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Mechanisms of collective cell migration at a glance

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Introduction

Collective cell movement occurs when two or more cells that retain their cell-cell junctions move together across a two-dimensional (2D) layer of extracellular matrix (ECM) or through a three-dimensional (3D) interstitial tissue scaffold (Friedl and Gilmour, 2009; Friedl et al., 2004; Lecaudey and Gilmour, 2006; Rorth, 2007). Time-lapse and morphological analyses suggest that collective cell movement is relevant for many processes in morphogenesis, tissue repair, and cancer invasion and metastasis (Christiansen and Rajasekaran, 2006; Friedl et al., 1995; Lecaudey and Gilmour, 2006; Vaughan and Trinkaus, 1966; Weijer, 2009). Collective cell dynamics give rise to complex changes in multicellular tissue structures, including epithelial regeneration, the sprouting of vessels and ducts in angiogenesis and branching morphogenesis, and the deregulated invasion of cell masses during cancer progression and consecutive tissue destruction.

Similarly to single-cell migration, collective cell movement results from actomyosin polymerization and contractility coupled to cell polarity; however, there are some key differences. Single-cell migration through interstitial tissue is a cyclical five-step process, comprising cell polarization and protrusion of the leading edge (driven by the actin cytoskeleton), followed by attachment of the leading edge to the substrate, proteolytic degradation of tissue components that physically confine the cell body, actomyosin contraction (leading to tension along the length axis) and, finally, forward sliding of the cell rear (Friedl and Wolf, 2009; Lauffenburger and Horwitz, 1996). Whereas these principles are retained in collective cell movement, the main modification is that the cells remain coupled by cell-cell junctions at the leading edge as well as in lateral regions and inside the moving cell group (Friedl et al., 2004; Lecaudey and Gilmour, 2006; Rorth, 2007). Consequently,

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1 Cell-cell interactions

The migrating cell group is held together by several types of cell-cell junctions.

Adherens junctions

Desmosomes and tight junctions

Gap junctions

2 Cell protrusions, force generation and ECM remodelling

Formation of lateral protrusions

Pericellular proteolysis and track generation

Anterior adhesion and force generation

Extracellular matrix

Tip cell (heterophilic stromal cell)

Tip cell (neoplastic)

Stromal cell

3 Interaction with the stroma

Collectively migrating cells and stromal cells cooperate to allow coordinated movement by means of:

- Interactions with stromal cells through cell-cell junctions
- Lateral matrix modification (secretion of basement-membrane components)

Cell-cell interactions

Lateral matrix modification

4 Front-rear asymmetry

Signalling by extracellular chemokines or growth factors

Track generation by tip cells

Leading-edge protrusions

Abbreviations: ALCAM, activated leukocyte cell adhesion molecule; cADP-ribose, adenosine 5'-cyclic diphosphate-ribose; CCL, chemokine; CCR, chemokine receptor; CX, connexin; FAK, focal adhesion kinase; GF, growth factor; GFR, growth-factor receptor; Ins(1,4,5)P₃, inositol (1,4,5) triphosphate; JAMs, junctional adhesion molecules; MMP, matrix metalloproteinase; MT-MMP, membrane-type MMP; PKC, phosphoinositide 3-kinase; TIMP-2, tissue inhibitor of metalloproteinase 2; uPA, urokinase-type plasminogen activator; uPAR, uPA receptor; ZO-1, zona occludens 1; ZO-2, zona occludens 2

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(See poster insert)

collective cell migration differs from single-cell migration in the simultaneous coordinated polarization of (often many) cells at the leading edge of the cell collective; the translocation of cells through physical coupling and drag force; the activity of actin-rich lamellae in multiple cells along or underneath the cell collective; the secondary remodelling of the extracellular matrix along the migration track, leading to the formation of a basement membrane or the widening of a 3D track (macropatterning) to encompass an increasing volume of the cell mass; and the coordinated retraction of multiple cells at the rear end of the group (Friedl and Gilmour, 2009).

