Decoding leader cells in collective cancer invasion

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Abstract | Collective cancer invasion with leader–follower organization is increasingly recognized as a predominant mechanism in the metastatic cascade. Leader cells support cancer invasion by creating invasion tracks, sensing environmental cues and coordinating with follower cells biochemically and biomechanically. With the latest developments in experimental and computational models and analysis techniques, the range of specific traits and features of leader cells reported in the literature is rapidly expanding. Yet, despite their importance, there is no consensus on how leader cells arise or their essential characteristics. In this Perspective, we propose a framework for defining the essential aspects of leader cells and provide a unifying perspective on the varying cellular and molecular programmes that are adopted by each leader cell subtype to accomplish their functions. This Perspective can lead to more effective strategies to interdict a major contributor to metastatic capability.

Invasion refers to the process of cancer cells penetrating neighbouring tissues and is an essential trait of metastasis. Multiple modes of cancer invasion, such as amoeboid or mesenchymal single cell invasion, multicellular streaming and collective invasion, have been observed[1]. It was shown long ago that aggregates or clumps of cancer cells can have a higher metastatic potential than individual cancer cells[2] and that cell clusters can invade through the extracellular matrix (ECM) in a coordinated manner[3]. Nevertheless, only recently has collective cancer invasion been recognized as a pivotal mechanism in the progression of solid tumours[4,5]. Histopathological analysis of specimens from cancer patients suggests that cancer cells invade collectively in the form of sprouts, strands, clusters and luminal or hollow structures[1]. Despite these diverse morphologies, emerging evidence now supports the idea that invading cancer cells are spatially organized in a manner similar to that which occurs during tissue morphogenesis and regeneration[6]. The invading fronts of tumour structures are typically populated by aggressive cells with actin-rich protrusions and matrix remodelling activity. These cells at the invading front, termed 'leader' cells, support various aspects of the collective invasion process such as sensing the physicochemical microenvironment, steering the invasion direction, creating a low-resistance invasion path and coordinating with follower cells[7].

Recently, much effort has been devoted to investigating the mechanisms of collective cancer invasion and deciphering the roles of leader cells, facilitated by technological advancements (BOX 1). These efforts have resulted in novel insights into the regulation and organization of leader and follower cells. For instance, it is now recognized that the formation of leader cells can be attributed to genetic heterogeneity[8], epigenetic states[9] and interaction with other cells in the tumour stroma[10] or some combination thereof. The leader cells can adopt wide-ranging and apparently context-dependent molecular programmes to support their role in cancer progression. Nevertheless, there is a paucity of central principles that can unify our understanding of the diverse molecular programmes associated with leader–follower organization.

In this Perspective, we review recent studies that interrogated the regulation and functions of leader cells during collective cancer invasion. We broadly define collective invasion as intrusion of cancer cells in a cooperative manner; this cooperation can involve ECM degradation and/or remodelling, cell–cell signalling and/or mechanical interactions. The adhesion between leader and follower cells can be either strong or weak. We limit the discussion to carcinoma, which is composed of cells with an epithelial origin and is the most common type of cancer[11]. We note that the topic of leader-like cells also arises in angiogenesis, in developmental contexts and in tissue repair; these are discussed elsewhere[6,12], and our focus here is exclusively on cancer progression. To organize this rapidly expanding field, we first propose a set of principal functions to define leader cells. We then focus on how four different categories of leader cell, based on cellular origin and detailed phenotype, carry out these functions (TABLE 1). By classifying the major categories of leader cells and examining the similarities and disparities in the molecular underpinnings of their common functional programmes, we provide a conceptual framework for understanding how cancer cells coordinate the invasion process under different scenarios.

Functional definition of leader cells

Significant effort has been devoted to the detailed characterization of leader cells in specific tumour types and in specific experimental contexts. Nevertheless, a universal definition of leader cells, focusing on their functional aspects, has not yet been formulated. Here we propose a set of key capabilities for a leader cell. First, leader cells are responsible for path generation: by either physical or biochemical means, they create low-resistance migration tracks through the ECM, tracks that then can be followed by cells not endowed with 'leadership' qualities[13]. Leader cells can create such migration tracks by matrix deposition, physical remodelling and proteolysis. Second, leader cells interact with and thereby coordinate the motion of follower cells via both biochemical and biomechanical mechanisms; this coordination enables the moving collective to stay together[7,14]. The interaction can involve direct effects on the motility machinery but can also modulate aspects such as metabolism that support specific leader or follower cell

functions. Taken together, these necessary capabilities mean that eliminating the leaders attenuates the collective invasion. A third aspect is often but not always present; namely, when leader cells arise directly from the tumour cells themselves (instead of from stromal cells), they often have adjunct properties such as stemness and/or treatment resistance that can enhance the overall survival and eventual metastasis of the tumour[15].

Types of leader cell

On the basis of the functional definition discussed above, several types of cell in the tumour microenvironment can act as leader cells. The diverse origins of leader cells can create confusion for the conceptual understanding of these cells and their role in collective cancer invasion. This section summarizes four major categories of leader cell based on their origins and reported characteristics. In particular, leader cells are categorized into tumour-derived, which include mesenchymal or hybrid epithelial–mesenchymal (EM) and basal leader cells, and stroma-derived, which include cancer-associated fibroblasts (CAFs) and tumour-associated macrophages (TAMs) (FIG. 1).

Mesenchymal and hybrid EM leader cells

The epithelial–mesenchymal transition (EMT) has long been associated with cancer invasion and progression[16]. Carcinoma cells undergoing EMT lose their apical–basal polarity and cell–cell adhesion, and acquire mesenchymal characteristics such as front–back polarity[16]. Mesenchymal cells exhibit strong matrix interactions, the ability to migrate through ECM and, often, increased resistance to chemotherapeutics, all of which are essential traits in the dissemination of cancer. In the traditional view, these mesenchymal cells migrate individually, appear as single cells in the circulation and directly initiate metastatic lesions by reverting back to a proliferating epithelial phenotype[17]. The renewed emphasis on collective cell migration has emerged as an alternative to this overly simplistic picture.

Induction of mesenchymal markers has been reported in several cancer types, such as non-small-cell lung cancer (NSCLC), breast adenocarcinoma, bladder carcinoma and head and neck squamous cell carcinomas (SCCs), spatially located at the invading fronts within 3D invasion assays, in vivo models and histopathological analyses[18–22]. These mesenchymal markers include biomechanical effectors such as vimentin and N-cadherin (also known as cadherin 2) and established master EMT-regulating transcription factors including TWIST1, SNAIL and ZEB1. For instance, in a 3D spheroid invasion model, cancer cells derived from human SCCs exhibited collective invasion mediated by leader cells, most of which expressed vimentin and N-cadherin but not epithelial markers cytokeratin or E-cadherin (also known as cadherin 1)[19]. Similarly, leader cells isolated by spatiotemporal genomic and cellular analysis (SaGA; BOX 1) in a 3D model of NSCLC expressed N-cadherin but were negative for E-cadherin[18]. In a 3D co-culture model of breast and prostate tumours, invasive cancer cells with mesenchymal characteristics served as leader cells and enabled the invasion of non-invasive epithelial cells[23]. Hence, it is clear that mesenchymal cancer cells can not only invade individually but also facilitate the dissemination of other cancer cells. In these systems, the mesenchymal nature of the leader cells directly contributes to modification of the ECM, effectively generating a path through the tissue. Also, although E-cadherin is lacking, there is presumably enough adhesion due to heterotypic cadherin interaction to maintain the leader–follower coordination; we will see this again in the case of fibroblast leader cells.

A full or complete EMT, however, is not an absolute requirement of leader cells (BOX 2). It has now been recognized

Box 1 | Emerging methods for studying leader cells in collective cancer invasion

High-resolution characterization methods that are compatible with advanced models are required to study leader cells as leader cells represent only a small subset of cells in the microenvironment.

