

Modes of invasion during tumour dissemination

Pahini Pandya[†], Jose L. Orgaz[†] and Victoria Sanz-Moreno

Tumour Plasticity Team, Randall Division of Cell and Molecular Biophysics, King's College London, UK

Keywords

actomyosin contractility; cancer metastasis; invasion; plasticity; Rho GTPases

Correspondence

V. Sanz-Moreno, Tumour Plasticity Team, Randall Division of Cell and Molecular Biophysics, King's College London, New Hunt's House, Guy's Campus, London SE1 1UL, UK
E-mail: victoria.sanz_moreno@kcl.ac.uk[†]Equal contribution

(Received 6 July 2016, revised 24 October 2016, accepted 28 October 2016, available online 9 December 2016)

doi:10.1002/1878-0261.12019

Cancer cell migration and invasion underlie metastatic dissemination, one of the major problems in cancer. Tumour cells exhibit a striking variety of invasion strategies. Importantly, cancer cells can switch between invasion modes in order to cope with challenging environments. This ability to switch migratory modes or plasticity highlights the challenges behind antimetastasis therapy design. In this Review, we present current knowledge on different tumour invasion strategies, the determinants controlling plasticity and arising therapeutic opportunities. We propose that targeting master regulators controlling plasticity is needed to hinder tumour dissemination and metastasis.

1. Cancer cell invasion and dissemination

Abnormal tumour cell migration and invasion underlies metastatic dissemination, a major clinical problem in cancer (Sanz-Moreno and Marshall, 2010). Metastasis is a multistage process involving cell migration and invasion, transit in the blood or lymph, extravasation and colonization in the secondary site. Acquisition of invasive behaviour involves activation of signalling pathways controlling cytoskeletal dynamics, as well as turnover of cell–matrix and cell–cell adhesions (Fig. 1; Friedl and Alexander, 2011). Cancer invasion is a heterogeneous and adaptive process involving changes in cell morphology and generation of cell polarity,

resulting in translocation of the cell body. Cancer cells display exceptional ability to adapt to different environmental conditions engaging in different migration strategies, as reviewed in Clark and Vignjevic (2015); Friedl and Alexander (2011); Sahai (2005). Cancer cells can migrate either individually in the absence of cell–cell junctions, or collectively upon retention of cell–cell adhesions (Friedl and Alexander, 2011; Fig. 1). In turn, cancer cells can use a number of strategies when migrating individually (elongated-mesenchymal, rounded-amoeboid, spike-mediated) or collectively (multicellular streaming, tumour budding, collective invasion; Fig. 1). Studies using histopathological human samples and intravital imaging of xenografted tumours in mice have shown that these strategies can be observed *in vivo*, as

Abbreviations

Cdc42, cell division cycle 42; ECM, extracellular matrix; EMT, Epithelial-to-mesenchymal transition; ERM, ezrin/radixin/moesin; ERULS, ezrin-rich uropod-like structure; ESCRT, endosomal sorting complexes required for transport; GAP, GTPase-activating proteins; GEF, guanine exchange factor; LIMK, LIM kinase; MAT, mesenchymal-to-amoeboid transition; MLCK, myosin light chain kinase; MMP, matrix metalloproteinase; MRCK, myotonic dystrophy kinase-related Cdc42-binding kinase; MYPT1, myosin phosphatase target subunit-1; NMII, nonmuscle myosin II; PAK, p21-associated kinases; PIG3, p53-induced gene 3 protein; Rac, Ras-related C3 botulinum toxin substrate; Rho, Ras homolog family member; RLC, regulatory light chain; ROCK, Rho-associated coiled-coil-containing protein kinase; ROS, reactive oxygen species; SDF-1, stromal cell-derived factor 1; uPA, urokinase plasminogen activator; uPAR, uPA receptor; ZIPK, zipper-interacting protein kinase.

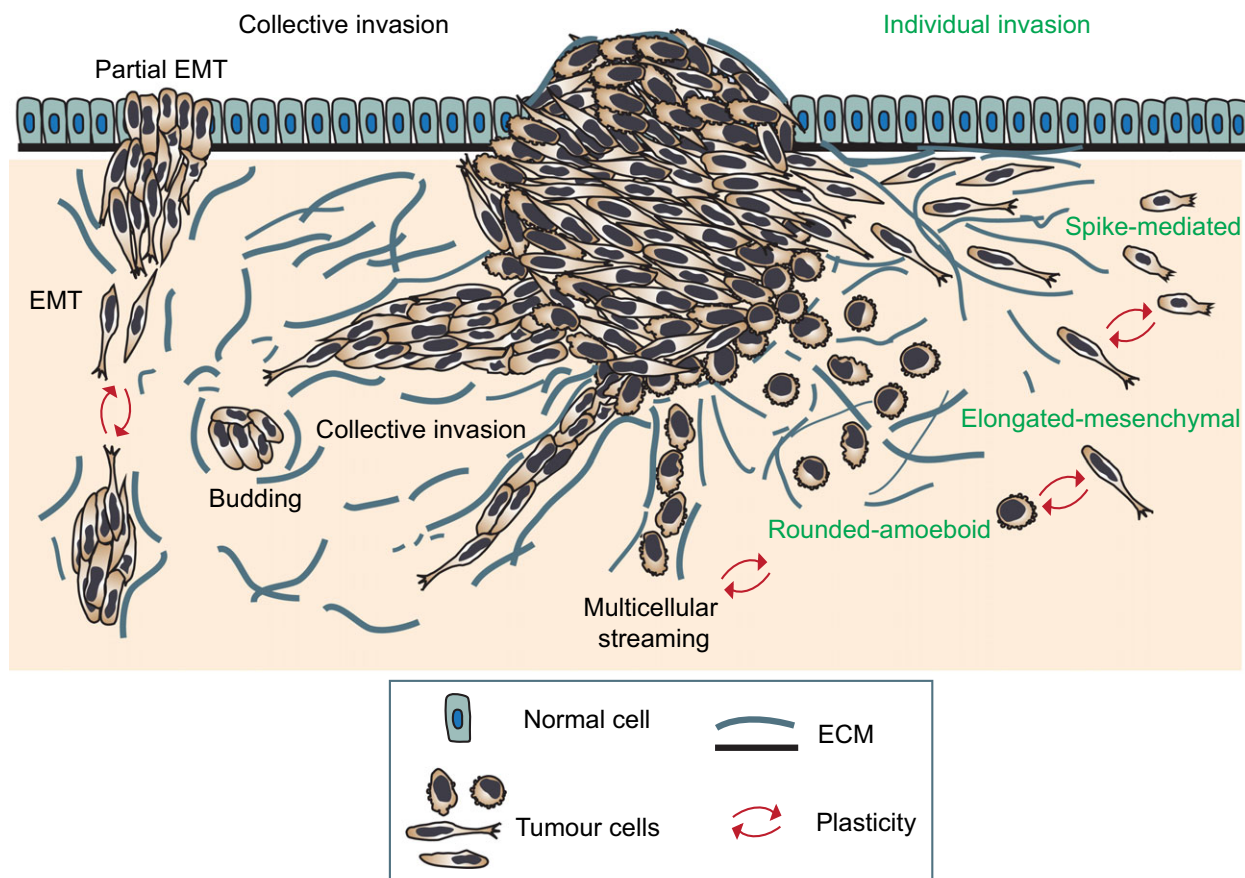


Fig. 1. Modes of invasion during tumour dissemination. Diagram showing the main individual and collective modes of tumour invasion and plasticity that allows interconversion between modes. Cells invading individually can use protrusion-based elongated-mesenchymal, bleb- and contractility-driven rounded-amoeboid and filopodial spike-mediated strategies. When cell–cell junctions are maintained, cells can move collectively as multicellular streams, budding or larger clusters (collective invasion). Migratory plasticity drives interconversion between the different modes.

reviewed in Clark and Vignjevic (2015); Friedl and Gilmour (2009); Friedl *et al.* (2012). While collective cell migration allows entry into the lymphatic system, individual cell migration is essential for entry into the bloodstream and dissemination to distant sites (Giamperri *et al.*, 2009).

In this Review, we describe the different individual and collective modes of invasion, the plasticity that cancer cells display, enabling them to switch between different migratory modes and the determinants of this plasticity. We also discuss the therapeutic challenges arising from migratory plasticity that could explain failure of some therapies, and the potential targets that could lead to a complete blockade in cancer cell migration and invasion. We propose that targeting master regulators controlling plasticity is needed to hinder tumour dissemination and metastasis. While this Review tries to cover the different modes of migration and key aspects of migratory plasticity during invasion

and metastasis, it is beyond the scope of this work to provide detailed insight into each section. Hence, throughout the Review, readers are directed to other excellent reviews that cover the relevant topic in depth.

2. Cell migration mechanisms

The molecular interactions between F-actin and non-muscle myosin II (NMII) govern the generation of mechanical forces across diverse length scales, and these are important not only for migration (Murrell *et al.*, 2015; Vicente-Manzanares *et al.*, 2009) but also for modulating cytokinesis (Green *et al.*, 2012) and tissue morphogenesis (Murrell *et al.*, 2015; Salbreux *et al.*, 2012).

During cell migration (Fig. 1), directional polarity is achieved by cells generating a leading edge at the front and a lagging edge at the back (reviewed in Ridley, 2015). Protrusion and adhesion of the leading edge

and retraction of the rear edge drive movement in the direction of locomotion (Richardson and Lehmann, 2010). The dynamics of cytoskeletal coupling with cell surface receptors that engage with surrounding tissue structures is the key process underlying all forms of migration (Friedl and Alexander, 2011).

Cell migration is a cyclic process (Friedl and Wolf, 2009; Lauffenburger and Horwitz, 1996) that begins with actin polymerization on one side of the cell resulting in actin-rich protrusion at the leading edge. Migration is facilitated by the forward movement of the cell, which is achieved by the engagement of cell surface receptors with the extracellular matrix (ECM); the formation of leading edge adhesions associated with proteolytic degradation of the ECM; and actomyosin contractility-mediated retraction of the rear edge of the cell.

Actin polymerization and organization into different cytoskeletal structures is regulated by the Rho family of proteins that play a central role in cell migration and has been extensively reviewed in Ridley (2015). Rho GTPases are molecular switches that cycle between active states when bound to GTP and inactive states when bound to GDP. This is regulated by activators or guanine exchange factors (GEFs) and inactivators or GTPase-activating proteins (GAPs; Ridley, 2015). By interacting with specific downstream effectors, active GTPases induce diverse actin rearrangements (Heasman and Ridley, 2008).

Three prototypical members of the family, Ras-related C3 botulinum toxin substrate (Rac), Ras homolog family member (Rho) and cell division cycle 42 (Cdc42), have been extensively linked to cell migration regulation (Ridley, 2015). Rho induces unbranched actin polymerization via formin mDia1, while Rho-associated coiled-coil-containing protein kinase (ROCK) promotes bundling of actomyosin filaments resulting in either stress fibres or an actomyosin cortex (Kimura *et al.*, 1996; Otomo *et al.*, 2005). Activation of ROCK downstream of Rho results in activating phosphorylation of myosin II (Amano *et al.*, 1996) and inactivation of myosin phosphatase target subunit-1 (MYPT1; Kimura *et al.*, 1996). Phosphorylated myosin II drives contraction of actin fibres in an ATP-dependent manner (Scholey *et al.*, 1980; Wang *et al.*, 2003). In addition to myosin II, ROCK can also phosphorylate ezrin/radixin/moesin (ERM), LIM kinases (LIMK1, LIMK2), α -adducin and several other proteins important for migration (Kimura *et al.*, 1996; Matsui *et al.*, 1998; Ohashi *et al.*, 2000).