For most types of collective cell migration, our understanding of specific molecular mechanisms and their cooperation is incomplete; however, if viewed in context, common themes emerge. In this poster article, we provide an overview of the cellular and molecular regulation of collective migration by combining known aspects of collective migration in cancer with aspects of collective migration in morphogenesis and epidermal regeneration. The aim is to generate one cohesive and thus 'idealized' model (see poster).

Settings for collective cell migration: morphogenesis, repair and cancer

Collective cell migration occurs in many physiological and pathological processes, including morphogenesis, tissue repair and cancer. In morphogenesis, all stages of the development of the multicellular organism show collective migration, including branching morphogenesis of the tracheal system (Ghabrial and Krasnow, 2006); the formation of mammary ducts in mouse and human explant models (Ewald et al., 2008); migrating border cells in the *Drosophila* ovary (Niewiadomska et al., 1999; Geisbrecht and Montell, 2002); and the migration of cells that form the lateral line primordium in zebrafish (Dambly-Chaudiere et al., 2007; Lecaudey et al., 2008; Weijer, 2009). During tissue repair, collective cell migration of epidermal sheets occurs across the provisional wound-bed, leading to epidermal wound closure (Farooqui and Fenteany, 2005; Poujade et al., 2007). Likewise, collective strands of endothelial cells penetrate the provisional wound bed and deliver neo-vessels into the regenerating neo-tissue (Schmidt et al., 2007).

Similarly to morphogenetic movements, collective movement occurs in many cancers in which cells are not completely de-differentiated, including rhabdomyosarcoma, oral squamous cell carcinoma and breast cancer (Christiansen and Rajasekaran, 2006; Friedl et al., 1995; Gaggioli et al., 2007), and in colorectal

carcinoma cells (Nabeshima et al., 1998). It is probable that most cancer types comprise invasive zones of intact cell-cell cohesion and collective invasion (Christiansen and Rajasekaran, 2006). Such collective invasion zones show expression of cell-cell adhesion molecules and gap junctions, which are characteristic of collective cell migration (see below) (Gavert et al., 2008; Hsu et al., 2000; van Kempen et al., 2000), strongly suggesting that the mechanisms of collective migration apply to invasive cancers (Friedl, 2004; Hashizume et al., 1996; Hegerfeldt et al., 2002; Langbein et al., 2003; Nabeshima et al., 2000). The molecular prerequisites for collective invasion in different types of cancer, its interdependence on other invasion modes (such as the epithelial-mesenchymal transition) and its contribution to cancer metastasis are currently unknown [discussed by Friedl and Gilmour (Friedl and Gilmour, 2009)].

Mechanisms of cell-cell cohesion and polarity within collectively migrating cell groups

Similarly to non-migrating epithelia, collectively migrating cell groups are connected by cell-cell junctions that mediate cell-cell cohesion, mechanical integrity, cell polarity and, probably, direct cell-cell signalling. The types of cell-cell junctions utilized are those that are known to occur in epithelia and endothelia; here they occur in the context of multicellular dynamics and tissue remodelling.

Adherens junctions

Adhesive cell-cell coupling in all known forms of collective cell migration is mediated by adherens-junction proteins, including cadherins and transmembrane proteins of the immunoglobulin superfamily. During branching morphogenesis in the mammary gland, luminal epithelial cells within elongating ducts elongate collectively while retaining E-cadherin along cell-cell interfaces (Ewald et al., 2008). In carcinoma cells, loss of expression of E-cadherin, together with upregulation of N-cadherin and neural cell adhesion molecules, results in the onset of collective migration in which cell-cell junctions are retained; this process is often referred to as incomplete epithelial-mesenchymal transition (Lee et al., 2006; Lehembre et al., 2008). Immunoglobulin family members, including activated leukocyte cell adhesion molecule (ALCAM, also known as CD166) and L1 cell adhesion molecule (LICAM), mediate homophilic cell-cell interactions in cell-cell junctions and are upregulated in cohesively invading melanoma (van Kempen et al., 2000) and colorectal carcinomas (Gavert et al., 2008; Weichert et al.,

2004). However, their role in collective cell dynamics still needs to be elucidated.

