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Plasticity of cancer cell invasion: Patterns and mechanisms

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ABSTRACT

Cancer cell migration and invasion are integral components of metastatic disease, which is the major cause of death in cancer patients. Cancer cells can disseminate and migrate via several alternative mechanisms including amoeboid cell migration, mesenchymal cell migration, and collective cell migration. These diverse movement strategies display certain specific and distinct hallmarks in cell-cell junctions, actin cytoskeleton, matrix adhesion, and protease activity. During tumor progression, cells pass through complex microenvironments and adapt their migration strategies by reversible mesenchymal-amoeboid and individual-collective transitions. This plasticity in motility patterns enables cancer cells disseminate further and thus limit the efficiency of anti-metastasis therapies. In this review, we discuss the modes and mechanisms of cancer cell migration and focus on the plasticity of tumor cell movement as well as potential emerging therapeutic options for reducing cancer cell invasion.

Contents

Abbreviations: ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; CTC, circulating tumor cells; ROCK, Rho-associated kinase; FAK, focal adhesion kinase; MMPs, matrix metalloproteinases; MAT, mesenchymal-amoeboid transition; AMT, amoeboid-mesenchymal transition; BM-MSCs, bone marrow-derived mesenchymal stem cells; CMT, collective-mesenchymal transition; CAT, collective-amoeboid transition; YAP, Yes-associated protein; TGFBR1, TGF-beta type-I receptor; MCT, mesenchymal-collective transition; ACT, amoeboid-collective transition.

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Introduction

Malignant cell migration and invasion are the main manifestations of tumor biology and are critical components of metastasis, which is the major cause of death in oncology patients [1]. Tumor cells need to gain malignant phenotypes to detach from the primary tumor mass. These cells then pass through the basal membrane, penetrate the extracellular matrix (ECM), and intravasate into circulation [2]. However, the molecular mechanisms underlying tumor cell migration and invasion through these different tumor microenvironments are still poorly understood.

Although epithelial-to-mesenchymal transition (EMT) is often presumed to be indispensable for tumor cell invasion, increasing evidence indicates the presence of additional dissemination mechanisms of tumor cells [3]. Tumor cells can disseminate as individual cells via mesenchymal or amoeboid modes, or move as collective groups [3]. Mesenchymal cells, exhibiting elongated morphology, can move forward by generating traction force via cytoskeletal contractility and integrin-mediated ECM-adhesion [4]. Proteolysis-dependent ECM degradation is also required for mesenchymal tumor cells to generate paths for their migration. EMT and hybrid EMT have been identified as key pathways for epithelial tumor cells to gain mesenchymal phenotypes [5]. Conversely, amoeboid cells with rounded and deformable morphology can squeeze through narrow spaces and smaller pores of the ECM in the absence of proteolysis-dependent ECM remodeling [6,7]. During this type of movement, the cells exhibit bleb-like protrusions driven by actomyosin contractility and maintain weak and dynamic cell adhesion to ECM, resulting in high-speed movement [8]. Distinct from single-cell motility, collective cell migration is a movement pattern of multiple cells that retain cell-cell connections and migrate coordinately [9,10]. This type of tumor cell movement depends on actin dynamics, integrin-based ECM adhesion, and proteolytic cleavage of ECM. The migrating cohesive groups comprise heterogeneous tumor cells that maintain a front-rear polarity and cooperate in a hierarchical manner [10].

The diversity in movement modes provides alternative dissemination mechanisms that allow tumor cells to retain their migration capability in different environmental challenges [11]. Clinically, collective and single-cell patterns are often detected within the same tumor microenvironment by histological examination [11]. As evidenced by immunohistochemistry in oral squamous cell carcinoma, cancer cells can migrate via EMT-mediated mesenchymal patterns, collective cell invasion, and combined intermediate phenotypes [12]. Similarly, both single circulating tumor cells (CTC) and multicellular CTC clusters have been detected in the circulation of patients with epithelial cancers [13]. Experimental studies using advanced techniques such as 3D culture models, computational models, and 3D microfluidic devices as well as intravital techniques, have provided more direct evidence for the plasticity of tumor migration modes [14]. Depending on tissue context or the mechanical and biochemical cues from the ECM, tumor cells may switch their migration modes including reversible collective-individual and mesenchymal-amoeboid transitions [15]. These transitions have also been observed in treatment with agents that target tumor invasion and metastasis, and in turn favor malignant metastasis, thus limiting the efficiency of clinical therapies [15,16].

In this review, we provide a broad overview of the modes and mechanisms of cancer cell migration and elucidate the mechanisms underlying the plasticity of tumor cell movement. We also focus on the mechanisms through which cancer cells switch their migration modes based on different tumor microenvironments. Additionally, we highlight the emerging challenges originating from the alternative mechanisms of cancer cell dissemination triggered by anti-metastasis therapies and discuss the potential emerging therapeutic options for addressing these issues.