Models

- 3D invasion assays such as spheroids, organoids and co-culture samples are used to study invading tumour models in 3D extracellular matrix scaffolds[157]. It is possible to incorporate varying biomechanical and biochemical properties and supporting cells (for example, cancer-associated fibroblasts). Implementing 3D invasion assays in microfluidic devices can mimic other extrinsic factors, such as chemical gradients, interstitial flow and gas or material exchange, in the tumour microenvironment.
- In silico models provide a mathematical representation of gene regulatory networks, signalling dynamics and biophysical interactions between cancer cells and tumour environments[158]. These models can focus on either signalling pathways or biophysical regulation. Computational models can elucidate complex relationships (for example, predicting intermediate hybrid epithelial–mesenchymal states) but are challenged by model parameters and limited understanding of gene networks.
- In vivo models such as xenografts and genetically engineered animals denote complex interactions between cancer cells and the host, such as vascular and lymphatic structures and immune components, that cannot be fully represented by in vitro models[159]. However, they can be expensive and time-consuming, and do not fully represent human physiology.

Characterization methods

- High-throughput sequencing including whole-genome sequencing, exome sequencing, RNA-sequencing, ChIP-sequencing and epigenetic sequencing are particularly useful for exploring markers and molecular programmes of leader cells. However, on their own, they do not provide spatial and temporal information on the sample.
- Spatiotemporal genomic and cellular analysis (SaGA) uses photoconvertible fluorophores to define rare cells in heterogeneous cell populations. Photoconverted cells can then be isolated using a cell sorter, selecting leader and follower cells based on phenotypic features, and allow the targeting, extraction and amplification of leader cells for further analysis[18].
- Live single cell biosensors are nanoengineered structures and molecular probes internalized in live cells for dynamic gene expression analysis, detected by displacement reactions or conformational changes of the fluorescent probes[160,161]. These techniques measure spatial and temporal dynamics of gene expression in tumour tissues and organoids at single cell resolution[136,162].
- Optical microscopy techniques are powerful methods for characterizing live or fixed tumours or cells. Two-photon excitation with second harmonic imaging microscopy can be used to visualize collagen structures and monitor matrix remodelling activities of leader cells. With 3D reconstruction, functional events at the invading edge, such as filopodia dynamics, can be revealed[74]. High-resolution, single molecule imaging can also be applied to study subcellular features of leader cells, such as protrusion-localized RNAs[111].
- Intravital imaging allows observation of tumour invasion in live animals with optical microscopy techniques[163]. The procedure typically involves surgical implantation of an imaging window. Imaging can be done in one or multiple imaging sessions. With a proper molecular probe (typically fluorescent), it is possible to resolve the invasion process at the tissue, single cell or subcellular resolution[163].

that EMT, instead of having two mutually exclusive states, is in fact a reversible transition process with multiple intermediate stages[24–27]. The intermediate states, known as partial or hybrid EM, can arise as a result of mutually inhibiting microRNA and transcription factor circuits[28,29]. In silico models were able to predict that additional hybrid states appear in the presence of different stressors[27,28] and led to directed experiments that confirmed the existence and importance of these states in multiple cancer cell lines[15]. Hybrid EM states promote the plasticity of cancer cells and allow cancer subpopulations to convert back and forth between states during cancer progression[30]. This plasticity helps to explain the fact that metastatic lesions in the secondary site typically display epithelial features instead of mesenchymal characteristics. As expected, cells in a hybrid EM state acquire some combinations of epithelial and mesenchymal characteristics, and indeed, in vivo analyses (for example, circulating tumour cells from breast cancer patients and mouse models of skin SCC and breast cancer) often reveal subpopulations of cancer cells with reduced epithelial markers and upregulated mesenchymal markers[31,32].

Leader cells that display hybrid EM phenotypes are observed in experimental models such as 3D spheroid invasion assays and mouse models, as well as in histological analyses of clinical samples[33,34]. In a 3D spheroid invasion model of breast cancer, leader cells at the invading fronts expressed both E-cadherin and N-cadherin along with other mesenchymal markers, including vimentin, ZEB1, SNAIL and TWIST, whereas follower cells expressed only epithelial markers[33]. As opposed to having a primary focus on path generation coupled with relatively weak heterotypic adhesions, hybrid EM leader cells can interact more directly with follower cells via their shared complement of adhesive proteins. Importantly, cancer cells in the hybrid EM state display enhanced capacity for growth at metastatic sites and hence this type of collective invasion may reflect an aggressive phenotype[31–33].

Basal leader cells

In normal tissue, basal epithelial cells, related to myoepithelial cells, are located at the bottom of the epithelium and above the basement membrane. Basal epithelial cells express specific cytokeratins (for example, KRT5 and KRT14) and connect to adjacent cells and ECM via junctional complexes known as desmosomes and hemidesmosomes[35]. Tumours with a basal molecular profile such as breast and bladder cancer are often more aggressive than other cancer subtypes[36,37]. In the wound-healing context, leader cells exhibiting basal signatures have been reported in various tissues, such as embryonic skin and corneal epithelia[38,39].

In a mammary organoid invasion model, leader cells displaying a basal epithelial profile with KRT14, P-cadherin (also known as cadherin 3), p63 and KRT5 have been reported[40]. These basal cells are usually considered to be purely epithelial, and their motion is not dependent on having undergone any form of EMT. Consistent with this assumed epithelial phenotype, the leader cells express E-cadherin, but not typical mesenchymal markers such as TWIST1, SLUG (also known as SNAI2) or vimentin. The leader cells also lacked markers of myoepithelial cells, including smooth muscle actin (SMA) and calponin 1 (REF.[40]). Another study has shown that cancer cells that express podoplanin (a marker of myoepithelial cells and basal cells[41]) in vitro, in histological sections of transgenic mice, and in human cancer biopsy samples, invade in the absence of any EMT-related signature[42]. Podoplanin promotes collective invasion by inducing cytoplasmic projections known as filopodia, and an invasive phenotype by downregulating activity of the RHO family of GTPases[42]. In a study of mouse organoid invasion in a microfluidic system, KRT14-expressing leader cells have been shown to respond to biochemical and biomechanical factors in the microenvironment and to polarize to the leading edge[43]. Taken together, these examples show that epithelial cells can accomplish all the functions expected of leader cells. At least in the breast cancer context, this capability seems to depend more heavily on the precise nature of the ECM; here the adhesion-mediated interaction with the followers is very strong and the path generation aspect rather weaker[44].

It is worth noting that the basal and mesenchymal molecular programmes are apparently not always mutually exclusive. KRT14-expressing leader cells can also co-express both epithelial and mesenchymal markers. In one specific mouse mammary tumour model, KRT14-expressing tumour cells acting as leaders also expressed E-cadherin, vimentin and αSMA (also known as ACTA2)[45]. In a 3D spheroid

Table 1 | **Molecular markers and functional programmes of leader cells in collective cancer invasion**