Desmosomes

Desmosomal proteins are markers of epithelial differentiation, and loss of their expression results in the epithelial-mesenchymal transition during morphogenesis and cancer progression (Lee et al., 2006; Chidgey and Dawson, 2007). During epidermal regeneration, migrating keratinocyte sheets retain desmosomal cell-cell junctions while closing a wound (Shaw and Martin, 2009). In addition, there is substantial evidence that membrane-localized desmosomal proteins are expressed during collective migration in advanced epithelial cancer (Christiansen and Rajasekaran, 2006). Expression of desmocollins 1 and 3, which are members of the desmosomal cadherin family, increases in invasion regions of colorectal adenocarcinomas, as detected by immunohistochemistry (Khan et al., 2006), and this is indicative of collective invasion. Squamous cell carcinomas of the skin retain functional desmosomes at cell-cell junctions, which does not seem to prevent aggressive tumour behaviour or risk of metastasis (Kurzen et al., 2003).

Integrins

Integrins are heterodimeric cell-surface receptors that are typically involved in cell-matrix interactions. The function of integrins in cell-cell interactions is poorly understood, but recent data suggest that integrins are also involved in formation of cell-cell contacts in collective cell migration. $\alpha 5 \beta 1$ integrin interacts with fibronectin along interfaces between ovarian carcinoma cells (Casey et al., 2001) or fibroblasts (Salmenpera et al., 2008), and blocking of $\beta 1$ -integrin function through the use of a function-perturbing antibody in migrating multicellular melanoma clusters leads to loss of cell-cell cohesion followed by cell detachment and the transition to amoeboid single-cell migration (Hegerfeldt et al., 2002).

Tight junctions

Tight junctions and tight-junction-related proteins (including claudins 1 and 4, occludin and zona occludens 1; ZO-1) are present in many invasion zones of squamous cell carcinomas (Langbein et al., 2003) as well as in melanomas *in vitro*, as detected by histopathological sections. ZO-1 colocalizes with N-cadherin in homophilic junctions between melanoma cells and in heterophilic junctions between melanoma cells and fibroblasts (Smalley et al., 2005), suggesting that expression of junction proteins favours

invasiveness of melanomas. Besides its function as a cell-adhesion molecule, the tight-junction protein junctional adhesion molecule C (JAM-C) can lead to activation of $\beta 1$ and $\beta 3$ integrins and promote collective migration of epithelial cancer cells across a 2D surface (Mandicourt et al., 2007).

Gap junctions

Gap junctions are present at cell-cell junctions in all epithelia and in most other cells, and mediate direct intercellular metabolic coupling and signalling across the plasma membranes of neighbouring cells. In many cancer cells, including melanoma and lung squamous cell carcinomas, the homotypic gap junctions between cancer cells themselves and the heterotypic gap junctions between cancer cells and dermal fibroblasts are mediated by connexins CX26 and CX43, respectively (Ito et al., 2006). Heterotypic gap-junction formation depends additionally on cadherin-mediated cell-cell adhesion (Hsu et al., 2000), but the role of connexins in supporting collective migration is unclear.

Growth factors and chemokines

Paracrine and autocrine secretion of growth factors and chemokines has a direct influence on cell polarization, migration initiation and persistence of migration in single cells (Friedl and Weigelin, 2008). Likewise, collective cell migration in morphogenesis and cancer strongly depends upon chemokine and growth-factor signalling to establish and maintain the collective cell polarity and migration (Friedl and Gilmour, 2009; Lecaudey and Gilmour, 2006). Soluble factors either stem from the cytokine network produced by adjacent stromal cells and act in a paracrine manner, or are released from cells within the group and act in an autocrine or juxtacrine fashion. In oral squamous cell carcinoma in vitro, collective invasion is stimulated by paracrine stromal-cell-derived factor 1 (SDF-1) and hepatocyte growth factor (HGF), which are produced by fibroblasts of the tumor stroma in response to cancer-derived cytokines such as interleukin-1 α (IL-1 α) (Daly et al., 2008). In sprouting angiogenesis, autocrine regulation of collective endothelial cell sprouting occurs through the secretion of endothelial-cell-derived secreted epidermal growth factor (EGF)-like domain-containing protein 7 (EGFL7), which is deposited into the ECM on the basal side of sprouts and supports outgrowth of nascent vessels (Schmidt et al., 2007). The autocrine release of fibroblast growth factor (FGF) along the axis of the primordium is required for the development of the lateral line (the anlage of the inner ear) in zebrafish (Aman and Piotrowski, 2008).