Rac and Cdc42 also regulate actin polymerization (Ridley *et al.*, 2003; Wojciak-Stothard and Leiper, 2008). Binding of Cdc42 to myotonic dystrophy kinase-related Cdc42-binding kinase (MRCK) results in

phosphorylation of myosin II, MYPT1, LIMK1, LIMK2 and moesin (Leung *et al.*, 1998; Nakamura *et al.*, 2000; Scott and Olson, 2007; Tan *et al.*, 2001b). The activation of LIM kinases by phosphorylation allows for the inactivating phosphorylation of actin-severing protein cofilin, which inhibits actin depolymerization (Maekawa *et al.*, 1999; Sumi *et al.*, 1999). Rac proteins interact with lamellipodin and the WAVE complex that, in turn, promote actin nucleation by the Arp2/3 complex (Law *et al.*, 2013; Ridley, 2015).

Another set of downstream effectors of Rac and Cdc42 include the p21-associated kinases (PAKs). PAK1 promotes motility by inducing rapid turnover of focal contacts at leading edge of cells via phosphorylation of paxillin (Brown *et al.*, 2002; Nayal *et al.*, 2006; Premont *et al.*, 2004). PAK-mediated actin remodelling also involves LIMK1 (Edwards *et al.*, 1999; Yang *et al.*, 1998).

Actin polymerization by Rho GTPases directs the forces generated by actomyosin contractility needed for migration to take place. F-actin polymers serve as the scaffold for myosin II motors and accessory proteins (Murrell *et al.*, 2015; Vicente-Manzanares *et al.*, 2009) that can walk along, propel the sliding of or produce tension on actin filaments via ATPase activity (Vicente-Manzanares *et al.*, 2009). Depending on the location of myosin with respect to the middle filaments, this can result in the contraction or extension of two bound actin filaments. The contractile activity of NMII can be regulated via reversible phosphorylation of Ser19 on the regulatory light chain (RLC; Hirata *et al.*, 2009) by ROCK, myosin light chain kinase (MLCK) and other kinases such as MRCK, citron kinase, LIMK, zipper-interacting protein kinase (ZIP kinase) and Ca^{2+} /calmodulin-dependent protein (Endo *et al.*, 2004; Kimura *et al.*, 1996; Madaule *et al.*, 1998; Poperechnaya *et al.*, 2000; Tan *et al.*, 2001a). Subsequent phosphorylation at Thr18 of the RLC further increases the contractile activity of myosin II (Hirata *et al.*, 2009; Umemoto *et al.*, 1989). For detailed function of myosin II, readers are referred to the review (Vicente-Manzanares *et al.*, 2009).

3. Collective modes of cancer invasion

While the models above tend to focus on cells migrating as separate entities, cancer cell invasion is not restricted to cells moving individually. Histopathological samples show invasion of normal tissue by compact groups or clusters of cells and strands or cords of connected tumour cells (Clark and Vignjevic, 2015; Friedl and Gilmour, 2009; Friedl *et al.*, 2012; Leighton *et al.*, 1960; Wang *et al.*, 2016; Willis, 1952). Likewise,

intravital microscopy and *in vitro* studies have shown that cancer cells can move as loosely/nonadherent ‘streams’ of cells or collective migration of cell strands and sheets (Alexander *et al.*, 2008; Clark and Vignjevic, 2015; Friedl *et al.*, 2012). At the invasive front (tumour border) of certain cancer types, such as some carcinomas, invasive cells are observed to migrate as collective groups (Christiansen and Rajasekaran, 2006; Friedl *et al.*, 1995, 2004). Furthermore, collective cancer invasion can be seen as a dysregulated recapitulation of key steps that occur in many physiological processes such as embryonic morphogenesis or regeneration and tissue repair after wounding (Friedl and Gilmour, 2009).

Transition from collective to single-cell invasion may enhance metastatic efficiency and has been reviewed in Friedl *et al.* (2012). However, intravasation into lymphatic vessels can be efficiently performed by cell groups or clusters (Byers *et al.*, 1995; Giampieri *et al.*, 2009; Hashizume *et al.*, 1996; Madhavan *et al.*, 2001). This is also supported by the existence of circulating tumour clusters from patient peripheral blood samples (Aceto *et al.*, 2014; Brandt *et al.*, 1996; Hart, 2009; Hou *et al.*, 2011; Kats-Ugurlu *et al.*, 2009; Khoja *et al.*, 2014).

Similar to single-cell migration, collective cell movement results from the coordinated actions of the actin cytoskeleton, actomyosin contraction, cell polarity and cell surface receptors that engage with surrounding tissue structures (Friedl and Alexander, 2011; Ridley *et al.*, 2003). While collective cell migration also follows the cyclical process described above for single-cell migration (Friedl and Wolf, 2009; Lauffenburger and Horwitz, 1996), in collective movement cells remain grouped by cell–cell junctions (Friedl *et al.*, 2004, 2012; Rorth, 2007). Protrusion extension and retraction are coordinated in a ‘supracellular manner’, in which cytoskeletal protrusion and contractility are mechanically mediated through cell–cell junctions and involve several cells (Friedl *et al.*, 1995; Hegerfeldt *et al.*, 2002; Hidalgo-Carcedo *et al.*, 2011; Tambe *et al.*, 2011). Therefore, collective cell migration involves coordinating cell movement with ‘supracellular’ polarity, cytoskeletal organization and cell–cell junction stability (Friedl and Gilmour, 2009; Friedl *et al.*, 2012).

Both histopathological studies of cancer tissues and those using intravital microscopy have shown distinct modes of collective cancer migration (Fig. 1), as reviewed in Clark and Vignjevic (2015); Friedl and Gilmour (2009); Friedl *et al.* (2012). These sometimes overlapping strategies are determined by a combination of parameters such as degree of cell–cell adhesion,

cellular morphology and supracellular coupling of cell–cell signalling (Friedl *et al.*, 2012).

3.1. Multicellular streaming

During multicellular streaming, cells move one after the other in the same path within the tissue (Fig. 1; Friedl *et al.*, 2012; Friedl and Wolf, 2003; Manning *et al.*, 2015). In this migratory mode, cells are typically guided by chemokine or morphogen gradients or ECM structures (i.e. ‘microtracks’; Friedl *et al.*, 1997; Haeger *et al.*, 2015). Hence, coordinated migration takes place as directed movement of small strands of single cells, multicellular streams and as diffuse infiltration (‘chain- or swarm-like’; Friedl and Alexander, 2011; Kedrin *et al.*, 2008; Patsialou *et al.*, 2013; Roussos *et al.*, 2011; Seftor *et al.*, 2002; Wyckoff *et al.*, 2004). These chains (‘Indian files’) have been observed in infiltrating breast carcinoma (Page and Anderson, 1987; Pitts *et al.*, 1991), ovarian cancer (Sood *et al.*, 2001) and melanoma (Friedl and Wolf, 2008; Seftor *et al.*, 2002). Importantly, in this mode of migration, each cells’ cytoskeleton acts independently to generate traction force on the matrix, while cell–cell adhesions are weak or short-lived (Friedl *et al.*, 2012), allowing velocities similar to those achieved by cells migrating individually ($1\text{--}2\ \mu\text{m}\cdot\text{min}^{-1}$ or even faster; Clark and Vignjevic, 2015; Friedl *et al.*, 2012). Streaming cells can display rounded-amoeboid or elongated-mesenchymal phenotypes (Clark and Vignjevic, 2015; Friedl and Alexander, 2011; Friedl *et al.*, 2012). Intravital studies have shown that cells that display rounded-amoeboid morphology *in vitro*, such as human and mouse melanoma cells, are more likely to migrate as single cells or as streams *in vivo* (Herraiz *et al.*, 2016; Manning *et al.*, 2015; Pinner and Sahai, 2008a,b; Sanz-Moreno *et al.*, 2008).

3.2. Tumour budding

Scattered clusters of approximately five cells (‘tumour buds’) located in close proximity ahead of the invasive front (Fig. 1) have also been observed in colorectal cancer (Brabletz *et al.*, 2001; Bronsert *et al.*, 2014; Carr *et al.*, 1986; Prall *et al.*, 2005) and carcinomas from the oesophagus, pancreas, lung and breast (reviewed in Grigore *et al.*, 2016). Studies using 3D reconstructions from 2D serial sections of colorectal (Carr *et al.*, 1986) and other cancer types (pancreatic, lung, breast; Bronsert *et al.*, 2014) demonstrated that tumour budding is a dynamic process by which the tumour mass extends several finger-like multicellular projections that, later, break away from the main

tumour mass as small cell clusters (tumour buds; Bronsert *et al.*, 2014; Carr *et al.*, 1986). Importantly, tumour budding has been associated with poor cancer outcomes (Grigore *et al.*, 2016).

3.3. Collective cell invasion

This mode involves compact and cohesive cell groups with two or more neighbouring cells (Fig. 1). Collective invasion is facilitated by long-lived cell–cell junctions (Alexander *et al.*, 2008; Friedl *et al.*, 1995, 2012). Cells may adopt different morphologies depending on cell type and number and the structure of the tissue invaded (Friedl and Alexander, 2011).

These groups can be composed of small clusters, solid strands or files (1–2 cells in diameter) up to broad masses (Wolf *et al.*, 2007) that can even form an inner lumen if epithelial polarity is maintained, as seen in some breast, prostate, pancreatic and colorectal tumours (Christiansen and Rajasekaran, 2006; Friedl and Gilmour, 2009; Friedl *et al.*, 2012; Nabeshima *et al.*, 1999). Protruding sheets and strands that remain in contact with the primary site and generate local invasion have been detected in invasive epithelial tumours such as oral squamous cell carcinoma and mammary carcinoma (Bell and Waizbard, 1986; Page and Anderson, 1987), colon carcinoma (Nabeshima *et al.*, 1999), basal cell carcinoma and others (Friedl and Wolf, 2003). Cell clusters or ‘nests’ that detach from the primary tumour and extend into surrounding tissue have been described in epithelial cancers, melanoma and rhabdomyosarcoma (Ackerman and Ragaz, 1984; Bell and Waizbard, 1986; Nabeshima *et al.*, 1999; Page and Anderson, 1987).

In the most cases, the leading edge of the multicellular group is composed of one or several leader cells with mesenchymal characteristics (Fig. 1). Leader cells extend actomyosin-mediated actin-rich protrusions that generate integrin-mediated forward traction (Hegerfeldt *et al.*, 2002) and pericellular proteolysis towards the tissue structure (Nabeshima *et al.*, 2000; Wolf *et al.*, 2007), which yields a re-aligned ECM that guides the group (Fig. 2A; Gaggioli *et al.*, 2007; Khalil and Friedl, 2010). Following cells are passively dragged behind along the established migration track by cell–cell adhesion (Fig. 2A; Friedl *et al.*, 1995). Nevertheless, follower cells reinforce this ECM alignment and increase the diameter of the invading strand (Friedl and Wolf, 2008).