Patterns of cancer invasion/migration

At present, tumor cell migration can be classified as either individual or collective cell migration [11]. This classification is originally based on the morphology of migrating cells and the molecular genetic parameters of cell-cell junctions as well as the actin cytoskeleton, matrix adhesion, and protease activity [11,17].

Individual (single) cell invasion/migration

According to different morphological features and molecular expression, single cell migration can be divided into two distinct morphological and functional movement types: mesenchymal and amoeboid [18]. This type of migration is characterized by loss of intercellular connections and dissemination as a single tumor cell.

Amoeboid cell migration

Amoeboid movement is so named because of the similarity with the behavior and movement of the single-celled organism, *Dictyostelium discoideum* [19]. This movement mechanism is considered the most primitive and, in some ways, the most efficient migration mode [6]. Amoeboid movement has certain distinctive features including high-velocity motion, roundish but highly deformable cell morphology, and weak cell-ECM interaction as well as a lack of intercellular adhesion and proteolytic degradation of the surrounding matrix (Fig. 1) [6].

Amoeboid cells have rapid deformability that is effective for penetrating through narrow gaps of the surrounding ECM [20]. This rapid deformability, generated by reorganization of the cortical actin cytoskeleton, allows the moving cells to expand and contract in high-speed cycles, resulting in relocation by changing their positions [7,21]. The deformation of the nucleus, the largest and one of the stiffer cell structures, also maintains amoeboid cell movement [22]. When tumor cells squeeze through pores smaller than their cell diameter, the nucleus can be deformed into a maximum compressed state [21,23]. Another key motivator for cell movement is the development of bleb-like protrusions of the cell membrane to the surrounding tissue structures [21]. These protrusions enable cells to sense the microenvironment by mechanotransduction and allow penetration through narrow spaces [21]. The bleb-like protrusions and cortical actin cytoskeleton dynamics are predominately regulated by the small GTPase RhoA as well as its effector, Rho-associated kinase (ROCK) [24].

This type of migration predominantly relies on changes in cell shape but not on the proteolytic degradation of ECM. Thus, amoeboid migration of tumor cells can occur without proteolytical ECM reorganization [25]. Another feature of amoeboid migration that is unique compared to other types of cell movement is the lack of strong cell-ECM interactions. It has been shown that integrin inhibition cannot abolish amoeboid movement [26]. Instead, these cells move at high velocities (2–30 mm/min) and interact with the substrate through short-lived and weak connections by way of a ‘crawling’ type of movement [19,27].

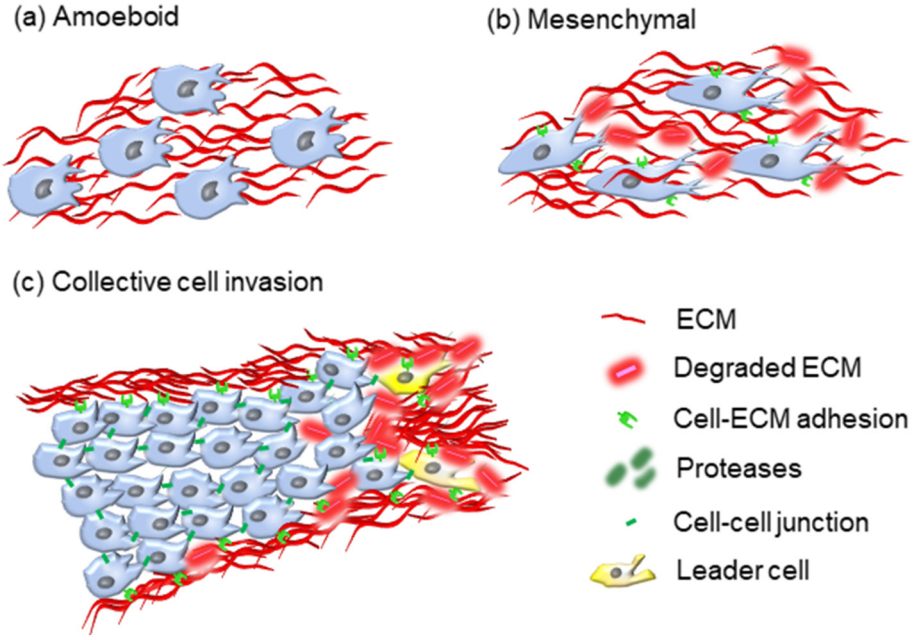

Fig. 1. Modes and mechanisms of cancer cell migration. (a) Amoeboid migrating cells are characterized by roundish and highly deformable cell morphology with bleb-like protrusions, high Rho-directed actomyosin contraction, weak cell-ECM interaction as well as lack of intercellular adhesion and proteolytic degradation of the surrounding matrix. (b) For movement, mesenchymal cells with elongated morphology require cytoskeletal contractility, integrins-mediated ECM-adhesion and pericellular proteolysis. (c) Collective migrating cell groups retain high intercellular adhesion and front–rear polarity. This type of motility depends on actin dynamics, integrin mediated cell-ECM adhesion and pericellular proteolysis mediated ECM reorganization.

