.7 μm/min streaming: ~1.2 μm/min	[58]
TN1 human breast cancer (from effusion)	~5% (66% of fields have ≥1 motile cell)	44% single-cell 56% multicellular streaming	Single: ~0.6 μm/min streaming: ~0.9 μm/min	[58]
CT26 mouse colon carcinoma	Most immobile	Single-cell (mesenchymal)	0.2 μm/min	[59], Unpublished data
A431 human squamous cell carcinoma		Single-cell (overexpression of E-cadherin to switch to collective)		[60]
HT-1080 human fibrosarcoma	90%	14% single-cell 86% collective (sheets and strands)		[61]
Spontaneous mouse breast tumors (MMTV-PyMT)	80% (if close to macrophage)		~3.9 μm/min (in proximity to macrophages; sig. slower away from macrophages)	[62]
Spontaneous mouse intestine tumors (NICD; *Trp53*$^{-/-}$;Villin-Cre^{ERT2})		Multicellular streaming collective (sheets and strands) occasional single-cell		[63••], Unpublished data

straight collagen bundles containing stromal cells surround collectively migrating sheets and strings of cancer cells. In contrast, regions with poorly organized, shorter and curly collagen fibers contain isolated cancer cells [63••]. This further supports the hypothesis that ECM alignment may promote collective migration. However, in these tumors, it is difficult to distinguish between the effects of the microenvironment and genetic/signaling differences, given the heterogeneity of these tumor cells. In the case of xenografted tumors, which are usually generated from established cell lines and thus genetically similar, the range of different migration modes appears to be limited (see Box 1), indicating that these migration modes are also heavily influenced by genetics and signaling cues. For example, in murine breast cancer cells, TGFβ signaling can promote a transition from an immobile epithelial-like state to individual amoeboid-like migration [56]. Future work will be required to address how the combination of microenvironment structure, genetics and signaling cues influences tumor cell migration modes and dynamics.

Although intravital imaging accurately captures cancer cell invasion in the native environment, such studies

Figure 3



Modes of cancer cell migration *in vivo*. (a, a') Two different areas in spontaneously growing murine intestinal tumors showing cell clusters and independently migrating cells **(a)** and cells migrating collectively as strands and small clusters **(a')**. Green: tumor cells (nuclei). Pink: collagen (by SHG). Blue: stromal cells (membrane). Modified from [63••]. **(b)** Amoeboid-like migration of MTLn3 rat breast cancer cells in the mouse mammary fat-pad. Green: cancer cells (myosin light chain). Red: collagen (SHG). Modified from [74]. **(c, c')** Mesenchymal migration of mouse colon cancer CT26 cells in the mouse dermis at successive time points. Green: cancer cells. Pink: collagen (SHG). Arrows and asterisks indicate the protrusion tips for two different cells. Courtesy of Sara Geraldo. **(d)** Streaming migration of MTLn3 cells in the mouse mammary gland. Red: cancer cells (photoconverted Dendra2). Green: cancer cells in the bulk of the tumor (non-photoconverted Dendra2). Modified from [54]. **(e)** Collective migration of B16F10 mouse melanoma cells in the mouse dermis. Green: blood vessels. Red: collagen (SHG). Blue: cancer cells (nuclei). Yellow: cancer cells (cytoplasm). Modified from [9].

remain primarily descriptive, owing to the difficulty in assaying and modifying properties of the tumor cells and microenvironment. Due to limited resolution, most intravital imaging setups cannot currently be used to study sub-cellular activity. In contrast, *in vitro* studies offer a more precise, controlled environment to test different aspects of cancer cell migration at high resolution.

Tumor cell migration modes *in vitro*

A number of techniques have been developed to study cell migration in 3D, in environments that closely resemble the *in vivo* setting [68,69]. As the number of intravital imaging studies has grown, it is becoming more apparent that the timescale of single-cell migration in cancer cells, both *in vivo* and *in vitro*, is ~μm/min, while collective migration modes are typically slower (see Box 1). Networks for 3D migration studies are typically comprised of ECM components like collagen-I or matrigel, a dense network of primarily collagen-IV and laminin [70]. Matrigel is more similar in composition to the basement membrane than the stroma and may not faithfully recapitulate the stromal microenvironment [2,71]. The properties of collagen-I networks (*e.g.*, fiber thickness, pore size, mechanical properties) depend on the polymerization conditions and can therefore be experimentally controlled [72].