From studies using intravital microscopy, breast cancer cells or fibrosarcoma cells with predominantly individual phenotypes (Alexander *et al.*, 2008;

Giampieri *et al.*, 2009; Roussos *et al.*, 2011) are more prone to switching between single-cell and collective migration modes (see ‘Plasticity during collective invasion’ section). Collective invasion is typically the slowest migratory mode (0.01–0.05 $\mu\text{m}\cdot\text{min}^{-1}$; Weigelin *et al.*, 2012). Looking for advantages of this slower mode of migration, it has been suggested that the large cell mass could secrete high concentrations of pro-migratory factors and matrix proteases and protect inner cells from immune clearing. In addition, more migratory clones within the group could promote invasion of less motile cells, thereby increasing overall tumour invasion (Friedl and Wolf, 2003).

4. Plasticity during collective invasion

Extrinsic and intrinsic factors determine the adaptation of tumour cells to modify their migration mechanism (Friedl, 2004; Friedl and Alexander, 2011). This adaptive, dynamic behaviour is termed plasticity of tumour cell migration, and it is a combination of specific morphologic and mechanistic entities (Fig. 1). However, cells often display heterogeneity and can exhibit multiple modes of migration in 3D tissues (Fig. 1; Friedl and Wolf, 2010; Wolf *et al.*, 2003). Furthermore, some cancer cells can spontaneously switch between different modes of migration (Sanz-Moreno *et al.*, 2008).

Extensive research has been performed in the last 15 years trying to understand the mechanisms supporting different types of migration and the signals and conditions that trigger tumour cell plasticity (Friedl and Alexander, 2011; Lauffenburger and Horwitz, 1996; Ridley *et al.*, 2003; Sanz-Moreno and Marshall, 2010). By understanding this complex array of extracellular and intracellular determinants, the general machinery governing most types of cancer migration could be identified holding promise to translation into therapeutic interventions.

4.1. Epithelial-to-mesenchymal transition (EMT) and partial EMT

In epithelial cancers, EMT is a molecular programme characterized by loss or weakening of cell–cell junctions, which disrupts apico-basal polarity and cell anchoring to the basement membrane (Thiery *et al.*, 2009). This leads, in turn, to individual cell migration with enhanced migratory and invasive capacity, increased resistance to apoptosis and augmented ECM production (Kalluri, 2009). EMT can be complete or partial depending on the degree of cell–cell adhesion (Fig. 1). Therefore, EMT-like dissemination without

the typical EMT-associated gene expression patterns has been observed (Christiansen and Rajasekaran, 2006; Gavert *et al.*, 2011; Wicki *et al.*, 2006). Colorectal carcinomas often display cohesive cells at the leading edge, small groups of cells and individual cells scattered without connection to the main tumour, indicative of different degrees of EMT (Brabletz *et al.*, 2001; Gavert *et al.*, 2007).

4.2. Collective-to-individual transition

When cell–cell and cell–ECM interactions are simultaneously weakened, a transition from collective invasion to single-cell migration takes place (Fig. 1; Friedl, 2004). In multicellular clusters invading away from melanoma explants, the inhibition of $\beta 1$ integrin by blocking antibodies abolishes collective movement by inducing the detachment of individual cells (Hegerfeldt *et al.*, 2002). This mechanism could involve an intermediate mesenchymal migration step that would later lead to rounded-amoeboid single-cell dissemination (Friedl, 2004). Collective invasion from fibrosarcoma and breast carcinoma spheroids can be abolished by proteolytic inhibition or by collagenase MT1-MMP knock-down, leading to nonproteolytic single-cell dissemination (Wolf *et al.*, 2007).

4.3. Determinants of plasticity

The ability to switch between various modes of migration is regulated by signalling pathways and sustained via transcriptional programmes. This, in turn, can facilitate efficient invasion and distant metastasis by conferring increased resistance to external stimuli and adaptability to different microenvironments. Plasticity requires integration of intracellular and extracellular physical and molecular cues (Friedl, 2004; Salbreux *et al.*, 2012). In this section, we describe how cancer cells translate extracellular signals into intracellular responses that impact the mode of migration.

Factors determining plasticity during collective migration include physical cues and molecular cues (Fig. 2).

4.3.1. Physical cues

The molecular and physical characteristics of the ECM, such as composition, geometry, porosity, alignment and stiffness, strongly contribute to cell adhesion, migration and invasion (Wolf and Friedl, 2011). As such, pericellular proteolysis generated by tumour- and stromal cell-derived proteases generates micro- and macrotracks (micro- and macropatterning, respectively; Friedl and Wolf, 2008) surrounded by collagen bundles that support collective invasion (Friedl *et al.*, 1997; Gaggioli *et al.*, 2007; Wolf *et al.*, 2007). In addition, force-mediated ECM remodelling favours collective breast carcinoma cell invasion (Provenzano *et al.*, 2008; Fig. 2A). Mechanical cues affecting modes of cell migration include confinement and topology, among other factors (Kurniawan *et al.*, 2016).

4.3.2. Molecular cues

4.3.2.1. Proteases

Tumour invasion and progression have been linked to upregulation of proteases (Egeblad and Werb, 2002; Wolf and Friedl, 2011) with highest levels of activated proteases expressed at the tumour–stromal interface (Sternlicht *et al.*, 2000). These proteases include matrix metalloproteinases (MMPs), ADAMs, cathepsins, urokinase plasminogen activator (uPA) and its receptor uPAR (Mason and Joyce, 2011; Rizki *et al.*, 2008). Proteases contribute towards ECM degradation and tissue remodelling to form ECM bundles as well as generation of active epitopes of ECM components (Gaggioli *et al.*, 2007; Kenny *et al.*, 2008). The localized cleavage of ECM fibres by proteases results in release of ECM-imposed confinement, allowing the relaxation of the nucleus and enhancing migration speeds (Wolf *et al.*, 2007, 2013). As a consequence, the degree of proteolytic cleavage of ECM determines the degree of deformation and the confinement experienced by the cell.

During collective migration, cells at the leading edge of collectively invading colorectal carcinomas show increased expression and activity of membrane-tethered MT1-MMP and secreted MMP2, leading to polarized ECM degradation (Nabeshima *et al.*, 2000;

Fig. 2. Signalling pathways controlling collective modes of invasion. (A) Diagram showing the key regulators of collective migration. The leading edge of the multicellular group comprises one (or several) leader cells with mesenchymal characteristics. Leader cells extend actomyosin-mediated actin-rich protrusions that generate integrin-mediated forward traction and pericellular proteolysis yielding a re-aligned ECM that guides the group. Following cells are passively dragged behind along the established migration track by cell–cell adhesion. (B) Diagram showing the intracellular pathways activated in response to external stimuli and proteolysis of ECM. Membrane receptors such as $\beta 1$ integrins control migration of individual elongated-mesenchymal cells. Rac activation at the leading edge allows for protrusion formation that is linked to a ‘supracellular’ cytoskeleton. Activation of myosin II-based contractile forces by Rho-ROCK and Cdc42-MRCK signalling allows for contraction of cell body and retraction of the rear.

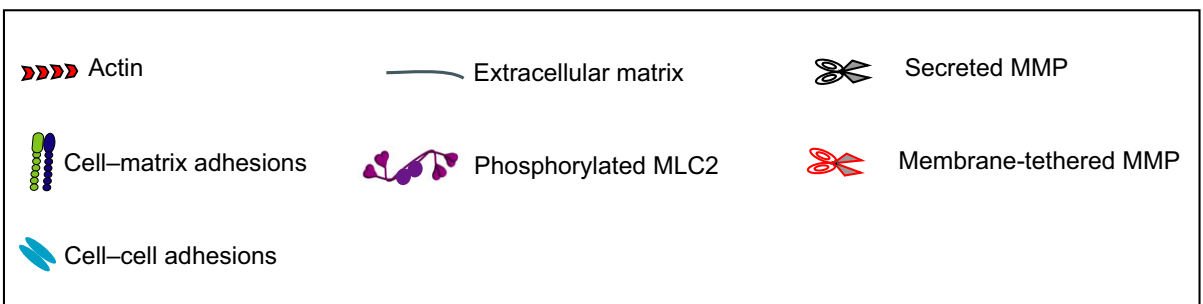
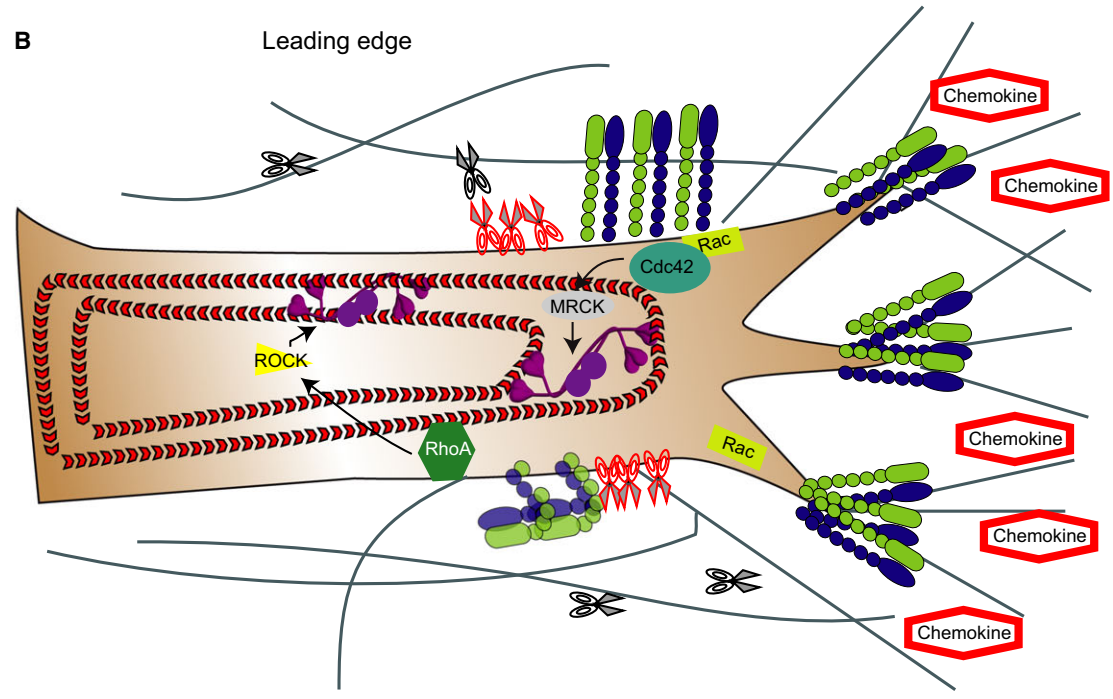
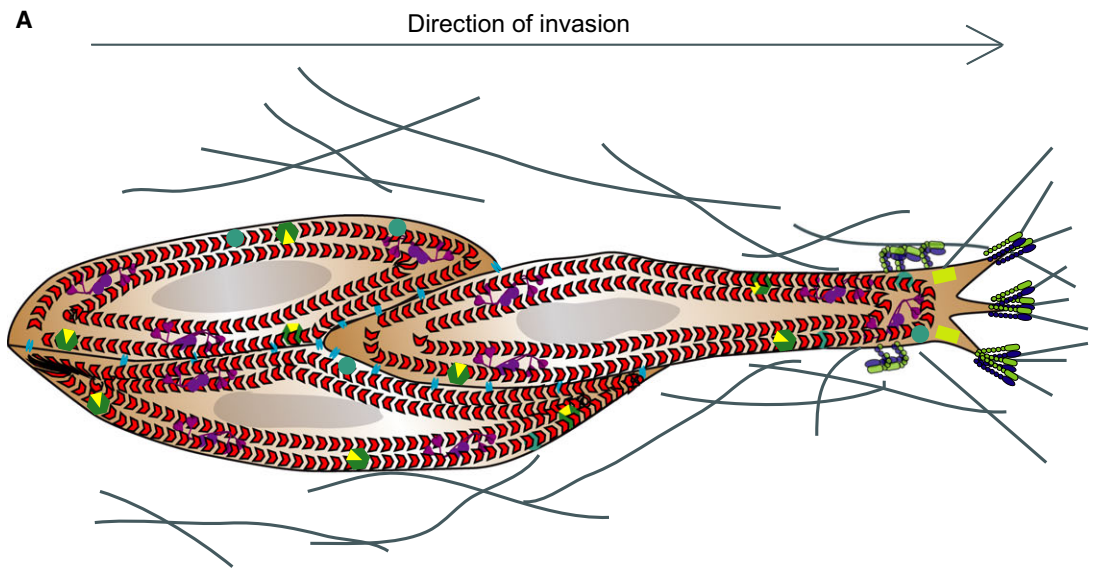


Fig. 2B). MT1-MMP is essential in collagen processing and multicellular strand formation during collective invasion of fibrosarcoma cells (Wolf *et al.*, 2007).