Mesenchymal cell migration

Mesenchymal cell migration is a typical movement pattern of fibroblasts, endothelial cells, and smooth muscle cells [9]. In tumors, mesenchymal movement is often found in tumors originating from connective tissues or the bone marrow, and from certain epithelial cancers that are poorly differentiated [27,28]. EMT was originally identified during embryonic development as a key process through which epithelial cells gain migration ability. Invasive tumor growth is often presumed to undergo EMT to detach single tumor cells from the primary tumor via downregulation of epithelial markers and loss of intercellular junctions along with upregulation of mesenchymal cell markers and increased cell motility [29,30]. Tumor cells showing mesenchymal migration histologically exhibit an elongated, spindle-like cell shape with the formation of pseudopod protrusions and filopodia [2].

Cytoskeletal contractility, integrins-mediated ECM-adhesion, and proteolytic degradation of the surrounding matrix are hallmarks of mesenchymal migration (Fig. 1) [4]. Focal adhesion kinase (FAK) and Src kinases control cytoskeletal reorganization and contractility by inducing the formation of focal ECM adhesion and contacts [31]. Cell movement is carried out by alternating cycles of RhoA-induced actomyosin contractility and turnover of integrin-mediated focal adhesions to the ECM [32]. When the cells move forward, Rac-induced cell elongation, migratory actin protrusions, and integrin-mediated cell-matrix adhesion generate pulling forces and tension toward the ECM at the leading edge, and Rho-mediated rear retraction simultaneously reduces anchorage of the cell rear to the ECM [33]. The slow focal contact formation and turnover result in relatively slow migration velocities with this type of movement [27].

During mesenchymal movement, integrin-mediated adhesion is essential for cell-ECM interaction, which plays critical roles in substrate recognition, attachment abilities, and direction choice. The integrin extracellular domain at adhesion sites can bond with the extracellular ligand, thereby transmitting outside-in and inside-out signals, which are important for the cells to adjust their cytoskeleton, maintain polarity, and steer cell migration [34]. Moreover, blocking of integrins in tumor cells results in the loss of their elongated shape and impairs migration speed, suggesting important roles of integrins in maintaining the elongated morphology of cells and in generating high traction forces for cell movement [35].

In contrast to amoeboid movement, mesenchymal migration depends on the proteolytic degradation of ECM [28,36]. Cells recruit surface proteases to an anterior adhesion site at the leading edge, which is an active zone for removing the ECM structures and barriers [37]. Matrix metalloproteinases (MMPs) and other proteases proteolytically digest the ECM molecules and generate cell migration tracks for disseminated cancer cells [28,36]. More directly, several studies have revealed that interference with these integrins or proteases by molecular inhibition can abrogate this type of migration or can push cancer cells to spread by amoeboid movement [38].

Collective cell migration

Collective cell migration is a movement pattern of multiple cells that retain cell–cell connections and migrate coordinately as multicellular groups [39]. This type of cell movement is a fundamental process exhibited during morphogenesis, wound healing, and cancer invasion [40]. Although EMT is often presumed to be indispensable for cancer dissemination and metastasis, it is difficult to observe spindle-shaped mesenchymal migration in vivo. A considerable body of evidence in human cancer pathology has revealed that most epithelial cancers primarily invade in a collective manner [41,42].

Collective cell migration is characterized by the movement of multicellular groups that retain cell-cell junctions as well as front-rear polarity and cooperate in a hierarchical manner (Fig. 1) [43]. The movement of these cells is dependent on actin dynamics, integrin-mediated cell-ECM adhesion, and pericellular proteolysis mediated ECM reorganization [44]. Cell-cell connections are stabilized by cadherins (e.g., E-, N-, P-cadherin), immunoglobulin superfamily members, and gap-junctional cell-cell connections [45]. These cell-cell junctions connect and coordinate the migrating cells as a multicellular functional unit by functional cooperativity based on coordinated actin dynamics, cell-substrate adhesion, and ECM remodeling [45]. Moreover, cell-cell communication secured by intercellular junctions is

required to sense and integrate external guidance cues and for traction force transmission across cell groups by mechanocoupling mechanisms [39,45].