The properties of collagen networks can influence 3D cancer cell migration. Both migration velocity and the requirement for proteolysis have been shown to depend on pore size [72]. High network density and/or inhibition of network degradation can promote contractility-dependent amoeboid-like migration [4,73,74], though the ability of cancer cells to migrate without degradation may depend on matrix cross-linking [75]. ECM network properties can also affect collectivity during migration. In a recent study, high collagen density was shown to favor proteolytic-dependent collective migration in cultured fibrosarcoma and melanoma cells, which display

mesenchymal phenotypes [76•]. Cells from fragments of primary breast carcinomas (both human and the mouse model MMTV-PyMT) are more likely to undergo collective migration when embedded in collagen-I compared with matrigel, which may reflect the differences in structure and/or composition between collagen-I and matrigel [77]. In collectively invading MMTV-PyMT fragments in 3D, leader cells display a distinctly elongated, mesenchymal phenotype and remain in close contact with follower cells [78].

Increased complexity in *in vitro* microenvironments will help to further study the role of the stroma in cancer cell invasion. Proteomics-based studies focused on determining the *matrisome* of different tissues (*e.g.* [79,80]) will help to design more relevant *in vitro* systems. As the ECM likely differs significantly between different cancer types, *in vitro* systems should be adapted to recapitulate the native stromal environment. In their microenvironment, tumor cells also interact with other tumor and stromal cells. Experimental systems combining cancer cells and stromal cells will help to understand the importance of heterologous cell-cell interactions during invasion and metastasis.

Conclusions and outlook

Pathology, intravital and *in vitro* studies point to a dynamic regulation of cell migration in tumor cells. However, it is unclear why human epithelial cancer cells predominantly migrate collectively, while most cells observed *in vivo* using intravital techniques and *in vitro* studies migrate as single cells. One potential explanation for this discrepancy is the difference in the stromal environment. In human cancers, the tumor and stroma evolve together over months and years. In most intravital imaging studies, where tumor cells are implanted, the stroma has not had sufficient time to progress, either in terms of structure or stromal cell population. In addition, many intravital imaging studies use Nude mice to allow for the growth of xenografted tumor tissue; the lack of a sufficient immune response could affect stromal network remodeling or the chemical signals in the tumor environment, which could bias migration mode and morphology. Alternatively, human and mouse stroma may differ significantly enough to change the pattern of cancer cell invasion. To account for some of these potential sources of bias, mouse models that form spontaneous tumors may be a good alternative to grafting cancer cells in mice. These models allow for the co-evolution of the tumor and stroma in non-immunodeficient mice, providing a more realistic microenvironment for cancer cell migration as well as a heterogeneous population of tumor cells that more closely reflects the disease state. New genome-editing techniques such as CRIPR/Cas9 will allow for more flexibility in labeling and genetically modifying tumor and stromal cells to test mechanisms of migration and metastasis in intravital studies.

Another challenge for the future of cancer cell migration research will be to more directly relate findings from intravital imaging studies to human cancer. Several studies have already related findings from intravital studies to human disease. For example, specific alignment of collagen fibers, termed tumor-associated collagen signature (TACS), has been identified as an independent prognostic factor for breast cancer patients [33••]. Similarly, cellular arrangements composed of a carcinoma cell, a macrophage, and an endothelial cell, termed tumor microenvironment of metastasis (TMEM), are suggested to serve as an independent indicator for metastasis development [81]. Higher density of TMEM correlates with a greater chance that a patient will develop distant metastasis. These and similar studies could allow for the development of treatments to prevent metastasis targeted toward the microenvironment rather than tumor cells themselves.

Cancer cell invasion is a flexible and multi-factorial process. How does the combination of stromal network structure, genetics and signaling cues determine how cancer cells migrate? How might migration mode influence cancer progression? Is collective migration a more efficient route to metastasis? Cells migrating collectively could have improved chemokine sensing through leader exchange or multicellular signal integration, as recently shown in 2D [82], allowing them to more efficiently reach the circulation by following chemical cues. Alternatively, collective migration could allow for a greater diversity in tumor cells seeding other organs. More migratory cancer cells could 'pull' cancer stem cells through the stroma and secondary organs, allowing the cancer stem cells to nest and proliferate at metastatic sites. Groups of cancer cells could also be more resistant to attack by the immune system or mechanical stress. Work toward addressing these questions will help not only to understand the basic cell biology of cell migration, but will also help to understand the escape mechanisms cancer cells use to metastasize to distant organs.

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- •• of outstanding interest

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