Cell origins		Path generation	Cell coordination and guidance		Survival and metastasis
Leader cell type	**Markers**	**Matrix remodelling**	**Cell mechanics**	**Cell signalling**	**Reprogramming**
Mesenchymal and hybrid EM	N-cadherin[19]; vimentin[19]; TWIST1, ZEB1, SNAIL[21,33]; ΔNp63α[46]; E-cadherin[33]	Fibronectin[18]; MYO10 (REF.[74]); MMPs[70]; cathepsin B[34]	Integrins[9], FAK[18,104]; RHO–ROCK, LIMK1, LIMK2 (REF.[107]); ACTR3-K240R[8]; E-cadherin[31,32]; E-cadherin–N-cadherin[19]	VEGFA[18]; CX43 (REF.[130]); DLL4 (REFS[18,70]), JAG1 (REF.[74]); PDH[147]	CD44 (REF.[33]); NANOG[33]
Basal	KRT14 (REF.[40]); podoplanin[42]; ΔNp63α[40]	AMIGO2 (REF.[45]); MMPs[40]	DDR2 (REF.[43]); E-cadherin[40]; P-cadherin[44]; desmosome[140]	DLL4 (REF.[136]); JAG1 (REF.[140]); CXCR4 (REF.[43])	CD44 (REF.[140])
CAF	N-cadherin[19]; vimentin[52]; TWIST1, ZEB1, SNAIL[85]	Fibronectin[63,64]; CD10 (REF.[85]); MMPs[82]	Integrins[10,63], FAK[149]; RHO–ROCK, LIMK1, LIMK2 (REFS[10,107]); E-cadherin–N-cadherin[53]	TGFβ[125]; PDGFR[85]; CCL6, CCL12 (REF.[149])	αSMA[152]; FAP[152]
TAM	CD68 (REF.[57])	SPARC[65]; MMPs[60,71]; cathepsin B[86]	Integrins[156]	NOTCH1-ENAH (invasive isoform)[139]	CD163 (REFS[57,154]); CD206 (REF.[57])

The table shows a selection of markers reported in the corresponding leader cell type, but does not represent an exhaustive list. αSMA, α-smooth muscle actin; ACTR3, actin-related protein 3; AMIGO2, amphoterin-induced protein 2; CAF, cancer-associated fibroblast; CCL, C-C motif chemokine; CX43, connexin-43; CXCR4, CXC-chemokine receptor 4; DDR2, discoidin domain receptor 2; DLL4, delta-like ligand 4; EM, epithelial–mesenchymal; FAK, focal adhesion kinase; FAP, fibroblast activation protein; JAG1, Jagged 1; KRT14, kertain 14; LIMK, LIM domain kinase; MMP, matrix metalloproteinase; MYO10, myosin X; PDGFR, platelet-derived growth factor receptor; PDH, pyruvate dehydrogenase; ROCK, RHO-associated protein kinase; SPARC, secreted protein acidic and rich in cysteine; TAM, tumour-associated macrophage; TGFβ, transforming growth factor-β; VEGFA, vascular endothelial growth factor A.

invasion model of basal-like breast cancer cells and lung SCC, ΔNp63α (an isoform of p63) promotes cell migration through the induction of a hybrid EM state[46]. The notion of exactly what constitutes EMT as a phenotypic state change and its connection to other indicators of cell behaviour and protein composition is still an area undergoing rapid development, especially given the accelerating increase in the availability of single cell data[47–49].

CAF leader cells

In addition to tumour cells, stromal cells can also function as leader cells. In normal tissue, fibroblasts have important functions in matrix remodelling and have a crucial role in the wound healing response[50]. During cancer progression, stromal fibroblasts can be activated by various factors in the tumour microenvironment, such as changes in the ECM, DNA damage, oxidative stress, redox imbalance and cell signalling[51]. These activated CAFs contribute to cancer progression via soluble factor secretion, metabolic effects, immune crosstalk and matrix remodelling[51]. Similar to mesenchymal leader cells, CAF leader cells in head and neck and lung cancer, for example, display mesenchymal markers including N-cadherin and vimentin, and EMT-related transcription factors both in vitro and in vivo[19,52]. CAFs are also characterized by the expression of αSMA and fibroblast activation protein (FAP)[51]. It is important to note that the matrix remodelling capability of CAFs allows them to generate tracks for cancer cells to disseminate from the primary tumour, and hence they function as leader cells. Again, there is the likelihood of some degree of heterotypic cadherin-based adhesion, which promotes cell–cell coordination and guidance; indeed, this has been seen directly in an experiment involving fibroblasts assisting tumour cells in leaving the primary tumour in vitro and in vivo[53].

TAM leader cells

TAMs, another type of stroma-derived leader cell, can also support collective invasion. Under normal physiological conditions, macrophages are known to influence the development, regeneration and repair of multiple tissue types[54]. Macrophages, in particular TAMs, comprise a significant subpopulation in solid tumours and are crucial to various aspects of the metastatic cascade[55,56]. Macrophages demonstrate phenotypic plasticity and exist in two broadly defined polarization states, namely M1 and M2 (REF.[57]). M1 (or classically activated) macrophages, which express IL-12 and IL-19, promote an inflammatory response against infection. M2 (or alternatively activated) macrophages, which express matrix metalloproteinases (MMPs; such as MMP2 and MMP9), CD163 and CD206, are immunosuppressive[57]. M2 macrophages participate in wound healing and tissue remodelling under normal physiological conditions. TAMs, which are typically in an M2-like phenotype, have important roles in communicating with cancer cells by producing various cytokines, chemokines and growth factors[57]. Macrophages can be recruited and activated by various signals in the tumour microenvironment[57,58]. For instance, mesenchymal breast cancer cells can polarize monocytes into TAMs via tumour-derived cytokines such as IL-10, transforming growth factor-β (TGFβ) and macrophage colony-stimulating factor (M-CSF; also known as CSF1)[58,59].

In a manner similar to CAFs, TAMs are known to create invasion paths with low resistance for follower cells[60], and typically lead to invasion by multicellular streaming, whereby leader and follower cells migrate and generate force on the matrix independently[61]. For example, TAMs in a 3D breast cancer spheroid invasion model created migration channels by proteolytic degradation and physical compaction of the matrix[60]. However, unlike the other leader cell categories, TAMs have only weak interactions with carcinoma cells. After formation of the migration channel, cancer cells can then invade individually or collectively in an MMP-independent manner. Given our formulation, it is only when TAMs govern true collective motion that they should be considered leader cells; otherwise, their actions are just equivalent to those of any other factors that remodel the ECM without actively guiding tumour cells through adjacent tissue.

We have seen that there is no unique source of cells that can act as leader cells in cancer metastasis. In other words, cells of various origins and phenotypes can potentially accomplish the tasks needed for effective leadership. Of course, certain mutations (in non-clonal populations) and/or certain epigenetic changes can

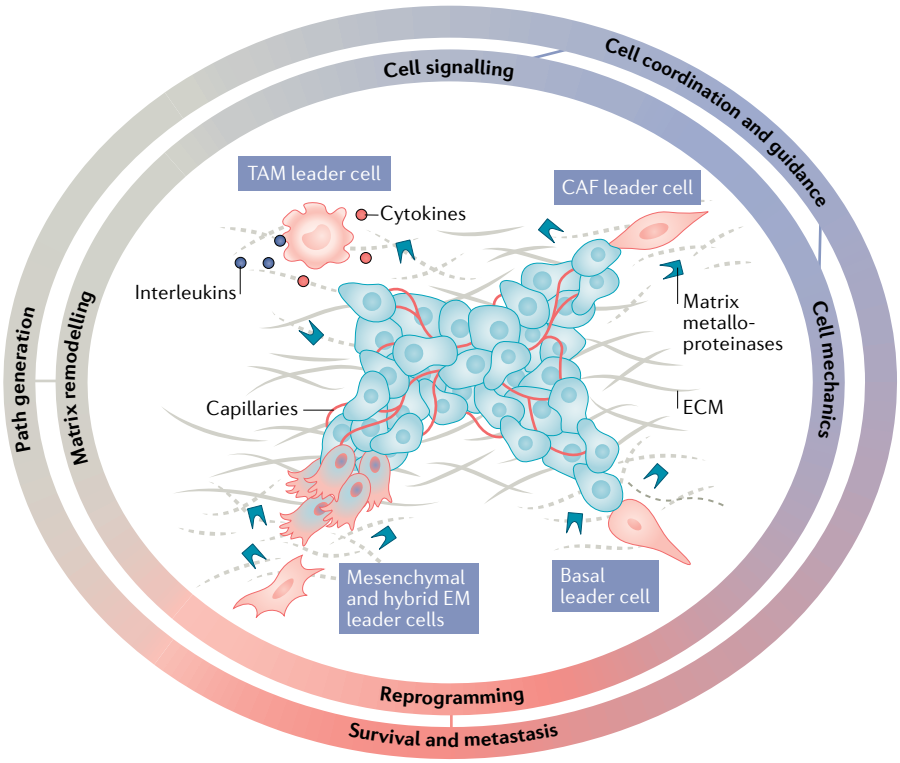

Fig. 1 | **Leader cell categories and key functions.** Mesenchymal and hybrid epithelial–mesenchymal (EM), basal, cancer-associated fibroblast (CAF) and tumour-associated macrophage (TAM) represent four major categories of leader cell that drive collective cancer invasion. Multiple leader cell types may arise in a tumour, though not necessarily all together. Key functions of leader cells include generating a migration path, coordinating with nearby cells to enable collective movement and enhancing the survival and metastatic capabilities of the tumour. Leader cells perform these functions using several molecular programmes such as matrix remodelling, cell mechanics and cell signalling, and cell reprogramming. ECM, extracellular matrix.