Collectively migrating multicellular units are groups of heterogeneous tumor cells that are polarized into the “leading edge” or “leading front” and the “trailing edge” [46]. Leader cells are a group of cancer cells at the invasive front of multicellular units and these have clearly different gene expression and morphology as well as proliferative, invasive, and metastatic abilities compared to the follower cells at the “trailing edge” [46]. Leader cells steer the migration of cancer cell groups by exploring the surrounding tissue environment via Rac-driven protrusions and integrin-mediated ECM adhesion [47]. At the cellular level, there are many similarities between collective migrating cells and single migrating cells [48]. For instance, actin contraction, ECM adhesion, and proteolysis-mediated matrix degradation are also indispensable for the collective invasion of cancer cells [47,49]. MMP-14 and cathepsin B are two important proteases that digest tissue barriers and generate tube-like tracks as the migration path for invading cancer cells [50]. These proteases are overexpressed in leader cells that are capable of generating ECM tracks and paving the way for their trailing follower cells [46]. Interestingly, cumulative evidence shows that stromal cells can take the leading position during collective migration of tumor cells [51,52]. In a three-dimensional “organotypic” invasion assay the leading cell is always a fibroblast that generates tracks by protease- and force-mediated matrix remodeling and thereby enable the collective invasion of the squamous cell carcinoma cells [51]. Stromal fibroblasts at the tumor margins and target organ can release chemoattractive factors such as CCL8 to drive invasion, intravasation and ultimately extravasation and seeding of cancer cells [53]. Similarly, another type of stromal cells, tumor-associated macrophages that can be recruited by cancer cells via CCR2 signaling, have been revealed to bring motile cancer cells into blood vessels via CXCL12 and CXCR4 signaling [54].

Plasticity of cancer invasion modes

Differentiated cancer cells do not typically retain a single mode during migration but adapt their migration mechanisms to different environmental challenges by switching between collective and single-cell dissemination [55]. Clinically, both individual and collectively migrating cancer cells have been detected in the same tumor microenvironment upon histological observation [11].

Mesenchymal-amoeboid transition (MAT) and amoeboid-mesenchymal transition (AMT)

Mesenchymal-amoeboid and amoeboid-mesenchymal transition in cancer cells can be triggered by the tumor microenvironment or pharmacotherapeutic intervention targeting RHO/ROCK signal pathways, integrin–ECM adhesion, and protease function (Fig. 2) [3].

RHO/ROCK signal pathways

The Rho family of small GTPases including RhoA, Rac1, and Cdc42 are key regulators of actin assembly, actomyosin contractility, and cytoskeletal dynamics during cancer cell migration. Specifically, Rac1 drives lamellipodia formation whereas RhoA and its downstream effector kinase ROCK, activate the formation of actin stress fibers and promote actomyosin contractility [24]. For the plasticity of migration modes, Rac signals can promote AMT through the SCAR/WAVE2 complex and conversely, Rho-kinase signaling drives MAT by inactivating Rac [56]. Upon treatment with specific inhibitors of Rac1 in glioblastoma, cancer cells show reprograming of amoeboid motility to mesenchymal movement by AMT whereas specific inhibitors of RhoA, a regulator with inverse interaction of Rac1, inhibit AMT [57]. Interestingly, this behavior of these cells can be observed in a 3D hydrogel model but not in 2D monolayer cultures [57].