The polarity of cells within a cluster is maintained by differential expression of growth-factor receptors in cells located at the front and rear of the cluster. FGF induces front-rear asymmetry by the differential expression of the SDF-1 α receptors CXCR4 and CXCR7 at front and rear regions, respectively. This maintains preferential sensitivity to SDF-1 α and, hence, collective forward migration in tip cells (Lecaudey and Gilmour, 2006; Aman and Piotrowski, 2008). Similarly, during branching morphogenesis of the developing trachea in *Drosophila* embryos, high expression of FGF receptor favors commitment to leader cell function, whereas cells with low FGF responsiveness take over trailing function (Ghabrial and Krasnow, 2006).

Cell-matrix interactions in collective cell migration

The molecular mechanisms of cell-matrix interactions in collective cell migration share many features with the migration of individual cells. These include the formation of actin-rich protrusions, force generation through the formation of cell-matrix adhesions, and focalized proteolysis. Below, we summarize how these three mechanisms function together to support collective cell migration.

Actin-rich protrusions

The mechanisms that control cell polarization and actin polymerization and lead to protrusion of a collective leading edge (i.e. a defined tip of cells that guides migrating cell groups and generates force) are most probably homologous to the polarity mechanisms of single cells (Vitorino and Meyer, 2008). Leading-edge protrusions are dynamic actin-containing cell structures that protrude in the direction of increased concentration of chemoattractants, growth factors and other extracellular ligands that define cell polarity and the location of cell-matrix interactions (Friedl and Weigelin, 2008). Cell protrusions are driven by polymerization at the barbed end of actin filaments (oriented toward the plasma membrane) and dissociation at the pointed end (in the cytoplasm) (Mattila and Lappalainen, 2008). In collective migration across a flat 2D substratum, the front row of cells contains continuous lamellipodia that cross the boundaries of multiple cells and drive the leading edge forward (Farooqui and Fenteany, 2005).

In vascular sprouting and collectively invading cancer cells, the leading edge frequently contains one or several pseudopodia and filopodia, which are cylindrical actin-rich protrusions with a finger-like shape (Inai et al., 2004; Wolf et al., 2007). Pseudopodia and filopodia develop in response to chemoattractant

stimuli, and contain multiple actin filaments in parallel orientation that push the plasma membrane forward. Actin dynamics in pseudopodia and filopodia are controlled by the Rho GTPase CDC42 and its downstream effectors Ena/VASP, mDia2/Drf3 and IRSp53, which enhance actin nucleation and deform the plasma membrane outward (Krugmann et al., 2001; Mattila and Lappalainen, 2008). Besides establishing directionality of cell polarization, actin-rich cell protrusions sense the environment, initiate cell attachment to adjacent tissue structures, and have a role in the maturation of E-cadherin-containing adherens junctions (Vasioukhin et al., 2000; Zaidel-Bar et al., 2007). Initiation and expansion of E-cadherin-mediated cell-cell contacts are dependent on the activity of RhoA and its downstream effector myosin II, as well as on the Rho GTPase Rac1 and the actin-nucleating ARP2/3 complex, which mediate concurrent lamellipodial protrusion and turnover, and the formation and remodelling of cell-cell junctions (Yamada and Nelson, 2007).

In addition to the anterior protrusions of leader cells, collectively migrating epithelial monolayer sheets generate multiple 'cryptic' actin-rich lamellipodia underneath each cell that generate traction against the underlying 2D substratum (Farooqui and Fenteany, 2005; Fenteany et al., 2000). Thus, despite the presence of E-cadherin-dependent cell-cell junctions in cell regions that are more distal to the substrate, the basolateral regions of moving cell sheets develop protrusive cytoskeletal activity, and this occurs even in cells that are multiple rows behind the leading edge (Farooqui and Fenteany, 2005). Thus, protrusive force generation occurs both in leading cells and in cells in the mid-regions of collectively migrating cell sheets, suggesting that cells translocate actively instead of passively throughout the cell group.