Box 2 | **Leader cells and epithelial–mesenchymal transition**

The existence of multiple types of leader cell may help to address recent controversies regarding the necessity of the conventional epithelial–mesenchymal transition (EMT) programme for metastasis and collective invasion[30,164,165]. Lineage tracing of fibroblast specific protein 1 (FSP1; also known as S100A4), a key indicator of EMT, and EMT inhibition by overexpression of miR-200 microRNA suggest that a classical EMT programme is not required for lung metastasis of breast cancer[164]. Genetic knockdown of SNAIL and TWIST1 also supports the idea that EMT is dispensable for systemic dissemination and metastasis of pancreatic ductal adenocarcinoma[165]. The ability of stromal cells such as cancer-associated fibroblasts and tumour-associated macrophages to function as leader cells may offer a mechanism of carcinoma cell dissemination that does not require EMT and certainly does not require it in its conventional 'binary' form. Similarly, the fact that collective invasion can be organized by basal leader cells without any obvious signs of EMT induction also offers a possible explanation for these findings, namely, that collective motility can be purely epithelial in nature as far as molecular signatures are concerned. Of course, it may also be the case that the discrepancy in the role of EMT may arise owing to the molecular complexity of EMT and the need for a more direct functional definition[49].

enable leadership, and hence cells with those advantages will become the leader cells, all else being equal. However, a wide variety of cells act effectively as leaders, if thrust into that position. In fact, collective migration can even occur without leader cells. For example, during morphogenesis of the *Drosophila* egg chamber, cells collectively rotate without the assistance of leader cells[62]. We next turn to a discussion of the molecular underpinnings of leader cell functions, involving both biomechanical and biochemical processes.

Cellular and molecular programmes

Path generation via matrix remodelling

Leader cells remodel the ECM to facilitate the dissemination of follower cells, which may have either strong or weak adhesion to the leader cells. Matrix deposition, force-mediated matrix deformation and pericellular proteolysis are interrelated strategies adopted by leader cells to remodel 3D matrices[13] (FIG. 2).

Matrix deposition. Enhanced secretion of matrix components such as fibronectin is observed in mesenchymal and CAF leader cells in lung, colon and prostate cancer both in vitro and in vivo[18,63,64]. The extent to which this occurs for basal leader cells is not known. The deposited matrix serves as a ligand for focal adhesion formation and integrin–focal adhesion kinase (FAK) signalling activation. This induced effect enhances the ability of follower cells to move through the ECM. As an example, CAF leader cells isolated from colon tumours assembled fibronectin to promote invasion of intestinal cancer cells in a 3D invasion assay[63]. Fibronectin deposition is required to induce cancer cell invasion, and the degree of invasion induced by CAF leader cells correlates with the amount of assembled fibronectin[63]. By contrast, TAMs enhance mammary carcinoma cell metastasis by synthesizing the ECM protein known as secreted protein acidic and rich in cysteine (SPARC) in a mouse model[65]. SPARC promotes fibronectin and vitronectin deposition to allow cancer cell migration by enabling the cells to exert tractional force on the matrix[65]. TAM-derived SPARC may represent a strategy for carcinoma cells to invade in a manner that allows them to evade SPARC-dependent repression[66], which can occur when SPARC is made directly by the cancer cells.

Force-mediated matrix remodelling. Cancer cells not only deposit matrix proteins but also physically remodel the matrix. Cell forces can generate orientational re-ordering of ECM to control cell motion by direct contact guidance, by allowing cells to elongate and by creating spaces between clustered matrix proteins[67,68]. Indeed, many cells in the tumour microenvironment are known to mechanically remodel the ECM[13,69]. Mesenchymal, CAF and TAM leader cells are capable of aligning and assembling matrix fibres; for instance, the alignment of collagen fibres by mesenchymal and TAM leader cells has been monitored in 3D invasion assays of breast cancer by time-lapse multiphoton imaging with second harmonic generation[70,71]. CAF leader cells isolated from prostate and colon cancer use integrins (for example, αv integrin and β3 integrin) for force-mediated fibronectin remodelling in a myosin II-dependent manner[63,64]. CAFs also exert contractile forces to alter the organization and physical properties of the basement membrane in 3D invasion assays and histological analyses of colon cancer[72]. In a breast cancer organoid model, KRT14-expressing basal leader cells have also been shown to increase the fibre density of collagen matrices[40].

Filopodia and other actin-rich structures (for example, lamellipodia and invadopodia), which are often observed in leader cells, are crucial mechanisms in matrix remodelling[73]. In a 3D spheroid invasion model of lung cancer, leader cells upregulated the filopodial motor protein myosin X (MYO10) via Jagged 1 (JAG1) signalling[74]. MYO10 then enhanced the persistence of filopodia in the invading front of leader cells and promoted micropatterning of extracellular fibronectin, organizing fibrils to align themselves along the direction of invasion. Recent work has shown that this alignment can directly determine the degree to which cancer cells successfully migrate in a fixed direction[75]. Finally, computational models focusing on how cell-generated forces might reorganize ECM structures have been developed[75,76]. However, in silico models typically either ignore chemical modification (for example, by proteases) or do not explicitly couple it to motility models. The interplay between mechanical and biochemical matrix remodelling and cell motility is an important direction for future research.

Proteolysis. Pericellular proteolysis is another matrix remodelling mechanism used by cancer cells[77]. Proteases such as MMPs and cathepsins modulate the tumour microenvironment by enzymatically digesting matrix proteins[78]. Proteases can also digest adhesion molecules to disrupt cell–cell adhesion (for example, vessel barriers)[78]. Membrane-associated proteases (for example, MMP14) localize proteolytic activity by tethering to the plasma membrane whereas secreted proteases (for example, MMP2 and MMP9) confine their proteolytic activity by binding to integrins or CD44 on cell membranes[79,80]. Cancer cells, as well as macrophages, can also accumulate proteases in invadopodia, which are matrix-degrading protrusions[81].

Proteolysis is used by major leader cell types[82]. For example, in 3D invasion assays of breast cancer, MMP9 was upregulated in mesenchymal leader cells[70], and MMP inhibition significantly reduced the invasion distance of cancer cells[23]. Hybrid EM leader cells in salivary adenoid cystic carcinoma in histological analysis and in 3D invasion assays expressed cathepsin B to promote collective cancer invasion[34]. In a 3D microfluidic invasion model, the invasion of CAF-led salivary gland adenoid cystic carcinoma was promoted by MMPs[82]. It is noteworthy that the proteolysis-associated collective invasion process appears to be context dependent and may involve multiple players and

steps. Distinct CAF subtypes with and without proteolytic activities have been identified in primary breast and colorectal tumours[83,84]. Immunohistochemical analyses revealed that CAF leader cells in SCC, but not basal cell carcinoma or malignant melanoma, upregulate a zinc-dependent metalloproteinase, CD10 (REF.[85]). In a 3D microfluidic invasion model of breast cancer cells, extravasation of mesenchymal cancer cells was promoted by pre-invasion of macrophages, which created microtracks in the ECM and destructed endothelial tight junction via an MMP9-dependent mechanism[71]. In a mouse model, both tumour cell-derived and macrophage-derived cathepsin B promoted lung metastasis of mammary cancer[86]. Another study showed that the invasion of fibrosarcoma and breast cancer cells in a 3D invasion assay included two consecutive modes of pericellular collagenolysis, starting with ECM micropatterning to create microtracks the size of individual cells and followed by microtrack widening by multiple trailing cells[87]. Both steps were MMP14 dependent and required the cooperation of multiple cancer cells.