4.3.2.2. Membrane receptors

Extracellular matrix-binding molecules also determine the mode of invasion. Integrins couple the ECM to the actin cytoskeleton and develop small focal complexes (Friedl and Wolf, 2003; Hynes, 2002), which allow Rho GTPase-mediated outside-in signalling (Geiger and Peepers, 2009; Grashoff *et al.*, 2010; Hodivala-Dilke *et al.*, 1999; Lee *et al.*, 2009; Ridley *et al.*, 2003; Fig. 2B).

$\beta 1$ Integrins can control migration of multicellular melanoma (Hegerfeldt *et al.*, 2002) and ovarian carcinoma (Casey *et al.*, 2001).

CD44 binds to different ECM proteins (Zoller, 2011) and connects to the actin cytoskeleton through the ERM complex and ankyrin, signalling also through Rho GTPases (Zoller, 2011). CD44 serves also as a co-receptor for other adhesion molecules such as integrins and podoplanin; the latter signals to enhance RhoA activity, increasing collective invasion of squamous cell carcinomas (Martin-Villar *et al.*, 2006).

DDR family of receptors interact with fibrillar collagen and signal through several intracellular pathways (STAT5, NF- κ B, p38 MAPK/ERK and Src-family kinases; Neuhaus *et al.*, 2011; Vogel *et al.*, 2006). When co-engaged with DDR1, E-cadherin signalling limits excessive actomyosin contractility along cell–cell junctions; this stabilizes junctions and, in turn, maintains collective invasion (Hidalgo-Carcedo *et al.*, 2011).

In addition to cell–matrix adhesion, collective migration is also enabled by cell–cell adhesions through different adhesion systems, such as cadherins, tight junctions, gap junctions and others (Friedl *et al.*, 2012; Hegerfeldt *et al.*, 2002; Hidalgo-Carcedo *et al.*, 2011; Fig. 2A). Loss or downregulation of E-cadherin expression that drives EMT seems to be tunable, therefore leading to complete or partial EMT. In the latter, different E-cadherin levels that do not confound migration may be retained, or alternative proinvasive cadherins including N- or VE-cadherin may be expressed (Yano *et al.*, 2004). Collective invasion with E-cadherin in cell–cell junctions can be facilitated upon upregulation of LI-CAM (Gavert *et al.*, 2011; Shtutman *et al.*, 2006) and upregulation of podoplanin, which activates RhoA (Wicki *et al.*, 2006).

4.3.2.3. Secreted factors

Extracellular chemokines, cytokines and growth factors secreted by tumour or stromal cells enable and

promote migration in a paracrine and autocrine fashion (Friedl and Alexander, 2011; Haeger *et al.*, 2015). In addition, ECM degradation allows the release of these factors that can also be processed by proteases resulting in their activation, inactivation or degradation (Dean *et al.*, 2008; Mu *et al.*, 2002; Shiao and Coussens, 2010; Sounni *et al.*, 2010).

Invasion-promoting chemokines, growth factors and their receptors engage intracellular signalling networks (JAK, PI3K, Src, ERK) and/or Rho GTPase activity (Friedl and Alexander, 2011; Fig. 2B). Collective invasion of oral squamous carcinoma cells is stimulated by stromal cell-derived factor 1 (SDF-1) and HGF secreted from stromal fibroblasts in response to tumour-derived IL-1 α (Daly *et al.*, 2008). Likewise, a paracrine loop between tumour-associated macrophages secreting EGF and breast carcinoma cells secreting CSF-1 drives cancer cell migration (Wyckoff *et al.*, 2004).

4.3.2.4. Intracellular signalling pathways

Effective collective migration requires supracellular coordination of the cytoskeleton, which is controlled by Rho GTPase signalling (Friedl and Alexander, 2011). Leader cells generate actomyosin- and integrin-mediated traction towards the ECM, controlling tensional regulation of ECM alignment (Hegerfeldt *et al.*, 2002). High Cdc42/MRCK- and ROCK-mediated actomyosin contractility levels are found at the edges of groups of invading cancer cells (Gaggioli *et al.*, 2007; Fig. 2B). Actomyosin contractility generates pulling forces between the substrate and the follower cells, which, together with cortical actomyosin at lateral regions of the groups, maintain coupling between cells and collective forward movement in melanoma (Hegerfeldt *et al.*, 2002) and squamous cell carcinoma (Gaggioli *et al.*, 2007; Hidalgo-Carcedo *et al.*, 2011). Cell contractility mediated by Rho/ROCK/MLCK is also required for retraction of the tail in migrating groups and for lateral mechanocoupling via cadherin-based adhesions (Vicente-Manzanares *et al.*, 2009).

5. Individual cancer cell invasion

Cancer cells can also invade individually in the absence of cell–cell junctions using a variety of strategies (Fig. 1).

5.1. Elongated-mesenchymal mode of invasion

On stiff 2D matrices and 3D matrices such as collagen I, adherent cancer cells arising from connective tissues,

such as sarcomas, gliomas and some epithelial cancers (Paulus *et al.*, 1996; Polette *et al.*, 1998; Wolf *et al.*, 2003), can adopt actin-rich protrusions for migration. During this mode of migration, cells have an elongated morphology (Fig. 3) that is characterized by focal adhesion formation, MMP activity and actomyosin contractility localized at the rear of the cells. The requirement of strong focal adhesion limits velocity for cells adopting elongated-mesenchymal mode of migration resulting in relatively slow speed ($0.1\text{--}2\ \mu\text{m}\cdot\text{min}^{-1}$ *in vitro*; Friedl, 2004).

Elongated-mesenchymal migration is a protrusion-dependent mode mediated by polarized signalling of GTPases Rac1 (Sanz-Moreno *et al.*, 2008; Yamazaki *et al.*, 2009) and Cdc42 (Nalbant *et al.*, 2004), which direct Arp2/3 to drive actin polymerization in branched filaments against the plasma membrane (Amann and Pollard, 2001; Giri *et al.*, 2013; Machesky *et al.*, 1999). Adhesion maturation is controlled by signalling activity of RhoA and effector proteins such as formin protein diaphanous homologs 1 and 2, while Rho-ROCK signalling is required for the contractile activity of actomyosin scaffold to retract the cell rear (Friedl and Wolf, 2009; Ridley *et al.*, 2003; Fig. 3).

5.2. Rounded-amoeboid mode of invasion

Cancer cells migrating across pliable matrices can use rounded-amoeboid strategies and squeeze through the matrix using small, unstable blebs present throughout the surface of the cell (Sahai and Marshall, 2003; Sanz-Moreno and Marshall, 2010; Sanz-Moreno *et al.*, 2008) except at the rear, due to the presence of ezrin-rich uropod-like structures (ERULS; Lorentzen *et al.*, 2011) that dictate cell polarity (Fig. 4). Blebs are a consequence of low membrane-cortex attachment, increased intracellular pressure, low degree of $\beta 1$ integrin-mediated adhesion, reduced focal adhesion size and force generation (Bergert *et al.*, 2015; Charras and Paluch, 2008; Charras and Sahai, 2014; Petrie *et al.*, 2012; Sahai and Marshall, 2003; Sanz-Moreno *et al.*, 2008; Wolf *et al.*, 2003). Due to low reliance on focal adhesions and their deformability, the average speed during rounded-amoeboid migration can be significantly faster ($2\text{--}25\ \mu\text{m}\cdot\text{min}^{-1}$ *in vitro*, $1\text{--}15\ \mu\text{m}\cdot\text{min}^{-1}$ *in vivo*) than the mesenchymal type of cell migration (Pankova *et al.*, 2010; Pinner and Sahai, 2008a; Sanz-Moreno *et al.*, 2008).

Rounded-amoeboid motility is supported by high levels of actomyosin contractility downstream of Rho-ROCK (Sahai and Marshall, 2003; Sanz-Moreno *et al.*, 2008; Wilkinson *et al.*, 2005; Yamazaki *et al.*, 2009). There is significant overlap in the RhoA- and

RhoC-mediated activation of actomyosin contractility. Nevertheless, the assembly of cortical actin as a consequence of formin FLMN2 activation seems to be specific to RhoC in rounded-amoeboid cells (Kitzing *et al.*, 2010). Furthermore, mDia2–Dip interaction induces the characteristic cell blebbing in rounded-amoeboid movement (Eisenmann *et al.*, 2007).

In rounded-amoeboid migrating cells, a local decrease in attachment of the cell membrane to the actin cortex or local rupture of the actin cortex initiates a cycle of bleb expansion and retraction that allows cell movement (Charras and Paluch, 2008). Bleb expansion appears to be a direct mechanical consequence of intracellular pressure pushing the membrane outwards in the direction of motion. Bleb expansion is then slowed down and inhibited by recruitment of membrane-cortex linker proteins that facilitate actin recruitment underneath the membrane. The retraction phase begins with rapid assembly of actomyosin filaments beneath the bleb membrane (Charras and Paluch, 2008). Bleb-based movement is generated by creating blebs at the leading edge and exerting force onto the substrate to translocate the cell body (Charras and Paluch, 2008). These forces could be achieved by weakly adhering to the ECM or to surrounding cells; by applying forces on the ECM perpendicular to the direction of movement; or through nonspecific substrate friction (Bergert *et al.*, 2015; Charras and Paluch, 2008). Tumour xenograft intravital imaging studies have shown that melanoma and breast cancer cells in the invasive fronts predominantly move using rounded-amoeboid strategies (Giampieri *et al.*, 2009; Herraiz *et al.*, 2016; Pinner and Sahai, 2008a,b; Sanz-Moreno *et al.*, 2008, 2011). Importantly, the invasive fronts of human melanoma primary tumours and metastases are enriched in rounded cells (Cantelli *et al.*, 2015; Orgaz *et al.*, 2014; Sanz-Moreno *et al.*, 2011).

5.3. Other modes of individual invasion

While elongated-mesenchymal and rounded-amoeboid modes of migration are extremes of the spectrum, intermediate modes of migration have been reported as cells transition between these modes (Yin *et al.*, 2013). Glioblastoma-initiating cells can efficiently invade exhibiting a round cell body aided by long or short protrusions (Ruiz-Ontanon *et al.*, 2013). Under confinement, breast cancer cells exhibit a mode of migration that is dependent on directed water permeation. This mode, termed the osmotic engine model, relies on aquaporin5 and Na^+/H^+ exchangers (Stroka *et al.*, 2014).