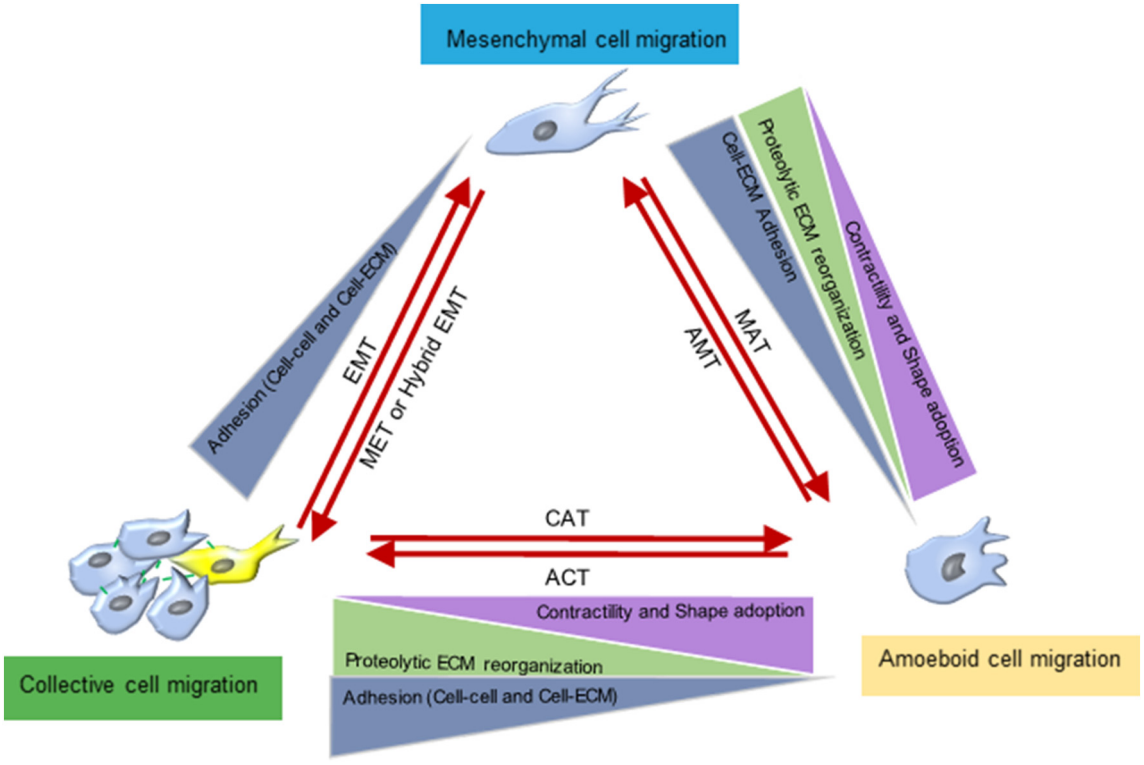

Fig. 2. Plasticity of cancer cell migration modes. Cancer cell can adapt their migration mechanism by reversible mesenchymal-amoeboid and collective-individual transitions based on different tumor microenvironments. Single-cell plasticity, including mesenchymal-amoeboid (MAT) and amoeboid-to-mesenchymal transitions (AMT), is mediated by protease function, cell-matrix interactions and Rho/Rac actomyosin dynamics. Reversible collective-individual transition, including collective-amoeboid, collective-mesenchymal, amoeboid-collective, and mesenchymal-collective transitions can be triggered by EMT, partial EMT or MET with alterations in cell-cell adhesions, cell-matrix interactions, cytoskeletal organization or pericellular proteolysis.

Previous studies have shown that pan-inhibition of ROCK can induce AMT in cancer cells. Inhibitors of Rho/ROCK signaling can restore the expression of alpha2beta1 integrin, enhance FAK autophosphorylation, and therefore, promote mesenchymal invasion [24]. However, ROCK isoforms have recently been shown to play different roles in regulating the migration phenotypes in mesenchymal carcinoma cells. For example, embryonic fibroblast cells derived from ROCK1(−/−) mice showed reduced cell shrinkage and detachment by increasing actin cytoskeleton stability, whereas those from ROCK2(−/−) mice revealed increased peripheral membrane folding through regulation of cofilin phosphorylation [58]. Notably, selective inhibition of ROCK1 and ROCK2 differentially regulates cell morphology and produces intermediate migration phenotypes that share some characteristics of both mesenchymal and amoeboid cells [59]. Mechanically, ROCK isoforms have been found to exert differential regulation of cell detachment, actin cytoskeleton, as well as actomyosin contractility, and ECM adhesion and degradation [59].

Different molecules have been demonstrated to modulate the migration mode of cancer cells by regulating the RHO/ROCK signal pathway. Tripartite motif 59 is overexpressed in breast cancer and regulates cell contractility as well as adhesion and transition between amoeboid and mesenchymal invasion via programmed cell death protein 10-mediated ROCK1 signaling suppression [60]. MAT induced by smurf1 inhibition also involves the activity of RhoA and ROCK as well as the phosphorylation of myosin light chain 2 [61]. Overexpression of guanine nucleotide exchange factor ECT2 can trigger an amoeboid phenotype transition in human astrocytoma cells by enhancing RhO/ROCK activity [62]. Similarly, EphA2 expression in melanoma cells can convert their migration patterns from mesenchymal to amoeboid motility. This nonproteolytic invasive program involves activation of the cytoskeleton, Rho-mediated rounding morphology, and retraction of cell protrusions, which enables cancer cells to penetrate the tissue barriers surrounding tumors [63]. Moreover, CD99 overexpression has been reported to promote the invasion of human malignant glioma cells with an increase in the proportion of amoeboid cells to mesenchymal cells. This CD99-induced transition is dependent on elevated Rho activity and decreased Rac activity but is independent of Akt, ERK, or JNK signaling pathways [64].

Integrin-mediated ECM adhesion

Integrin-mediated ECM adhesion dynamics are crucial for the mesenchymal migration but are dispensable for amoeboid migration. Experimental inhibition of cell-matrix adhesion in melanoma cells by integrin antagonists converts their elongated shape into a spherical and flexible morphology [26]. Simultaneously, integrin-independent mesenchymal migration is abrogated and the cells disseminate by alternative amoeboid motility [34]. In this type of movement, cancer cells pass through the ECM via cell deformation but without tissue remodeling or integrin-dependent ECM interaction.