Adhesion and force generation

As does individual cell migration, collective cell migration through 3D interstitial tissue depends upon integrins, which connect the ECM to the intracellular actin cytoskeleton. Integrins bind to extracellular ligands by clustering in the plasma membrane and recruiting several cytoskeletal adaptor proteins (including paxillin, talin, tensin and vinculin) with their cytoplasmic tail (Zaidel-Bar et al., 2007). In collective migration of melanoma cells from primary explant culture, $\beta 1$ integrins cluster preferentially at cell-matrix interactions, and are required to generate traction force at the leading edge and to maintain high migration speed (Hegerfeldt et al., 2002). Fibroblast-led collective invasion of squamous carcinoma cells (see below) depends on the

function of integrins $\alpha 3$ and $\alpha 5$ in fibroblasts, which generate force and tube-like migration tracks through which the cancer cells follow collectively (Gaggioli et al., 2007).

Focalized proteolysis

In 3D tissues, collective cell migration is more space-consuming than single-cell migration (Friedl et al., 1997; Friedl et al., 2004). To generate sufficient space to accommodate the volume of several cell diameters, collective cell migration through a 3D matrix is highly dependent on local matrix degradation and on the generation and widening of paths of least mechanical resistance (Gaggioli et al., 2007; Wolf et al., 2007). Whereas single cancer cells generate small microtracks, collective invasion strands form macrotracks of varying width (up to several hundreds of micrometres, or more) (Wolf et al., 2007). In migrating cell groups such as colon adenocarcinoma cells (Nabeshima et al., 2000) and fibrosarcoma cells (Wolf et al., 2007), several proteases including the matrix metalloproteinases (MMPs) MT1-MMP and MMP-2 are preferentially localized to the leading edge. This implicates ECM degradation as an early event in collective cell movement. MT1-MMP is a cell-surface-localized multifunctional protease that is required for the activation of other MMPs, such as MMP-2. It is also required for the degradation of fibrillar collagen, which leads to migration-path formation and secondary widening during collective invasion of sarcoma and epithelial-cancer cells (Sabeh et al., 2004; Wolf et al., 2007; Wolf and Friedl, 2008).

Cross-talk with the surrounding stroma during collective cell migration

For the initiation or maintenance of collective migration, the migrating cell group interacts with the adjacent stroma physically, and through soluble or matrix-deposited factors (chemically). The crosstalk with the tissue stroma generates migration trails, leads to the formation of basement membranes that act as tracks, and supports the migration process through heterologous cell-cell contacts with stromal cells.

Forming a migration track

The ability of squamous cell carcinoma cells to collectively invade into connective tissue is supported by adjacent activated fibroblasts, which generate migration tracks through an MMP- and adhesion-force-dependent process (Gaggioli et al., 2007). Fibroblast-led collective invasion requires RhoA- and ROCK-dependent actomyosin activity for MMP-dependent collagen remodelling by fibroblasts, and further requires CDC42-

mediated force generation in the cancer cells that follow the tracks (Gaggioli et al., 2007). In other systems, such as fibrosarcoma cells invading 3D tissue in vitro, proteolytic tip-cell function can be provided by the cancer cells themselves. Here, an individual 'leader' cell utilizes focalized proteolysis by the surface-collagenase MT1-MMP to generate tracks of least mechanical resistance that enable the cell mass to undergo subsequent collective invasion (Wolf et al., 2007).

Deposition of a basement membrane

Besides its role in guiding the direction of cell migration, the migration track has structural and molecular properties that could serve additional functions. For instance, during collective cell migration into primordial tissue, the newly secreted basement membrane might promote the maintenance of collective front-rear polarity. In addition to providing a smooth scaffold along which cells glide in a continuous fashion, the basement membrane (through its interaction with adhesion receptors) triggers cell polarization into basolateral and apical compartments; this occurs during the formation of both sprouting epithelial ducts (such as mammary ducts) and blood vessels.