Another mode of migration described in the recent years is the lobopodial mode of migration. This pressure-based mode involves the use of the nucleus as a piston to generate intracellular pressure that drives forward a blunt cylindrical protrusion termed lobopodia (Petrie *et al.*, 2012, 2014). This mode is characterized by nonpolarized distribution of active Rac1 at the plasma membrane and RhoA-driven actomyosin contractility at the front of the nucleus. Actomyosin contraction pulls the nucleus towards the front, which poses a diffusion barrier and results in increased intracellular pressure that pushes the leading edge forwards (Petrie *et al.*, 2012, 2014). However, this mode of migration has only been described in fibroblasts and its role in cancer cell invasion remains to be established.

Furthermore, filopodial spike-based cancer cell invasion has also been recently described (Paul *et al.*, 2015; Fig. 1). In this mode, $\alpha 5\beta 1$ integrin recycling promotes RhoA-ROCK-FHOD3-driven invasion independently of Arp2/3 activity.

6. Plasticity during individual cell invasion

6.1. Mesenchymal-amoeboid plasticity

As noted earlier, ECM degradation and tissue remodelling by secreted proteases regulate invasion (Friedl and Alexander, 2011; Mantovani *et al.*, 2008). Importantly, pioneer work in the cell migration field showed that upon inhibition of pericellular proteases, elongated-mesenchymal cells still invaded as rounded-amoeboid cells both *in vitro* and *in vivo* (Sahai and Marshall, 2003; Wolf *et al.*, 2003; Wyckoff *et al.*, 2006) while undergoing mesenchymal-to-amoeboid transition (MAT; Friedl, 2004; Wolf *et al.*, 2003). This plasticity most likely contributes to the failure of therapies targeting proteases (see section ‘Therapeutic challenges posed by migratory plasticity’).

As both actin assembly and the actomyosin machinery can regulate cell morphology, modulation of actin organization can predict the type of protrusions formed by migrating cells (Bergert *et al.*, 2012; Derivery *et al.*,

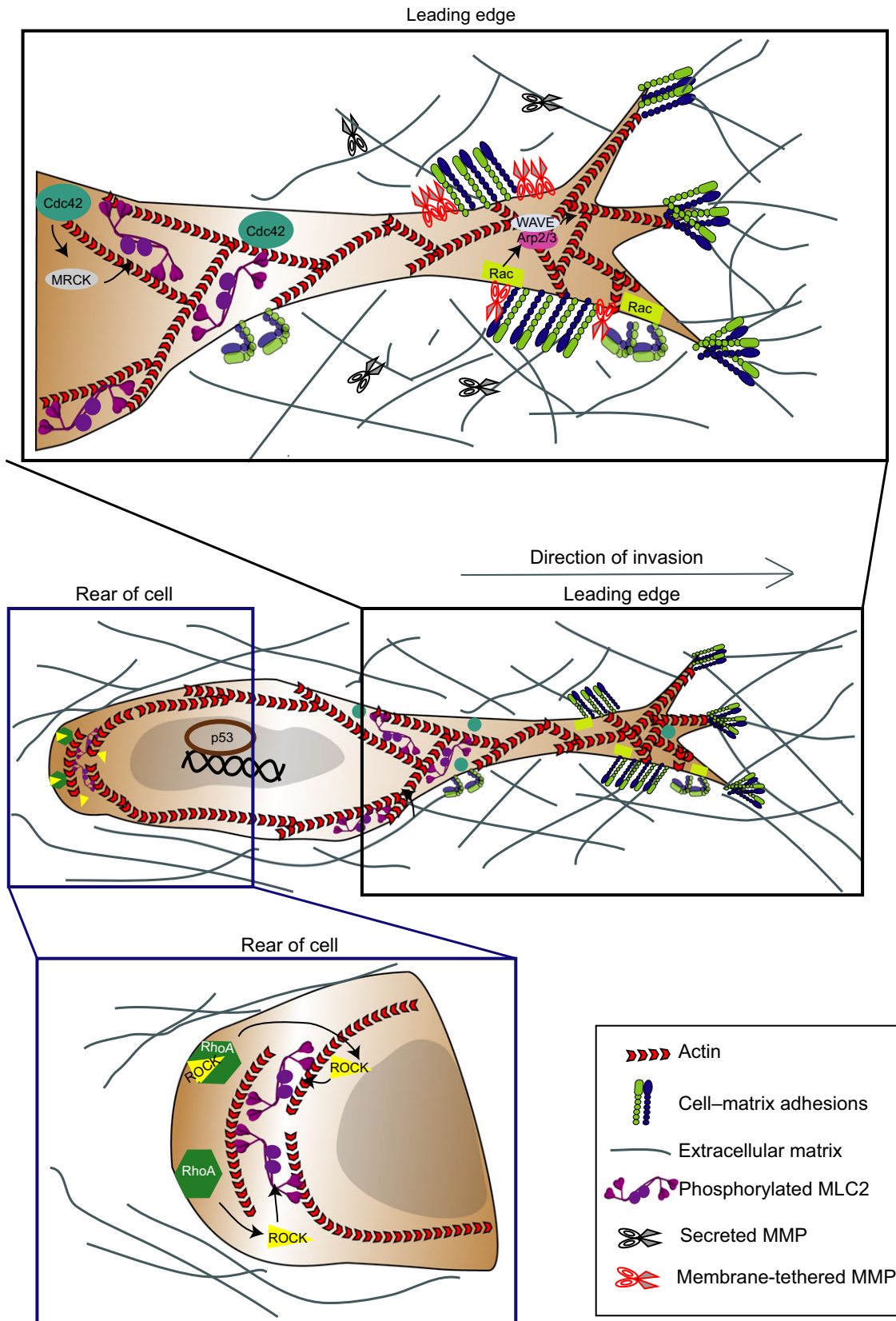
2008; Langridge and Kay, 2006; Mierke, 2015). These changes in actin structures have been shown to be highly dependent on two key pathways that play compensatory roles and inhibit each other and that regulate the switch between rounded-amoeboid and elongated-mesenchymal migratory states (Fig. 1). The activation of Rac1-WAVE2-Arp2/3 drives elongated-mesenchymal adhesive movement, while RhoA/C-ROCK1/2 pathways drive rounded-amoeboid migration (Sanz-Moreno *et al.*, 2008; Yamazaki *et al.*, 2009) although some degree of Rho-ROCK-driven contractility is required also for elongated-mesenchymal migration (Friedl and Wolf, 2009; Vicente-Manzanares *et al.*, 2009). Cdc42 is required for both elongated-mesenchymal and rounded-amoeboid movement depending on engagement of different effectors (Calvo *et al.*, 2011; Gadea *et al.*, 2008). Interestingly, loss of the Ras regulator RasGRF2 in melanoma cells induces MAT (Calvo *et al.*, 2011).

6.2. Single-to-collective tumour invasion

In fibrosarcoma and breast carcinoma 3D-spheroids, a spontaneous transition from individual mesenchymal invasion towards multicellular strands (Fig. 1) occurs in follower cells along the microtracks generated by leader cells (Wolf *et al.*, 2007). These microtracks are occupied by following coupled cells and therefore, tracks increase in width, ultimately resulting in strand-like collective invasion (Friedl and Wolf, 2008; Wolf *et al.*, 2007).

The microenvironment, in particular ECM porosity, can regulate tumour plasticity and single-to-collective transition. Cell jamming is a collective mode of invasion of mesenchymal tumour cells that is imposed by tissue confinement. Dense matrix induces cell–cell interactions, leader–follower cell behaviour and collective migration as an obligate protease-dependent process (Haeger *et al.*, 2014). The conversion to collective invasion with increasing ECM confinement supports the concept of cell jamming as a guiding principle for melanoma and fibrosarcoma cells into dense tissue (Haeger *et al.*, 2014; Sadati *et al.*, 2013; Vedula *et al.*, 2012). In addition, confinement modelled with

Fig. 3. Signalling pathways controlling elongated-mesenchymal mode of invasion. Diagram showing key regulators of elongated-mesenchymal mode of migration in cells. During this mode, cells adopt an elongated morphology that is characterized by actin-rich protrusions, focal adhesion formation, matrix metalloproteinase (MMP) activity and actomyosin contractility localized at the rear of the cells. Top inset: signalling activity at the leading edge of cells exhibiting elongated-mesenchymal migration. Polarized signalling of GTPase Rac1 directs Arp2/3 via WAVE2 to drive actin polymerization in branched filaments against the plasma membrane. Bottom inset: signalling activity at the rear of cells exhibiting elongated-mesenchymal migration. Rho-ROCK signalling is required for the contractile activity of actomyosin scaffold to retract the cell rear. Transcription driven by p53 promotes elongated-mesenchymal strategies.



micropillar arrays can also force collective migration of breast carcinoma cells (Wong *et al.*, 2014).

Single-to-collective migration can also be induced by gradients or changes in adhesion molecules. For example, when individual cells become attracted by the same chemotactic source, they may first undergo multicellular streaming with short-lived, dynamic cell–cell junctions. When cell–cell adhesion molecules are then upregulated, the cells may join each other and convert to a collective migration mode (Friedl and Alexander, 2011).

6.3. Determinants of plasticity

Determinants of plasticity in cells exhibiting individual mode of migration include physical and molecular cues (proteases, membrane receptors, secreted factors and intracellular signalling pathways), which are broadly highlighted in Figs 3 and 4.

6.3.1. Physical cues

Migration in discontinuous 3D substrates that allow cell–matrix adhesion results in a highly polarized spindle-shaped morphology in elongated-mesenchymal cells (Charras and Sahai, 2014; Starke *et al.*, 2014; Fig. 3A). However, within discontinuous 3D matrices, if availability of small surface areas for attachment is low, such surfaces might not support adhesion formation and bleb-based modes of migration are favoured (Petrie *et al.*, 2012; Tozluoglu *et al.*, 2013).

Another characteristic of ECM is porosity, which determines the confinement of migrating cells. Tissue confinement can also promote single-to-collective transitions such as cell jamming (Haeger *et al.*, 2014; Sadati *et al.*, 2013; Vedula *et al.*, 2012). During individual migration, increasing confinement and decreasing adhesion result in increased deformability of the cell and MAT (Liu *et al.*, 2015; Tozluoglu *et al.*, 2013). The switch in these modes of migration is regulated by a delicate balance between adhesion and actomyosin contractility (Bergert *et al.*, 2012).

While the cell cytoplasm is readily deformable in confined conditions, the nucleus is 2–10 times stiffer than the cytoplasm, thus generating a deformability barrier (Wolf *et al.*, 2013). The deformability of the nucleus is dependent on the stiffness of nuclear lamina, which is regulated by lamin A/C levels (Lammerding *et al.*, 2004, 2006). While low levels of lamins result in increased nuclear deformability, excessive softness of nuclear lamina decreases cell survival. In fact, cancer cells migrating in confined spaces experience nuclear envelope ruptures that result in DNA damage, which is solved using DNA repair machinery and endosomal sorting complexes required for transport (ESCRT; Denais *et al.*, 2016; Raab *et al.*, 2016). Cancer cells capable of resealing nuclear envelope rapidly could benefit from greater nuclear deformability, increased migration and survival. On the other hand, DNA damage responses induced by reactive oxygen species (ROS) dramatically reduce rounded-amoeboid invasion *in vitro* and *in vivo*, by suppressing actomyosin contractility (Herraiz *et al.*, 2016). In migrating cells, how different types of DNA damage are sensed and repaired will be an important question to solve.