What accounts for the difference in these various strategies and molecular components involved in the path-generating function of leader cells? We expect that both intrinsic factors (for example, genetic and epigenetic programmes) and extrinsic factors (matrix properties) may influence the specific matrix remodelling strategies employed by leader cells[88–90]. Physical properties of the ECM, such as cell–matrix adhesion, pore size, matrix stiffness and mechanical plasticity, are also known regulators of cancer cell invasion[91–94]. The cell nucleus is the stiffest cellular component and therefore represents a cell-intrinsic, rate-limiting parameter in protease-independent motility[95]. When the pore size of the matrix is small or when mechanical matrix remodelling is not possible, cancer cells may choose proteolysis-based invasion mechanisms[96,97]. If these are not possible, for example, owing to limited metabolic resources, collective motility may break down and cells may revert to single-cell amoeboid migration. Future investigations are required to clarify the intrinsic and extrinsic determinants of matrix remodelling strategies for different leader cells in various tissue contexts.

Coordination and guidance via biomechanics

Leader cells interact directly with the surrounding matrix and can biomechanically coordinate with the follower cells. Exactly what form these mechanical interactions take will depend on the ECM properties as they relate to cell motility. The net outcome of this coordination is the formation of coherently moving groups of cells. This occurs via a cell mechanics cascade that involves cell–ECM interactions, actomyosin contractility and intercellular junctions[98,99] (FIG. 3a).

Cell–ECM interactions. Cell–ECM interactions are essential for various forms of collective cell migration[100,101]. Integrins constitute a significant component of focal adhesions and physically link ECM structures to the cellular cytoskeleton[102]. Many different integrin subunits are expressed in leader cells, depending on the cancer type and stage[102]. In breast cancer, α11 integrin is upregulated in mesenchymal leader cells and is required for collective cancer invasion both in vitro and in vivo[9]. αv and β3 integrins were found to be expressed in CAFs isolated from patients with colon cancer and contributed to matrix remodelling in CAF-led carcinoma invasion models[63]. By contrast, KRT14-expressing basal leader cells isolated from mouse models of breast cancer metastasis use the discoidin domain receptor 2 (DDR2) to direct migration in response to mechanical cues, such as physical properties of matrix and interstitial fluid flow, in a microfluidic invasion assay[43]. Again, we see at work a general concept in which similar phenotypes can be supported by alternative sets of molecular components.

The bidirectional cell–ECM interaction is mediated largely by FAK and other signalling pathways (for example, PI3K–AKT and RAS–MAPK), which transduce tension in focal adhesions into biochemical signals[102]. FAK signalling, in turn, regulates multiple aspects of the cancer cell, such as cell adhesion, migration, protease expression, survival and proliferation[103]. During collective invasion of lung and breast cancer in vitro and in vivo, FAK is upregulated and activated at the leading fronts of leader cells to maintain cell polarity and regulate follower cells during collective invasion[104,105]. For example, *LKB1* mutant lung adenocarcinoma cells upregulate FAK at the invasive front, and FAK inhibition suppresses the collagen-associated collective invasion process in a genetically engineered mouse model[104].

Actomyosin contractility. The intracellular tension in the cell mechanics cascade originates from actomyosin contractility. RHO GTPases (for example, RHOA, cell division control protein 42 (CDC42) and RAC1) together with their downstream effectors such as RHO-associated protein kinase (ROCK) and LIM domain kinases 1 and 2, modulate actomyosin contractility by controlling F-actin polymerization and regulating the phosphorylation of the myosin light chain[106,107]. ROCK, in particular, is a crucial driver of contractile force for matrix deformation during cancer invasion[108]. Computational modelling describes the emergence of contractility and protrusion, and possible oscillations between them in terms of interconnected feedbacks between RHOA, RAC1 and the ECM[109,110]. RHO signalling is often upregulated in mesenchymal and CAF

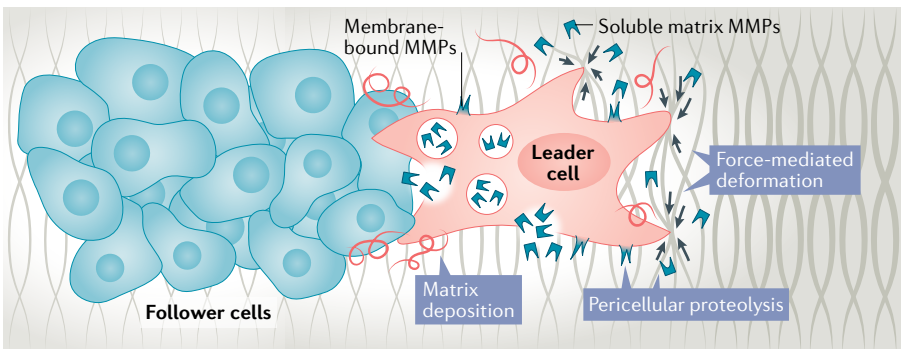

Fig. 2 | **Path generation.** Leader cells remodel the extracellular matrix to create a low-resistance invasion path for follower cells. Leader cells can deposit matrix proteins to facilitate the invasion of follower cells (migration path indicated through darkened gradient in the surroundings). Leader cells can also remodel the matrix through force-mediated interactions. Additionally, leader cells use pericellular proteolysis (soluble and membrane-bound matrix metalloproteinases (MMPs)) to rearrange and degrade the matrix in the surroundings. The specific matrix remodelling strategy used depends on both the leader cell properties and the extracellular matrix.