Protease function

Unlike mesenchymal invasion, amoeboid motility is independent of the proteolytic activity required for ECM reorganization and the turnover of integrin-dependent adhesions. When treated with an MMP inhibitor, cancer cells can compensate for the loss of this proteolysis-dependent mesenchymal migration by adapting an amoeboid migration mode [25]. Inhibition of protease function in HT1080 cells can trigger MAT, which is associated with weakened integrin-mediated adhesion and inhibition of FAK autophosphorylation [24]. In 3D cultures, HT-1080 fibrosarcoma and MDA-MB-231 carcinoma cells showed mesenchymal motility phenotypes including beta 1 integrin-dependent ECM adhesion and MT1-MMP–dependent proteolytic degradation tracks. When treated with inhibitors of MMPs or other proteases, the cells exhibited round amoeba-like morphology and squeezed through preexisting matrix gaps in the absence of matrix degradation [34]. This fundamental adaptation provides tumor cells with a non-proteolytic migration strategy upon treatment with pharmacotherapeutic protease inhibitors.

Tumor microenvironment

Physical ECM barriers can induce mesenchymal-amoeboid transition during cancer cell invasion. Cancer cells invade through narrow 3 μm-wide microchannels using amoeboid movement with the features of blebbing and smooth leading edge profiles compared with mesenchymal migration through 10 μm channels [20]. Using microfabrication techniques, Vedula et al. observed increased speeds of migrating cell sheets accompanied with a decrease in the width of the strips [65]. Further investigation revealed that both the Rho/ROCK and Rac pathway were required for amoeboid invasion in the confined environments [20]. Confinement-induced amoeboid invasion involves local protrusion or myosin-II-dependent cortical flow [66]. In a viscoelastic gel-strip model, amoeboid-mode migration can be elicited in low-resistance environments with loss of adhesion and increased cell contraction. In contrast, mesenchymal migration is observed in high-resistance environments [67]. A study of a hydrogel-based microchannel platform demonstrated that channel stiffness and width have a synergistic effect on the migration speed of cancer cells and that increased channel stiffness triggers mesenchymal-amoeboid transition [68].

Tumor stroma has been reported to play an important role in determining tumor invasion modes during tumor progression. When tumor cells invade through compliant and deformable ECM, they can adopt an amoeboid phenotype, whereas firm matrices stimulate invadopodia formation and mesenchymal motility [69]. Cancer-associated fibroblasts in tumor stroma can promote EMT programming in prostate cancer and simultaneously recruit endothelial progenitor cells to further shift mesenchymal migration toward amoeboid motility [70]. A high amount of matrix-associated plasminogen activator inhibitor type-1 in the tumor microenvironment has been reported to trigger MAT by activating the RhoA/ROCK1/MLC-P pathway [71]. Important roles of stem cells in MAT have also been indicated between bone marrow-derived mesenchymal stem cells (BM-MSCs) and osteosarcoma. Upon coculture with osteosarcoma cells, BM-MSCs have been shown to differentiate into cancer-associated fibroblasts, which in turn increased monocyte chemoattractant protein-1, growth-regulated oncogene-alpha levels, as well as IL-6 and IL-8 levels to promote MAT via activation of the small GTPase, RhoA [72].

Collective-individual transition

This type of migration plasticity includes collective-mesenchymal transition (CMT) and collective-amoeboid transition (CAT). Collectively migrating cell groups of epithelial cancers can convert into mesenchymal single cells via disruption of cell-cell connections and acquisition of mesenchymal phenotypes; they can also convert into amoeboid-like cells via loss of protease function or through weakening of integrin adhesion (Fig. 2) [15].

EMT

EMT can weaken E-cadherin-mediated cell-cell connections and enable cancer cells to acquire mesenchymal phenotypes, which usually trigger CMT during cancer cell invasion. For example, elevated cytoplasmic leukemia inhibitory factor and its receptor in nasopharyngeal carcinoma trigger the EMT program and convert the invasive mode of cancer cells from collective to invadopodia-associated mesenchymal migration through modulation of Yes-associated protein (YAP)1-FAK/Paxillin signaling [73]. Twist1-induced EMT drives single cell invasion in carcinoma cells by activating its downstream effector, ZEB1, which requires activation of TGF-beta type-I receptor (TGFBR1). When TGFBR1-activation is inhibited, the Twist1-induced invasion mode is switched from individual motility to collective movement [74]. Concomitantly, during breast cancer cell dissemination, activation of TGFbeta signaling switches cohesive migration into individual cell movement by activating Smad4, EGFR, and RhoC [75]. In the absence of TGF-beta signaling, fibroblasts in breast cancer drive the collective invasion of cancer cells instead of single cell invasion, which involves several EMT pathways [76].

For amoeboid migration, TGF-beta has been revealed to reshape melanoma cells into a rounded and membrane blebbing morphology by

controlling Smad2 and CITED1-mediated contractile forces [77]. However, a study on ovarian cancer by Gao et al. showed that all TGF-beta isoforms stimulated collective tumor cell migration by inducing N-cadherin expression as well as by retaining epithelial shape and E-cadherin expression [78].