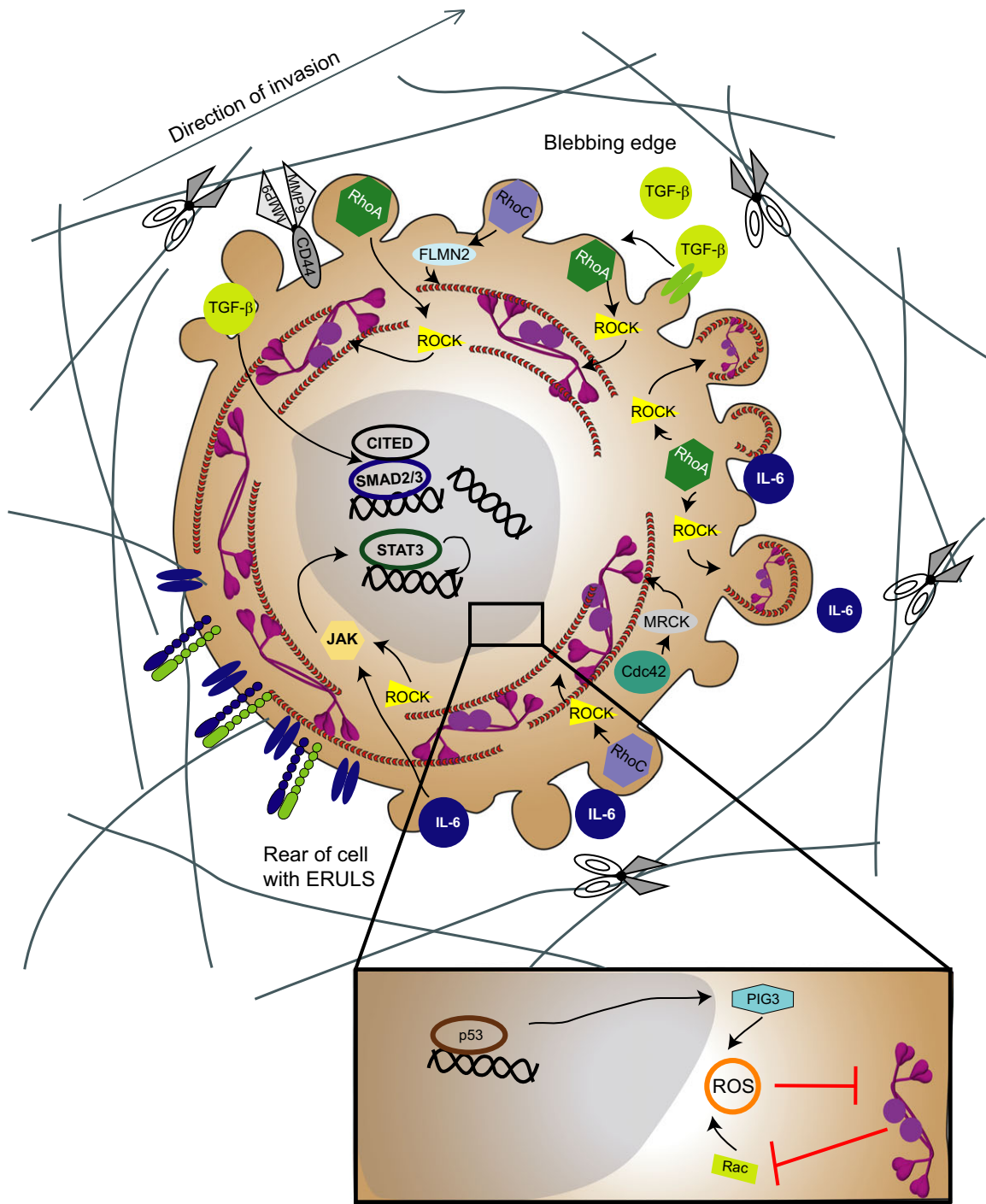
In addition to ECM properties, mechanical perturbations such as interstitial flow can also affect cell migration. In fact, inflammation in cancer can dramatically increase fluid flow between the blood and lymphatic system (Dafni *et al.*, 2002; Shieh and Swartz, 2011), causing an increase in migration speed of breast cancer cells (Haessler *et al.*, 2012). Interestingly, for breast cancer cells able to migrate using both rounded-amoeboid and elongated-mesenchymal motility within 3D collagen type I matrix, interstitial flow favours a switch towards rounded-amoeboid motility (Huang *et al.*, 2015).

6.3.2. Molecular cues

6.3.2.1. Proteases

While pericellular proteolytic inhibition in elongated-mesenchymal cells drives MAT and cells keep invading (Sahai and Marshall, 2003; Wolf *et al.*, 2003),

Fig. 4. Signalling pathways controlling rounded-amoeboid mode of invasion. Diagram showing key regulators of rounded-amoeboid mode of migration. Rounded-amoeboid cells squeeze through the matrix using small, unstable blebs present throughout the surface of the cells except at the rear, due to the presence of ezrin-rich uropod-like structures (ERULS) that determine polarity. Blebs are a consequence of low membrane–cortex attachment, increased intracellular pressure, high actomyosin contractility, low degree of $\beta 1$ integrin-mediated adhesion, reduced focal adhesion size and force generation. Rounded-amoeboid motility is supported by high levels of actomyosin contractility downstream of Rho-ROCK. While there is significant overlap in the RhoA- and RhoC-mediated activation of actomyosin contractility, the assembly of cortical actin as a consequence of formin FLMN2 activation seems to be specific to RhoC. Maintenance of rounded-amoeboid movement is driven by IL-6 family of cytokines and the transcription factor STAT3. Conversely, ROCK can activate JAK/STAT3 signalling generating a positive feedback loop. TGF- β promotes rounded-amoeboid migration, which is perpetuated via SMAD2/CITED1-mediated transcription. In addition, Rho/ROCK suppresses p53/PIG3-mediated ROS production. On the other hand, Rac suppresses actomyosin contractility via ROS generation.



Actin	Cytoskeleton-membrane attachment	phosphorylated MLC2
Cell-matrix adhesions	Extracellular matrix	Secreted MMP

rounded-amoeboid melanoma cells are able to degrade the matrix (Hooper *et al.*, 2006), in some cases even more efficiently than elongated-mesenchymal melanoma cells (Orgaz *et al.*, 2014). This may be due to a higher secretion of certain MMPs such as MMP13 and MMP2. Furthermore, melanoma cells use MMP9 non-catalytic functions to sustain rounded-amoeboid invasion (Orgaz *et al.*, 2014) via regulation of actomyosin contractility.

6.3.2.2. Membrane receptors

Membrane receptors such as $\beta 1$ integrins can also control migration of individual elongated-mesenchymal cells (Ahn *et al.*, 2012; Friedl, 2004; Wolf *et al.*, 2007). Furthermore, CD44 has been shown to be required for individual rounded-amoeboid invasion (Orgaz *et al.*, 2014). CD44 forms a complex with MMP9, which results in the activation of actomyosin contractility in melanoma (Orgaz *et al.*, 2014).

6.3.2.3. Secreted factors

Melanoma cells secrete high levels of IL-6 family cytokines that promote individual rounded-amoeboid invasion (Sanz-Moreno *et al.*, 2011). HGF receptor Met-driven signalling has also been implicated in MAT via Rho-ROCK pathway (Laser-Azogui *et al.*, 2014). Therefore, extracellular ligands govern how integration of signals is achieved in migrating cells travelling through different tumour microenvironments.

6.3.2.4. Intracellular signalling pathways

Actin dynamics determine the type of protrusions. Promotion of actin polymerization in carcinoma cells drives the formation of actin-rich lamellipodia, whereas blebbing requires both actin polymerization and depolymerization (Bergert *et al.*, 2012; Bovellan *et al.*, 2014; Derivery *et al.*, 2008; Langridge and Kay, 2006; Mierke, 2015).

The balance between antagonistic RhoA and Rac1 signalling determines the mode of migration and lies at the core of tumour cell plasticity in individual migration of several cancer cell types (Sanz-Moreno *et al.*, 2008; Yamazaki *et al.*, 2009). Downstream of $\beta 3$ integrin, adaptor NEDD9 activates Src signalling (involving also p130Cas, Crk) and the Rac GEF DOCK3 (Ahn *et al.*, 2012; Carragher *et al.*, 2006; Kiyokawa *et al.*, 1998; Sanz-Moreno *et al.*, 2008). In turn, active Rac signals through WAVE-2 promoting Arp2/3-dependent actin assembly and protrusion formation, driving elongated-mesenchymal migration (Sanz-

Moreno *et al.*, 2008; Yamazaki *et al.*, 2009; Fig. 3B). WAVE-2 suppresses rounded-amoeboid movement by inhibiting actomyosin contractility (Sanz-Moreno *et al.*, 2008; Yamazaki *et al.*, 2009).

Conversely, the Rac-specific GAPs ARHGAP22 and ARHGAP24 (also known as FilGAP), which are activated by high actomyosin contractility, maintain low levels of Rac activity in rounded-amoeboid cells (Saito *et al.*, 2012; Sanz-Moreno *et al.*, 2008). MAT can be induced through the inhibition of Rac activity (Sanz-Moreno *et al.*, 2008), or indirectly activating Rho by engaging EphA2 (Parri *et al.*, 2009). Lowering the levels of RhoA-negative regulator p27^{Kip1} (Besson *et al.*, 2004) also promotes rounded-amoeboid migration (Berton *et al.*, 2009). The antagonistic interplay between Rho-ROCK and Rnd3 (RhoE) at the cell membrane that regulates blebbing also drives cell plasticity. Absence of PDK1 allows for inhibitory binding of RhoE to ROCK leading to impaired actomyosin contractility and rounded-amoeboid motility (Pinner and Sahai, 2008b). Importantly, Cdc42 has a dual role as it supports rounded-amoeboid migration via DOCK10 and the Cdc42 effectors NWASP and PAK2 (Gadea *et al.*, 2008). Supporting these data, blocking the Cdc42-negative regulator and Ras GEF RasGRF2 ablates amoeboid invasion and metastatic colonization (Calvo *et al.*, 2011). On the other hand, in elongated-mesenchymal cells, Cdc42 promotes Rac activity by activating and recruiting ubiquitin ligase SMURF1 to the leading edge via a PAR6-aPKC polarity complex (Osmani *et al.*, 2010).

Regulation of protein levels and protein localization drives plasticity. As such, downregulation of SMURF1, which targets RhoA for localized proteasomal degradation in Rac-dependent protrusions, results in MAT (Sahai *et al.*, 2007). Rab5-dependent endocytosis regulates Rac localization to protrusions supporting therefore elongated-mesenchymal movement (Palamidessi *et al.*, 2008).

6.3.2.5. Transcriptional programmes

While individually invading cells can switch between blebs and protrusions in short timescales (Bergert *et al.*, 2012), maintaining cell motility programmes requires a tight temporal coupling of actin dynamics and transcriptional activity (Olson and Nordheim, 2010). Hence, it is no surprise that several transcriptional factors have been implicated in different modes of migration and cellular plasticity. Loss of p53 function via mutant p53 overexpression results in MAT in melanoma cells (Gadea *et al.*, 2007). Transcription

driven by p53 further suppresses fast rounded-amoeboid migration via induction of its transcriptional target p53-induced gene 3 protein (PIG3). PIG3 is an oxidoreductase that produces ROS and further suppresses Rho activity via regulation of ARHGAP5 (Herraiz *et al.*, 2016).