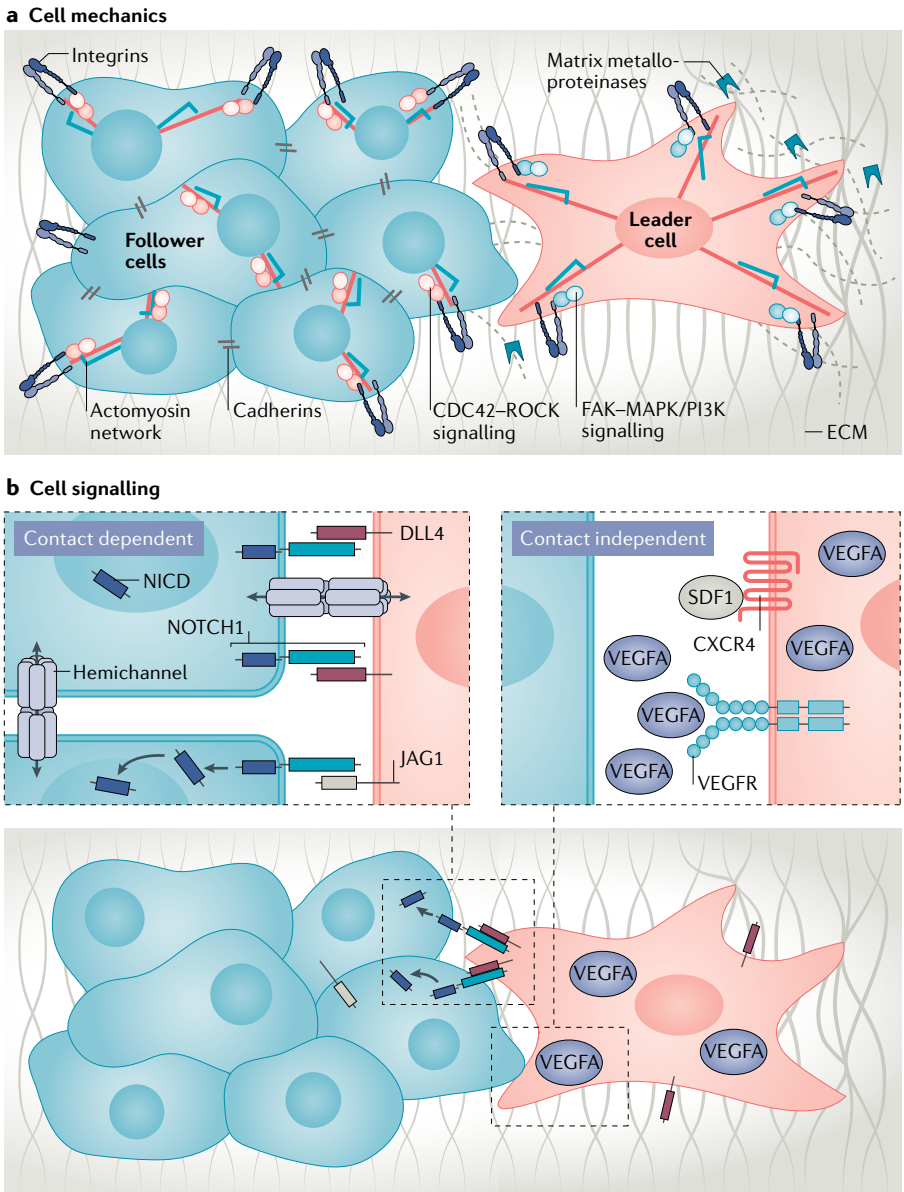

Fig. 3 | **Cell–cell coordination and guidance. a** | Leader cells communicate with other cells and their environments mechanically. This illustration highlights the key components of the cell mechanics cascade for cell–cell and cell–environment coordination. Leader and follower cells modulate RHO signalling according to their cell type and matrix density; this can in turn activate other pathways such as mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K) and transforming growth factor-β (TGFβ) pathways in leader cells. **b** | Leader cells coordinate with follower cells and other stromal cells in various ways including autocrine, paracrine and juxtacrine signalling programmes. Examples include vascular endothelial growth factor A–vascular endothelial growth factor receptor (VEGFA–VEGFR) vascular signalling, connexin-based gap junctions and hemichannels, and Notch signalling. These pathways can enhance the survival and invasion capabilities of cancer cells. CDC42, cell division control protein 42; CXCR4, CXC-chemokine receptor 4; DLL4, delta-like ligand 4; ECM, extracellular matrix; FAK, focal adhesion kinase; JAG1, Jagged 1; NICD, Notch intracellular domain; ROCK, RHO-associated protein kinase; SDF1, stromal cell-derived factor 1.

leader cells[10,107]. The importance of this pathway can be seen in a 3D invasion model of SCC, in which the inhibition of RHO GTPases in CAF leader cells, but not in follower cells, prevents invasion[10]. The carcinoma follower cells instead activate CDC42 and the MRCK family of serine/threonine kinases to follow the invasion tracks[10]. By contrast, podoplanin-expressing basal leader cells drive collective invasion by downregulating RHOA and RAC1 both in vitro and in vivo[42]. This is presumably owing to the need for a balanced amount of contractility, above the typical level of CAFs but below that of basal cells. A detailed deconstruction of how this works awaits the development of more powerful cell mechanics simulators.

In addition to RHO GTPases, other cytoskeletal proteins and components are also important in the formation and function of leader cells. For example, RAB13, a member of the RAB family of small GTPases, and NET1, a guanine nucleotide exchange factor for the RHOA GTPase, are regulators of cell migration and cancer invasion. Hybrid EM leader cells have been shown to localize *RAB13* and *NET1* RNAs at the protrusive fronts of a 3D breast cancer invasion model[111], and local translation of *RAB13* RNA was shown to be required for efficient migration of mesenchymal cancer cells and fibroblasts[112]. SaGA isolation shows that leader cell behaviour in NSCLC can be enhanced by a mutation in the gene encoding actin-related protein 3 (ACTR3; also known as ARP3)[8]. In particular, an *ACTR3* mutation (K240R) promotes leader cell behaviours, by stabilizing mutant ACTR3 compared with the wild-type protein; introducing ACTR3-K240R into follower cells was sufficient to induce the invasive leader phenotype in these cells.

Intercellular junctions. We have already mentioned cadherins as a major component of cell–cell interactions. Cadherins are a superfamily of transmembrane proteins that promote cell–cell adhesion and cadherin-based junctions, mechanically link cancer cells and transduce force fluctuations into biochemical signals that control motility, growth and EMT[99,113]. Physical modelling of cadherin-based cell–cell adhesion has successfully reproduced various cellular migration patterns observed on soft and rigid substrates, although the coupling of these mechanical models with the underlying signalling dynamics is largely unexplored[114]. For instance, leader cells with hybrid EM phenotypes maintain E-cadherin expression to connect with follower cells[31,32]. E-cadherin also promotes survival and proliferation of breast cancer cells, which may contribute to the enhanced metastatic potential of cancer cell clusters led by hybrid EM leader cells[115]. Loss of E-cadherin increases migration in vitro, but has been shown to be detrimental for breast cancer metastasis in vivo[115,116]. E-cadherin expression has been identified in M2 macrophages isolated from mouse models of infection and inflammation[117]; however, the involvement of E-cadherin in TAM-led collective invasion of carcinoma has not been established.

Other cadherins such as N-cadherin and P-cadherin are also implicated in collective invasion and can contribute to the leader–follower organization[118,119]. CAF leader cells and mesenchymal leader cells have been shown to use heterotypic N-cadherin and E-cadherin adhesion to physically guide carcinoma follower cells in vitro and in vivo[19,53]. Whether purely mesenchymal leaders (either cancer cells that have undergone a complete EMT or CAFs) typically exhibit weak or strong adhesive couplings to follower cells via this heterotypic interaction is not yet clear; the fact that single mesenchymal cells can leave the tumour mass individually argues that at least in some cases, residual adhesion must be weak. In these cases, ECM density may have an important role[120]: low density could lead to individual cell dissemination whereas high density and lower porosity may give rise to these same cells acting instead as path generators and enabling follower cells to migrate behind them. Furthermore, P-cadherin is a marker of basal epithelial cells[121]. P-cadherin can control sheet migration by activating CDC42 to regulate cell polarity and force anisotropy[122], and basal leader cells express P-cadherin in mouse mammary tumour organoids[40]. Consistent with this, loss of P-cadherin disrupts branching morphogenesis of mammary organoids and promotes the dissemination of individual cells in collagen matrix[44].

As the term 'collective' cell invasion implies, follower cancer cells must couple to leader cells and then have their invasion organized by their interaction with the leader cells. These needs are met by various molecular mechanisms that ensure that the cells can generate the needed traction, can transit through ECM and can stay together. Different collectives meet these needs differently, an interesting type of convergent phenotypic evolution.

Coordination and guidance via cell signalling

In addition to cell mechanical coupling, leader cells can also use other signalling mechanisms to coordinate the invasion process. In turn, the leader cells are affected by signals from the followers. These mechanisms include both contact-independent and contact-dependent communications (FIG. 3b). This coupling can also lead to metabolic specialization.