Integrin-mediated ECM adhesion

Integrin adhesion is required for cohesive cells to exert cell-substrate interaction and generate traction force, whereas this function is not essential in amoeboid motility. In melanoma explants beta1 integrin is preferentially expressed in a subset of promigratory cells at the leading edge of multicellular clusters. Upon beta1 integrin inhibition, collectively migrating cell groups lose cluster polarity and cohesion and switch into amoeboid cell migration. Hence, integrins targeting can achieve relapse-free radiosensitization and prevent metastatic escape [26,47]. In three-dimensional ECM scaffolds, E-cadherin-positive triple-negative breast cancer cells migrate via collective motion, which can be inhibited and switched to single cell movement by beta1 integrin inhibition. Mechanistically, this switch involves activation of TGFbeta signaling, which leads to a disbalance between miR-200 and ZEB2, further disrupting the E-cadherin junctions [79].

Tumor microenvironment

Migrating cells can adapt their migration phenotypes to the ECM composition. Collagen IV stimulates collective migration by activating actomyosin dependent adhesion whereas fibronectin favors individual motility by promoting Arp2/3-dependent protrusive actin [80]. Cancer cells move individually on substrates with low collagen concentrations and formation of cell clusters increases at high collagen concentrations [81]. When encountering fibronectin, cancer cells migrate via individual cell motility with increased Rac1 activity and smaller adhesions [82]. Laminin-coated nanofiber scaffolds can switch the migration patterns of glioblastoma stem cells from collective to single cell migration by increasing the spatial anisotropy of migration [83].

The nano-scale topography of the substrate can strongly affect the speed and morphology of epithelial sheets by triggering hybrid and complete EMT. This behavior involves YAP and two YAP-mediated feedback mechanisms that trigger EMT by E-cadherin downregulation and enhancement of cell migration abilities by regulation of Rho GTPase family proteins [84]. In multicellular systems, stochastic behavior between migratory and non-migratory cells can influence the migration strategy of cancer cells. Yamao et al. modeled physical interactions between cells in a crowded space as noise in a bio-physical model [85]. In the environment of strong noise from migratory cells, tumor cells invade collectively, whereas they move as individual cells on strong noise from non-migratory cells [85].

Collective-amoeboid transition in cancer cells can be induced by metabolic challenges such as hypoxia. Hypoxia-induced amoeboid dissemination in breast or head and neck cancer is driven by HIF-1-mediated E-cadherin downregulation and Twist-induced EMT accompanied by the production of heterogeneous cell subsets [86]. A study on a microfluidic platform demonstrated that under the condition of low oxygen tension, breast cancer cells consistently switched their mesenchymal migration mode into amoeboid motility due to an increase in HIF-1 expression [14].

Individual-collective transition

This type of migration plasticity includes mesenchymal-collective transition (MCT) and amoeboid-collective transition (ACT). Individual-collective transition can be triggered by specific tumor microenvironments such as tissue confinement or by hybrid EMT programming (Fig. 2) [15,87].

EMT and hybrid EMT

EMT programming is considered an important mechanism of cancer cell mesenchymal migration, and tighter cell-cell junctions are a hallmark of collective cell movement. However, acquisition of mesenchymal characteristics has been observed in collective cell migration, and EMT is also involved in the conversion of epithelial cancer cell groups to collectively migrating cells. For example, loss of epithelial markers keratin 8 and 18 can promote collective invasion in epithelial cancer cells, along with EMT activation [5]. Moreover, the EMT transcription factor Snail, has been shown to promote stable cell-cell contacts while eliciting collective invasion of squamous cell carcinoma by inducing the expression of claudin-11, Src activation, and inhibition of RhoA activity [88]. The EMT program is also involved in both galectin-1-induced and AKT-induced collective cell migration of squamous cell carcinoma cells, along with activation of cdc42 and Rac, or Snail and Slug respectively [89,90].

There is increasing evidence that EMT and MET are neither linear nor binary programs but rather successive processes with a range of intermediate phenotypes that possess different epithelial and mesenchymal traits simultaneously [30]. The notion that cancer cells are in a variety of hybrid or incomplete EMT states is still being debated but is gaining increasing and wide appreciation. These intermediate phenotypes have been generally accepted and are being used to illuminate the mechanisms of collective motility in cancer cells. In a lineage-labeled mouse model of pancreatic ductal adenocarcinoma, most tumor cells have been shown to lose the epithelial phenotype through “partial EMT” programming and to migrate as clusters [29]. In fact, during collective migration cell-cell adhesion is precisely regulated by the internalization of N-cadherin. The reduction of membrane N-cadherin in neural crest cells only elicits hybrid EMT-underlying collective migration instead of generating single or full mesenchymal movements [91]. This state of co-existence for epithelial and mesenchymal traits in a single cell is more favorable for collective cell movement compared to complete mesenchymal phenotypes [29]. In salivary adenoid cystic carcinoma, cancer cells at the invasion front preserve the expression of epithelial marker E-cadherin and also express mesenchymal markers, N-cadherin and vimentin, suggesting that leader cells at the invasion front gain an incomplete EMT phenotype [92]. Similarly, Konen et al. used a spatiotemporal genomic and cellular analysis technique to purify leader and follower cell lines and found that leader cells showed increased staining of the mesenchymal protein vimentin but decreased expression of N-cadherin, thereby indicating a partial EMT in leader cells [93].