In contrast, maintenance of rounded-amoeboid movement is driven by IL-6 family of cytokines and the transcription factor STAT3. ROCK can activate JAK/STAT3 signalling generating a positive feedback loop (Sanz-Moreno *et al.*, 2011). As a result of high levels of STAT3 activity, rounded-amoeboid melanoma cells secrete higher levels of most secreted MMPs (Orgaz *et al.*, 2014).

MRTF- and SRF-driven transcription can sustain high actomyosin contractility levels to promote metastasis in melanoma and breast carcinoma cells (Medjane *et al.*, 2009). In breast cancer models, TGF- β /SMAD induces transcriptional changes that promote a cohesive-to-single invasion (Giampieri *et al.*, 2009). Those transcriptional changes include genes that control actomyosin contractility (Giampieri *et al.*, 2009). In melanoma, TGF- β promotes rounded-amoeboid migration, which is perpetuated via SMAD2/CITED1-mediated transcription of LIF, JAK and the Rho GEF ARHGAP5 (Cantelli *et al.*, 2015).

7. Therapeutic challenges posed by migratory plasticity

Plasticity or adaptability in terms of cell migration modes likely underlies the failure of some therapies aimed at blocking cancer invasion and metastasis. Several therapies targeting pericellular matrix-degrading proteases were developed (Coussens *et al.*, 2002; Overall and Kleinfeld, 2006; Overall and Lopez-Otin, 2002). However, extensive phase III clinical trials not only failed but even worsened metastatic processes (Coussens *et al.*, 2002; Fingleton, 2003; Overall and Lopez-Otin, 2002; Zucker *et al.*, 2000). Such failure was attributed in part to the different roles of specific MMPs (Lopez-Otin and Matrisian, 2007). However, the MAT that occurs upon pericellular proteolysis inhibition (Friedl, 2004; Sahai and Marshall, 2003; Wolf *et al.*, 2003; Wyckoff *et al.*, 2006) would add up to the reasons why therapies broadly targeting MMP functions were not successful. In addition, noncatalytic regulation of cell signalling (Orgaz *et al.*, 2014) could be an additional reason for the failure of MMP inhibitor-based therapies (Dufour and Overall, 2013; Overall and Kleinfeld, 2006; Zucker *et al.*, 2000). Therefore, targeting specific proteolytic and nonproteolytic functions of certain MMPs may provide better results in the clinic.

While targeting MMPs offers a singular therapeutic focal point, it is crucial to keep in mind that the tumour microenvironment presents a heterogeneous and discontinuous environment with varying matrix geometries and degree of stiffness. As a consequence, cells could exhibit MAT spontaneously in response to localized changes in stiffness and this plasticity can impact tumour dissemination *in vivo*. Thus, effective therapies should focus on blocking plasticity by inhibiting multiple intracellular and extracellular drivers of this mode of drug resistance.

8. Potential therapeutic targets to block migratory plasticity and tumour cell invasion

Adaptation of cancer cells to different environmental conditions is exemplified by the wide variety of invasion strategies they can adopt. More striking is their ability to switch from one strategy to another to keep on invading. This adaptability is complex, as tumour cell migration plasticity may not need fixed genetic drivers, but it may be aided by accumulated DNA damage in migrating cancer cells.

Such adaptability of cancer cells to change their mode of migration could be considered a type of drug resistance. Therefore, therapies should be aimed at targeting cytoskeletal regulators involved in multiple modes of migration, or combination of drugs aimed at different key targets (Figs 3 and 4). This goes in line with combinational therapies that are currently in clinical trials to stop primary tumour growth. Some key regulators could be β 1 integrin, which controls single and collective invasion and the switch from one to another. Several therapeutic interventions are being clinically tested in patients with solid tumours, including peptide ATN-161, which inhibits binding of α 5 β 1 to fibronectin (Cianfrocca *et al.*, 2006; Thundimadathil, 2012), and α 5 β 1-blocking antibody volociximab (Ricart *et al.*, 2008). These therapies are also aimed to block tumour angiogenesis (Cianfrocca *et al.*, 2006; Ricart *et al.*, 2008).

ROCK lies at the core of cytoskeletal regulation in virtually all modes of migration, therefore appears as a good therapeutic target. Interestingly, a pan-AGC kinase inhibitor that very effectively targets ROCK (Sadok *et al.*, 2015) is being clinically evaluated in advanced solid tumours (ClinicalTrials.gov Identifier: NCT01585701).

Cdc42 or its effectors could be also suitable candidates given their involvement of both rounded-amoeboid and elongated-mesenchymal invasion strategies. A combination of drugs targeting cell adhesion and

the actomyosin core machinery could also be considered.

Furthermore, careful attention should be given to targeting transcriptional programmes that self-perpetuate invasion strategies (JAK/STAT3, TGF- β /SMAD) and control processes such as tumour promoting inflammation and immunosuppression. Given the pro-tumorigenic roles of the JAK/STAT3 pathway, inhibition of JAK/STAT3 in solid tumours is currently being evaluated (Buchert *et al.*, 2016). Moreover, several inhibitors of the TGF- β pathway are being developed and clinically tested for a number of cancers (Neuzillet *et al.*, 2015). However, the dual role of TGF- β as tumour suppressor or prometastatic (Massague, 2008) anticipates that targeting its transcriptional targets and/or regulators might be a better approach to block only its prometastatic effects.

9. Concluding remarks

Tumour cells usually encounter heterogeneous and discontinuous microenvironments. As a consequence, cancer cells need to adapt spontaneously in response to localized physical and chemical changes. The minimum machinery required to drive all different types of migration comprises the actomyosin cytoskeleton. Differential regulation of actomyosin machinery is what drives plasticity and different modes of migration, blockade of which is essential to prevent cancer invasion and metastasis. Thus, future therapies for preventing metastasis should focus on selective pharmacological inhibition of actomyosin machinery within cancer cells.

Acknowledgements

This work was supported by CRUK C33043/A12065 (VS-M, JLO) and Royal Society RG110591 (VS-M). PP is supported by King's Overseas Scholarship.

References

- Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, Yu M, Pely A, Engstrom A, Zhu H *et al.* (2014) Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell* **158**, 1110–1122.
- Ackerman AB and Ragaz A (1984) *The Lives of Lesions*. Masson Publishers, New York, NY.
- Ahn J, Sanz-Moreno V and Marshall CJ (2012) The metastasis gene NEDD9 product acts through integrin beta3 and Src to promote mesenchymal motility and inhibit amoeboid motility. *J Cell Sci* **125**, 1814–1826.
- Alexander S, Koehl GE, Hirschberg M, Geissler EK and Friedl P (2008) Dynamic imaging of cancer growth and invasion: a modified skin-fold chamber model. *Histochem Cell Biol* **130**, 1147–1154.
- Amann KJ and Pollard TD (2001) The Arp2/3 complex nucleates actin filament branches from the sides of pre-existing filaments. *Nat Cell Biol* **3**, 306–310.
- Amano M, Ito M, Kimura K, Fukata Y, Chihara K, Nakano T, Matsuura Y and Kaibuchi K (1996) Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). *J Biol Chem* **271**, 20246–20249.
- Bell CD and Waizbard E (1986) Variability of cell size in primary and metastatic human breast carcinoma. *Invasion Metastasis* **6**, 11–20.
- Bergert M, Chandradoss SD, Desai RA and Paluch E (2012) Cell mechanics control rapid transitions between blebs and lamellipodia during migration. *Proc Natl Acad Sci USA* **109**, 14434–14439.
- Bergert M, Erzberger A, Desai RA, Aspalter IM, Oates AC, Charras G, Salbreux G and Paluch EK (2015) Force transmission during adhesion-independent migration. *Nat Cell Biol* **17**, 524–529.
- Berton S, Belletti B, Wolf K, Canzonieri V, Lovat F, Vecchione A, Colombatti A, Friedl P and Baldassarre G (2009) The tumor suppressor functions of p27(kip1) include control of the mesenchymal/amoeboid transition. *Mol Cell Biol* **29**, 5031–5045.
- Besson A, Gurian-West M, Schmidt A, Hall A and Roberts JM (2004) p27Kip1 modulates cell migration through the regulation of RhoA activation. *Genes Dev* **18**, 862–876.
- Bovellan M, Romeo Y, Biro M, Boden A, Chugh P, Yonis A, Vaghela M, Fritzsche M, Moulding D, Thorogate R *et al.* (2014) Cellular control of cortical actin nucleation. *Curr Biol* **24**, 1628–1635.
- Brabletz T, Jung A, Reu S, Porzner M, Hlubek F, Kunz-Schughart LA, Knuechel R and Kirchner T (2001) Variable beta-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. *Proc Natl Acad Sci USA* **98**, 10356–10361.
- Brandt B, Junker R, Griwatz C, Heidl S, Brinkmann O, Semjonow A, Assmann G and Zanker KS (1996) Isolation of prostate-derived single cells and cell clusters from human peripheral blood. *Cancer Res* **56**, 4556–4561.
- Bronsart P, Enderle-Ammour K, Bader M, Timme S, Kuehs M, Csanadi A, Kayser G, Kohler I, Bausch D, Hoepfner J *et al.* (2014) Cancer cell invasion and EMT marker expression: a three-dimensional study of the human cancer-host interface. *J Pathol* **234**, 410–422.
- Brown MC, West KA and Turner CE (2002) Paxillin-dependent paxillin kinase linker and p21-activated