In branching morphogenesis of mammary ducts, basal myoepithelial cells secrete components of the circumferential basement membrane (particularly laminin I, the secretion of which is a prerequisite for both elongation of ducts and maintenance of the polarity of the luminal epithelial cell layer of the acinar structures) and move along the basement membrane (Gudjonsson et al., 2002). ZO-1 is apically expressed towards the luminal surface, which is suggestive not only of front-rear, but also of apico-basal, polarity during sprouting (Ewald et al., 2008). Likewise, sprouting blood vessels are laterally stabilized by a newly secreted basement membrane (Brachvogel et al., 2007). Perivascular basement membrane consists of nidogen-1, perlecan, several laminins and collagen IV, which are jointly deposited by endothelial cells and pericytes (Brachvogel et al., 2007). In the skin, dermal fibroblasts cooperate with epidermal keratinocytes to build the basement membrane by jointly depositing laminins 1 and 5, collagen IV and nidogen (Nischt et al., 2007; Smola et al., 1998). Such basement-membrane deposition is an early event during wound healing of the skin, whereby a keratinocyte monolayer moves across provisional wound matrix and deposits a basement membrane in cooperation with dermal fibroblasts (Friedl and Gilmour, 2009). Likewise, in epithelial cancers such as oral cancer and basal cell carcinoma, collective invasion occurs along

an intact basement membrane, yet the role of the basement membrane in favouring or counteracting collective invasion is not clear (Bauer et al., 2008; Boyd et al., 2008).

The role of E-cadherin

Besides its function in maintaining epithelial morphology and inhibiting invasive behaviour, the adherens-junction protein E-cadherin has a role in supporting collective cell migration by mediating adhesion and force generation between the migrating cell group and adjacent resident tissue cells. During oogenesis in *Drosophila*, border cells form a cohesive cluster of six to ten cells that moves by means of heterologous E-cadherin-E-cadherin interactions between migrating cells and nurse cells that are present in the stroma of the primordial ovary (Geisbrecht and Montell, 2002; Niewiadomska et al., 1999).

Conclusions and perspectives

Collective cell migration links hallmarks of single-cell movement with the process of cell-cell communication, apical and basal polarity, and multicellular tissue functions, all of which have previously been understood to be incompatible with cell-migration dynamics. Whereas the overall framework of collective cell migration is now becoming sufficiently clear, many of the mechanisms remain insufficiently defined by direct evidence, particularly the mechanisms of cell-cell cohesion and intercellular communication, as well as the role of tissue-derived factors that guide collective migration in a time-, space- and tissue-confined manner. Moreover, because of the diversity in cell type and tissue context in which collective cell migration can occur, many different molecular combinations are likely to substantially extend the few principles described here. As an example, if a cell group invades an epithelium, E-cadherin mediates not only the junctions within the moving group but also – with faster dynamics – the interaction with the surrounding tissue cells. By contrast, if a cell group invades ECM-rich interstitial tissue, homologous and heterologous interactions are mediated by E-cadherin and integrins, respectively. Moreover, if different cell and tissue compartments become transmigrated by the collective group (such as in cancer invasion), sequential engagement of different signalling, adhesion and protease systems might contribute to collective invasion and, possibly, to metastatic dissemination. Thus, rather than representing a uniform process, collective cell migration must be understood as diverse and plastic, and dependent on both the cell type from which the group originates and the tissue that is transmigrated.

With the advent of live-cell and live-animal microscopy, novel types of collective cell dynamics might be identified and classified in the future. A challenge will be to distinguish collective cell migration more clearly from other processes of multicellular translocation, including tissue drift and folding, the slow kinetics of cell-cell networks in live tissues, and the coordinated streaming of single, loosely connected cells. In addition, an understanding of common rules in, and differences between, collective invasion in different contexts will enable us to define strategies either to interfere with overgrown benign or neoplastic collective invasion or to enhance insufficient collective movement in wound healing. Finally, it is possible that, with advancing knowledge about tissue dynamics during formation and regeneration, many tissue regions that thus far are understood to be stable or even static structures will emerge as slowly moving cell convolutes that fulfil some, if not all, of the principles of collective migration.

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