Tumor microenvironment

During migration and metastasis, tumor cells are confronted with different extracellular structures. The migration efficacy as well as mode of plasticity are determined by the composition, density, geometry, and flexibility of ECM, as well as by stromal cells.

Cell jamming imposed by tissue confinement is a known contributor for individual-collective transition. Invasive breast cancer cells exhibit a variety of migration patterns in different geometrical confinements and Matrigel concentrations [94]. The dense matrix of collagen can promote cell-cell interaction, leader-follower polarization as well as joint guidance along migration tracks during collective migration. Increased collagen density switches mesenchymal tumor cells from single-cell to the collective invasion mode, which is dependent on the proteolytic reorganization of collagen, but independent of matrix stiffness [95]. The cell jamming caused by geometrical constraints and physical crowding can integrate actin-dependent deformability, cell-to-cell connection, cell-substrate adhesion, and intercellular force transmission to maintain collective movement [96]. During cell migration, inelastic collisions between neighboring cells cause mutual alignment of the cell velocities and promote cell-cell adhesion thereby leading to collective migration [97]. Similarly, tactile interactions caused by contact inhibition of locomotion can also reshape the cells and induce the transition to coherent motion [98]. According to a study involving a computational model of Madin-Darby canine kidney cells, contact inhibition caused by high cell density is a consequence of mechanical interaction and constraint rather than interfacial contact alone [99].

The ECM composition and its remodeling can trigger individual-collective transition of cancer cells. Corneal fibroblasts and dermal fibroblasts migrate on fibrin matrices with a fusiform morphology and an interconnected meshwork compared to a more dispersed morphology on collagen matrices [100]. During morphogenesis, tissue stiffening is necessary and sufficient to trigger collective cell migration in vivo through a mechanosensation mechanism that involves EMT activation mediated by

the integrin-vinculin-talin complex [87]. MT1-MMP-mediated multi-step pericellular proteolysis in fibrosarcoma and breast cancer cells is required for the transition from individual to collective invasion. During this multi-step pericellular proteolysis, the fibers are realigned into microtracks of single-cell caliber and further expanded into macrotracks by the degradation of lateral ECM [15].

Implications for anticancer therapy

The tendency of cancer cells to transit between a range of motility strategies in different contexts, including triggers by therapies targeting cancer metastasis, has been reported as a potential mechanism underlying the limited efficiency of therapeutic approaches [101]. Attempts to solve these issues may provide new insights into cancer therapeutic strategies.

Additional therapeutic benefits of the dual targeting of mesenchymal and amoeboid motility have been demonstrated by in vitro studies [102,103]. Neural precursor cells expressing developmentally downregulated 9 (NEDD9), a scaffolding protein, has been reported to promote both mesenchymal and amoeboid migration in breast cancer via VAV2-dependent Rac1 activation and the regulation of downstream RhoA signaling effectors, respectively. Simultaneous targeting of mesenchymal and amoeboid migration by a combination of NEDD9 depletion and ROCK inhibition reduced cancer cell invasion significantly, thereby indicating that this combination therapy can be an efficient therapeutic approach to hinder the invasion and metastasis of breast cancer [102]. Similarly, administration of serine protease inhibitor alone did not efficiently reduce carbon-ion radiation-induced invasiveness of a pancreatic cancer cell line, PANC-1, because a mesenchymal-amoeboid transition program was elicited. However, invasiveness has been reduced by the combination of a ROCK inhibitor and serine protease inhibitor, which has been reported to abolish amoeboid movement [103].

The use of small molecules that target mechanisms shared by several cancer cell migration modes is an effective approach for inhibiting malignant metastasis. Tropomyosin is an actin-associating protein that regulates several effectors of actin filament dynamics. Its isoform, Tm5NM1, has been reported to inhibit mesenchymal migration. In addition, Tm5NM1 can downregulate Src kinase activity and impair amoeboid migration by reducing the formation of pseudopodia and inhibiting rounded or elliptical morphology. In vitro studies have shown that Tm5NM1 can block mesenchymal motility without inducing transition to the amoeboid form [101]. Similar to Tm5NM1, inhibition of ROCK activity can reduce breast cancer cell invasion by 60% via decreasing both the ROCK-mediated amoeboid and ECM proteolysis-mediated mesenchymal modes [16].

CRediT authorship contribution statement

Wu Jiashun: Conceptualization, Writing - original draft preparation. Jiang Jian: Writing - original draft preparation, Validation. Chen Bingjun: Software. Wang Ke: Artwork. Tang Yaling: Writing - reviewing and editing. Liang Xinhua: Writing - reviewing and editing.

Declaration of competing interest

No conflict of interest to declare.

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