Autocrine and paracrine signalling. Transcriptomic analysis by high-throughput sequencing of SaGA-isolated cells suggests that leader cells are engaged in many signalling programmes[18]. For instance, mesenchymal leader cells activate vascular signalling and express a high level of vascular endothelial growth factor A (VEGFA) compared with follower cells[18]. VEGFA functions in both an autocrine and a paracrine manner, and triggers various tumour activities, such as adhesion, survival, migration and invasion[123]. Leader cells may induce these activities and serve as signalling centres during collective invasion. In agreement with this view, anti-VEGF antibody treatment and knockdown of the VEGF receptor 2 (VEGFR2) suppressed the formation of collective invasion packs in NSCLC tumour organoids[18]. Moreover, VEGFA is known to promote breast cancer invasion by upregulating CXC-chemokine receptor 4 (CXCR4), which is a receptor of the chemokine stromal cell-derived factor 1 (SDF1; also known as CXCL12)[124]. Similarly, in a microfluidic assay, basal leader cells expressed CXCR4 and used this chemokine receptor to direct migration along SDF1 gradients under hypoxic conditions, thereby supporting the function of leader cells in steering the invasion direction[43].

Stromal cells such as CAFs and TAMs are also known to modulate cancer cell invasion via paracrine signalling, independently of other leader cell functions. Coordination and communication between stromal cells and cancer cells are discussed in detail elsewhere[51,55,56]. To give a few examples, CAFs isolated from primary breast cancer tissues upregulated ΔNp63α expression in multiple breast cancer cell lines via TGFβ signalling, leading to a partial EMT programme in the cancer cells[125]. Furthermore, CAF leader cells in human basal cell carcinoma and SCC express platelet-derived growth factor receptors (PDGFRs), which are implicated in tumour angiogenesis and control of fibroblast migration modes[51,85]. Moreover, in a microfluidic breast cancer invasion model, TAMs were shown to destruct endothelial tight junctions by reducing levels of the tight junction proteins ZO1 and OCLN, via MMP9 (REF.[71]).

Gap junctional communication. Cancer cells can exploit gap junction channels for cell–cell and cell–matrix communication. In normal tissue, connexin-based gap junction channels and hemichannels regulate tissue homeostasis and multi-cellular contractility of cardiomyocytes, endothelial cells and myoepithelial cells of the mammary gland[126]. Gap junctional intercellular communication (GJIC) is associated with cancer progression, and gap junction channels can prevent cancer cells from undergoing a full EMT[127]. Cancer cells also communicate with other cells in the tumour microenvironment, such as endothelial cells and osteogenic cells via GJIC during the metastatic cascade[128,129]. In a study of breast cancer invasion, connexin-43 (CX43; also known as GJA1) was expressed in mesenchymal and hybrid EM leader cells in vivo and in vitro[130]. Collective invading cancer cells communicated by GJIC through CX43, and the leader cell functions were promoted by the CX43 hemichannel. The CX43-dependent signalling loop maintains the functions of leader cells and induces collective cancer invasion via release of purine derivatives (for example, ATP and ADP) into the extracellular space via the CX43 hemichannel. Degradation of ATP and ADP into adenosine within the extracellular space engages the adenosine A1 receptor (ADORA1) and AKT signalling. The roles of gap junction channels and GJIC in other leader cell types remain to be clarified.

Juxtacrine signalling. Leader cells can use juxtacrine (that is, contact-dependent) chemical communication to coordinate adjacent cells. A crucial example of this comes from the Notch signalling pathway, which is known to control various aspects of cancer and is a therapeutic target for cancer treatment[131,132]. The process of Notch signalling for cell coordination has been extensively studied in tip–stalk organization during angiogenesis[133–135]. The NOTCH1 receptor, along with its ligands delta-like ligand 4 (DLL4) and JAG1, regulates angiogenic sprouting and the resulting vascular structure. In a sprouting vessel, lateral inhibition between NOTCH1 and DLL4 limits the formation of tip cells while the pro-angiogenic JAG1 competes with DLL4 to antagonize NOTCH1–DLL4 signalling both in vitro and in vivo.

In a similar way, DLL4 upregulation has been observed in mesenchymal, hybrid EM and basal leader cells[18,70,136,137]. In 3D invasion models of breast and bladder cancer, *DLL4* mRNA is upregulated in leader cells and promotes collective cancer invasion[70,136]. In SaGA-isolated lung cancer cells, transcriptome profiling reveals that, here too, leader cells upregulate DLL4, and follower cells express NOTCH1 (REF.[18]). However, Notch inhibition did not enhance the formation of leader cells in this study but instead blocked the formation of invasion chains. Perhaps NOTCH1 is needed for followers to respond properly to signals emerging from the leaders and without followers there can be no collective motion.

In an in vitro experiment using a live single cell biosensor to identify DLL4-expressing leader cells, removal of these cells by laser ablation results in a transient reduction of cell migration followed by the emergence of new leaders, also with elevated levels of DLL4 (REF.[138]). The emergence of new leader cells agrees with the lateral inhibition of Notch signalling and again shows the nascent leader ability in many cells.

Functionally, Notch signalling regulates invadopodium formation by upregulating JAG1 in leader cells. In a 3D invasion assay of human NSCLC, JAG1 is upregulated in some leader cells and promotes MYO10-driven filopodial persistence for fibronectin micropatterning[74]. JAG1 knockdown is sufficient to reduce MYO10 expression and suppress collective invasion. Nevertheless, overexpression of JAG1 in follower cells does not induce MYO10. Thus, JAG1-mediated effects may be specific to leader cells. TAMs, likewise, mediate transendothelial migration of cancer cells by invadopodia formation via NOTCH1-ENAH (invasive isoform) signalling in a contact-dependent manner[139]. In a mouse model of breast cancer, JAG1 was among the top upregulated genes in KRT14$^+$ basal leader cells, and its level positively correlated with that of KRT14 (REF.[140]). For basal cell leader cells, there seems to be an overall consistent picture. There is evidence that basal cells in the developing mammary gland are relatively higher in delta-like ligands, and that the presence of NUMB in myoepithelial cells suppresses Notch signalling[141,142]. However, NOTCH1–JAG1 signalling has also been predicted computationally to lead to formation of collectively migrating clusters with hybrid EM phenotypes[143]. These results suggest that Notch signalling may regulate the formation and function of leader cells in non-canonical and context-dependent ways. Further investigations are warranted to clarify the regulation of Notch signalling in various types of collective invasion.

Metabolic shifts. Cancer cells alter their metabolic activities compared with normal tissues, and metabolic heterogeneity is commonly observed among cancer cells[144–146]. Leader cells and follower cells coordinate and engage in metabolic shifts to facilitate cancer invasion[147,148]. In the collective invasion of NSCLC in vitro, leader cells express pyruvate dehydrogenase (PDH) to engage in higher mitochondrial respiration, presumably to maximize the needed ATP production, while follower cells upregulate glucose transporter 1 (GLUT1; also known as SLC2A1) and enhance glucose uptake to support proliferation[147]. In contrast, in a breast cancer invasion model, mesenchymal leader cells displayed enhanced uptake of glucose compared with follower cells[148]. The disparity between these two studies might be attributed to the differences in cell type, matrix environment and migration model. Here, metabolism must support the energy required for matrix remodelling, and the energy requirement suggests a mechanism for coordinating leader–follower phenotypic switching during invasion. In this scenario, the follower cell gains the leading role when the leader cell depletes its energy for the energy-intensive invading process. Stromal cells such as CAF in the microenvironment can contribute to the regulation of metabolic shifts[149]. Also, the exact signals that coordinate these metabolic responses is unknown. Metabolic remodelling of leader cells is a relatively understudied area, and additional effort should be focused on elucidating the metabolic programmes used by leader and follower cells during collective cancer invasion.

Survival and metastasis via cell reprogramming

Cancer cells can undergo reprogramming to acquire tumour-initiating capabilities, treatment resistance and altered metabolic activities[150] (FIG. 4). Recent evidence suggests that hybrid EM cells are most likely to undergo this radical shift. It has also been shown that cancer stem-like cells preferentially migrate to the invading front and lead collective invasion in a 3D invasion assay, suggesting that cancer stem-like cells can function as leader cells[33]. This would therefore be consistent with hybrid EM phenotypes for these leader cells. But reprogramming towards a more stem-like state may be more general, as mesenchymal and basal leader cells in breast cancer may

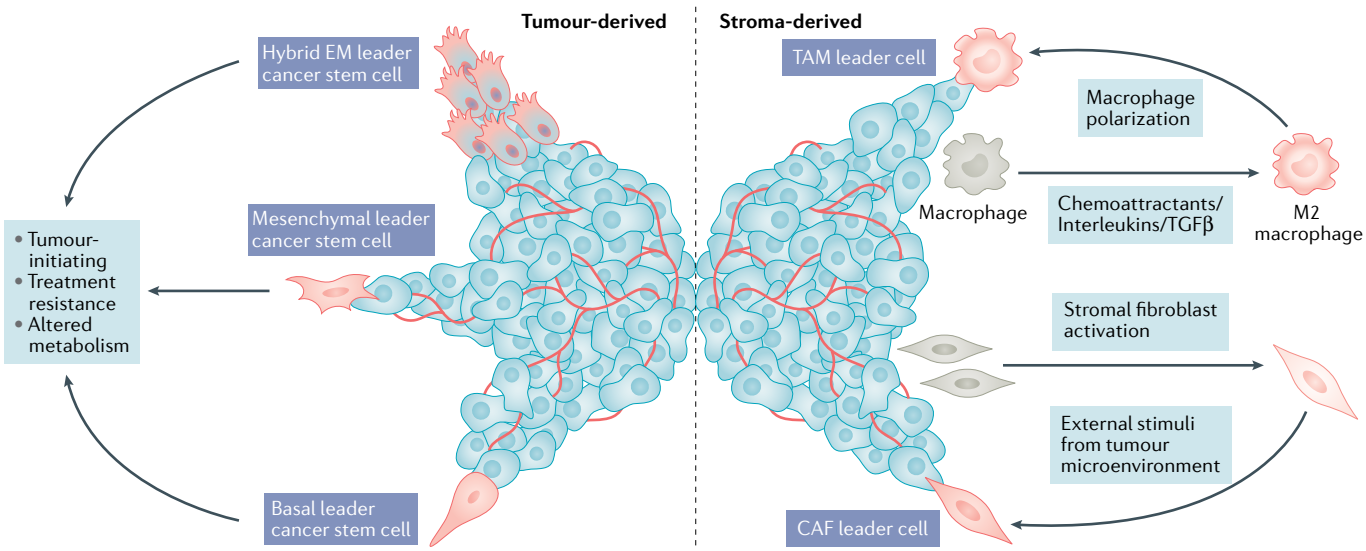

Fig. 4 | **Survival and metastasis.** Leader cells are often reprogrammed and specialized in facilitating collective cancer invasion. There are various types of reprogramming depending on the leader cell category. Tumour-derived leader cells often display tumour-initiating capabilities, treatment resistance and altered metabolism. Stroma-derived leader cells can also be reprogrammed through various external stimuli, such as changes in the extracellular matrix, DNA damage, oxidative stress, redox imbalance and tumour-derived cytokines. CAF, cancer-associated fibroblast; TAM, tumour-associated macrophage; TGFβ, transforming growth factor-β.

also exhibit cancer stem-like markers, such as CD44 positivity, CD24 negativity and NANOG expression[33,151]. The intrinsic properties of stem-like leader cells would then facilitate the metastatic process; however, this possibility would depend on the tumour and the leader cell category, for example, tumour cell-derived or stromal cell-derived.

Reprogramming can also be important for stroma-derived leader cells. Stromal fibroblasts can be activated to become CAFs by external stimuli in the tumour microenvironment[51]. Activated fibroblasts are characterized by αSMA and FAP expression[152]. In histological tumour samples, activated fibroblasts are observed in both intratumoural stroma and invasive fronts and may be acting as leaders in the latter location[152]. Likewise, macrophages can be polarized to the M2 phenotype and express MMP2, MMP9, CD163 and CD206 (also known as MRC1)[153]. $CD163^+$ TAMs are predominantly located at the invasive front of colorectal cancer, and these TAMs behave as leader cells in several 3D invasion assays[60,154]. Taken together, it appears that leader cells often arise via cellular reprogramming that affects a number of their intrinsic properties. Unravelling the mechanisms responsible for the reprogramming of leader cells, undoubtedly including epigenetic, genetic, transcriptional and post-transcriptional processes, represents an outstanding question in the field.

Examining the functions and characteristics of leader cells reveals many molecular programmes and signalling modalities that can be associated with collective cancer invasion. These programmes have significant overlap with other programmes that alter cell physiology, such as EMT, stemness and metabolic shifts. There is a similar set of intertwined networks controlling these programmes, including, for example, NOTCH1–DLL4–JAG1, mechanotransduction through FAK, paracrine VEGF communication and the NANOG-based stem cell pathway. Recent results have suggested that different cell types can mix and match these various ingredients to properly respond to their local environment and thereby effectively carry out needed tasks.

Future outlook

Collective invasion enabled by the formation of leader cells is increasingly recognized as a major mode of cancer invasion, but the diverse molecular programmes used by leader cells have created challenges in understanding this process. Systems biology frameworks that integrate high-throughput analysis, single cell analysis and advanced invasion models are warranted to further refine the concept of leader cells and provide a framework for the understanding of leader–follower organization. Biophysical models will help us to understand how cell–matrix interactions and matrix remodelling lay the foundation for leader cell selection. Models of gene regulatory networks and signalling dynamics will uncover the mutual connection between EMT, intracellular and intercellular signalling and the metabolism of cancer cells. Integrating the processes that drive cell motility and invasiveness at varying scales from molecular to systemic is a novel and exciting challenge that will help to decode the principles of collective invasion. In this Perspective, we have tried to lay the groundwork for these necessary advances. However, one should not expect there ever to be a precise signature of 'leadership' visible through ever more detailed single cell omics; apparently, leader cells are not driven by a single molecular pathway or network.

If leader cells are not driven by a common molecular programme, a fundamental question remains: what drives leader cell formation during collective cancer invasion? One possibility is that leader cells are driven by the competitive advantages among cells in the tumour microenvironment. The competitive advantages may arise because of epigenetic or genetic heterogeneity, differentiation states (for example, hybrid EM and basal differentiation), cellular origin (CAFs and TAMs) and molecular programmes in normal physiology (for example, tissue morphogenesis and wound healing). Extrinsic factors such as matrix properties may also contribute to the emergence of leader cells. Some subpopulations of cells may have higher invasiveness or specialized properties that can facilitate metastasis in a particular environment. We should also not lose track of the fact that follower cells may be heterogeneous, which may enhance their metastatic potential. The cooperation between these cancer cells may provide an effective approach for invasion, escape from immunosurveillance, dormancy and eventually tumour growth in other organs.

Conclusion

It is clear that leader cells are necessary for the collective migration of tumour cells, and collective migration is a very potent strategy for reaching potential metastatic sites. This then suggests a possible treatment approach whereby leader cells are targeted. Unfortunately, our analysis has shown that this may be difficult. Many cells can act as leaders and killing off one set runs the risk of having them replaced by equally capable alternatives; such a strategy would possibly just speed up the swapping-out process that occurs naturally. Finding and contravening the one key molecule needed for leader cell function is an idea whose time is rapidly passing. A more promising approach could rely on making the task of the leader cell harder to accomplish. One example pointing to this possibility is the lowered metastatic potential of tumours around which the ECM fibres wrap circumferentially[155]. Presumably, nascent leader cells are prevented by contact guidance from moving systematically away from the primary tumour. Restricting the ability of leader cells to garner the necessary metabolic resources for their energy-intensive tasks is another potential strategy. Finally, one could also speculate that leader cells require followers, and targeting the signals that keep the collective together is worth exploring. All of these ideas will need to be revisited as we continue to learn more and more about how primary cancer cells undergo the multiscale changes needed for effective invasion and eventual metastatic spread.

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