- kinase localization to focal adhesions involves a multistep activation pathway. *Mol Biol Cell* **13**, 1550–1565.
- Buchert M, Burns CJ and Ernst M (2016) Targeting JAK kinase in solid tumors: emerging opportunities and challenges. *Oncogene* **35**, 939–951.
- Byers SW, Sommers CL, Hoxter B, Mercurio AM and Tozeren A (1995) Role of E-cadherin in the response of tumor cell aggregates to lymphatic, venous and arterial flow: measurement of cell-cell adhesion strength. *J Cell Sci* **108**(Pt 5), 2053–2064.
- Calvo F, Sanz-Moreno V, Agudo-Ibanez L, Wallberg F, Sahai E, Marshall CJ and Crespo P (2011) RasGRF suppresses Cdc42-mediated tumour cell movement, cytoskeletal dynamics and transformation. *Nat Cell Biol* **13**, 819–826.
- Cantelli G, Orgaz JL, Rodriguez-Hernandez I, Karagiannis P, Maiques O, Matias-Guiu X, Nestle FO, Marti RM, Karagiannis SN and Sanz-Moreno V (2015) TGF-beta-induced transcription sustains amoeboid melanoma migration and dissemination. *Curr Biol* **25**, 2899–2914.
- Carr I, Levy M and Watson P (1986) The invasive edge: invasion in colorectal cancer. *Clin Exp Metastasis* **4**, 129–139.
- Carragher NO, Walker SM, Scott Carragher LA, Harris F, Sawyer TK, Brunton VG, Ozanne BW and Frame MC (2006) Calpain 2 and Src dependence distinguishes mesenchymal and amoeboid modes of tumour cell invasion: a link to integrin function. *Oncogene* **25**, 5726–5740.
- Casey RC, Burleson KM, Skubitz KM, Pambuccian SE, Oegema TR Jr, Ruff LE and Skubitz AP (2001) Beta 1-integrins regulate the formation and adhesion of ovarian carcinoma multicellular spheroids. *Am J Pathol* **159**, 2071–2080.
- Charras G and Paluch E (2008) Blebs lead the way: how to migrate without lamellipodia. *Nat Rev Mol Cell Biol* **9**, 730–736.
- Charras G and Sahai E (2014) Physical influences of the extracellular environment on cell migration. *Nat Rev Mol Cell Biol* **15**, 813–824.
- Christiansen JJ and Rajasekaran AK (2006) Reassessing epithelial to mesenchymal transition as a prerequisite for carcinoma invasion and metastasis. *Cancer Res* **66**, 8319–8326.
- Cianfrocca ME, Kimmel KA, Gallo J, Cardoso T, Brown MM, Hudes G, Lewis N, Weiner L, Lam GN, Brown SC *et al.* (2006) Phase I trial of the antiangiogenic peptide ATN-161 (Ac-PHSCN-NH(2)), a beta integrin antagonist, in patients with solid tumours. *Br J Cancer* **94**, 1621–1626.
- Clark AG and Vignjevic DM (2015) Modes of cancer cell invasion and the role of the microenvironment. *Curr Opin Cell Biol* **36**, 13–22.
- Coussens LM, Fingleton B and Matrisian LM (2002) Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science* **295**, 2387–2392.
- Dafni H, Israely T, Bhujwala ZM, Benjamin LE and Neeman M (2002) Overexpression of vascular endothelial growth factor 165 drives peritumor interstitial convection and induces lymphatic drain: magnetic resonance imaging, confocal microscopy, and histological tracking of triple-labeled albumin. *Cancer Res* **62**, 6731–6739.
- Daly AJ, McIlreavey L and Irwin CR (2008) Regulation of HGF and SDF-1 expression by oral fibroblasts—implications for invasion of oral cancer. *Oral Oncol* **44**, 646–651.
- Dean RA, Cox JH, Bellac CL, Doucet A, Starr AE and Overall CM (2008) Macrophage-specific metalloelastase (MMP-12) truncates and inactivates ELR+ CXC chemokines and generates CCL2, -7, -8, and -13 antagonists: potential role of the macrophage in terminating polymorphonuclear leukocyte influx. *Blood* **112**, 3455–3464.
- Denais CM, Gilbert RM, Isermann P, McGregor AL, te Lindert M, Weigel B, Davidson PM, Friedl P, Wolf K and Lammerding J (2016) Nuclear envelope rupture and repair during cancer cell migration. *Science* **352**, 353–358.
- Derivery E, Fink J, Martin D, Houdusse A, Piel M, Stradal TE, Louvard D and Gautreau A (2008) Free Brick1 is a trimeric precursor in the assembly of a functional wave complex. *PLoS ONE* **3**, e2462.
- Dufour A and Overall CM (2013) Missing the target: matrix metalloproteinase antitargets in inflammation and cancer. *Trends Pharmacol Sci* **34**, 233–242.
- Edwards DC, Sanders LC, Bokoch GM and Gill GN (1999) Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. *Nat Cell Biol* **1**, 253–259.
- Egeblad M and Werb Z (2002) New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* **2**, 161–174.
- Eisenmann KM, Harris ES, Kitchen SM, Holman HA, Higgs HN and Alberts AS (2007) Dia-interacting protein modulates formin-mediated actin assembly at the cell cortex. *Curr Biol* **17**, 579–591.
- Endo A, Surks HK, Mochizuki S, Mochizuki N and Mendelsohn ME (2004) Identification and characterization of zipper-interacting protein kinase as the unique vascular smooth muscle myosin phosphatase-associated kinase. *J Biol Chem* **279**, 42055–42061.
- Fingleton B (2003) Matrix metalloproteinase inhibitors for cancer therapy: the current situation and future prospects. *Expert Opin Ther Targets* **7**, 385–397.
- Friedl P (2004) Prespecification and plasticity: shifting mechanisms of cell migration. *Curr Opin Cell Biol* **16**, 14–23.

- Friedl P and Alexander S (2011) Cancer invasion and the microenvironment: plasticity and reciprocity. *Cell* **147**, 992–1009.
- Friedl P and Gilmour D (2009) Collective cell migration in morphogenesis, regeneration and cancer. *Nat Rev Mol Cell Biol* **10**, 445–457.
- Friedl P, Hegerfeldt Y and Tusch M (2004) Collective cell migration in morphogenesis and cancer. *Int J Dev Biol* **48**, 441–449.
- Friedl P, Locker J, Sahai E and Segall JE (2012) Classifying collective cancer cell invasion. *Nat Cell Biol* **14**, 777–783.
- Friedl P, Maaser K, Klein CE, Niggemann B, Krohne G and Zanker KS (1997) Migration of highly aggressive MV3 melanoma cells in 3-dimensional collagen lattices results in local matrix reorganization and shedding of alpha2 and beta1 integrins and CD44. *Cancer Res* **57**, 2061–2070.
- Friedl P, Noble PB, Walton PA, Laird DW, Chauvin PJ, Tabah RJ, Black M and Zanker KS (1995) Migration of coordinated cell clusters in mesenchymal and epithelial cancer explants in vitro. *Cancer Res* **55**, 4557–4560.
- Friedl P and Wolf K (2003) Tumour-cell invasion and migration: diversity and escape mechanisms. *Nat Rev Cancer* **3**, 362–374.
- Friedl P and Wolf K (2008) Tube travel: the role of proteases in individual and collective cancer cell invasion. *Cancer Res* **68**, 7247–7249.
- Friedl P and Wolf K (2009) Proteolytic interstitial cell migration: a five-step process. *Cancer Metastasis Rev* **28**, 129–135.
- Friedl P and Wolf K (2010) Plasticity of cell migration: a multiscale tuning model. *J Cell Biol* **188**, 11–19.
- Gadea G, de Toledo M, Anguille C and Roux P (2007) Loss of p53 promotes RhoA-ROCK-dependent cell migration and invasion in 3D matrices. *J Cell Biol* **178**, 23–30.
- Gadea G, Sanz-Moreno V, Self A, Godi A and Marshall CJ (2008) DOCK10-mediated Cdc42 activation is necessary for amoeboid invasion of melanoma cells. *Curr Biol* **18**, 1456–1465.
- Gaggioli C, Hooper S, Hidalgo-Carcedo C, Grosse R, Marshall JF, Harrington K and Sahai E (2007) Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. *Nat Cell Biol* **9**, 1392–1400.
- Gavert N, Sheffer M, Raveh S, Spaderna S, Shtutman M, Brabletz T, Barany F, Paty P, Notterman D, Domany E *et al.* (2007) Expression of L1-CAM and ADAM10 in human colon cancer cells induces metastasis. *Cancer Res* **67**, 7703–7712.
- Gavert N, Vivanti A, Hazin J, Brabletz T and Ben-Ze'ev A (2011) L1-mediated colon cancer cell metastasis does not require changes in EMT and cancer stem cell markers. *Mol Cancer Res* **9**, 14–24.
- Geiger TR and Peeper DS (2009) Metastasis mechanisms. *Biochim Biophys Acta* **1796**, 293–308.
- Giampieri S, Manning C, Hooper S, Jones L, Hill CS and Sahai E (2009) Localized and reversible TGFbeta signalling switches breast cancer cells from cohesive to single cell motility. *Nat Cell Biol* **11**, 1287–1296.
- Giri A, Bajpai S, Trenton N, Jayatilaka H, Longmore GD and Wirtz D (2013) The Arp2/3 complex mediates multigeneration dendritic protrusions for efficient 3-dimensional cancer cell migration. *FASEB J* **27**, 4089–4099.
- Grashoff C, Hoffman BD, Brenner MD, Zhou R, Parsons M, Yang MT, McLean MA, Sligar SG, Chen CS, Ha T *et al.* (2010) Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. *Nature* **466**, 263–266.
- Green RA, Paluch E and Oegema K (2012) Cytokinesis in animal cells. *Annu Rev Cell Dev Biol* **28**, 29–58.
- Grigore AD, Jolly MK, Jia D, Farach-Carson MC and Levine H (2016) Tumor budding: the name is EMT. Partial EMT. *J Clin Med* **5**, 1–23.
- Haeger A, Krause M, Wolf K and Friedl P (2014) Cell jamming: collective invasion of mesenchymal tumor cells imposed by tissue confinement. *Biochim Biophys Acta* **1840**, 2386–2395.
- Haeger A, Wolf K, Zegers MM and Friedl P (2015) Collective cell migration: guidance principles and hierarchies. *Trends Cell Biol* **25**, 556–566.
- Haessler U, Teo JC, Foretay D, Renaud P and Swartz MA (2012) Migration dynamics of breast cancer cells in a tunable 3D interstitial flow chamber. *Integr Biol (Camb)* **4**, 401–409.
- Hart IR (2009) New evidence for tumour embolism as a mode of metastasis. *J Pathol* **219**, 275–276.
- Hashizume R, Koizumi H, Ihara A, Ohta T and Uchikoshi T (1996) Expression of beta-catenin in normal breast tissue and breast carcinoma: a comparative study with epithelial cadherin and alpha-catenin. *Histopathology* **29**, 139–146.
- Heasman SJ and Ridley AJ (2008) Mammalian Rho GTPases: new insights into their functions from in vivo studies. *Nat Rev Mol Cell Biol* **9**, 690–701.
- Hegerfeldt Y, Tusch M, Bocker EB and Friedl P (2002) Collective cell movement in primary melanoma explants: plasticity of cell-cell interaction, beta1-integrin function, and migration strategies. *Cancer Res* **62**, 2125–2130.
- Herraiz C, Calvo F, Pandya P, Cantelli G, Rodriguez-Hernandez I, Orgaz JL, Kang N, Chu T, Sahai E and Sanz-Moreno V (2016) Reactivation of p53 by a cytoskeletal sensor to control the balance between DNA damage and tumor dissemination. *J Natl Cancer Inst* **108**, 1–14.

- Hidalgo-Carcedo C, Hooper S, Chaudhry SI, Williamson P, Harrington K, Leitinger B and Sahai E (2011) Collective cell migration requires suppression of actomyosin at cell-cell contacts mediated by DDR1 and the cell polarity regulators Par3 and Par6. *Nat Cell Biol* **13**, 49–58.
- Hirata N, Takahashi M and Yazawa M (2009) Diphosphorylation of regulatory light chain of myosin IIA is responsible for proper cell spreading. *Biochem Biophys Res Commun* **381**, 682–687.
- Hodivala-Dilke KM, McHugh KP, Tsakiris DA, Rayburn H, Crowley D, Ullman-Cullere M, Ross FP, Collier BS, Teitelbaum S and Hynes RO (1999) Beta3-integrin-deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival. *J Clin Invest* **103**, 229–238.
- Hooper S, Marshall JF and Sahai E (2006) Tumor cell migration in three dimensions. *Methods Enzymol* **406**, 625–643.
- Hou JM, Krebs M, Ward T, Sloane R, Priest L, Hughes A, Clack G, Ranson M, Blackhall F and Dive C (2011) Circulating tumor cells as a window on metastasis biology in lung cancer. *Am J Pathol* **178**, 989–996.
- Huang YL, Tung CK, Zheng A, Kim BJ and Wu M (2015) Interstitial flows promote amoeboid over mesenchymal motility of breast cancer cells revealed by a three dimensional microfluidic model. *Integr Biol (Camb)* **7**, 1402–1411.
- Hynes RO (2002) Integrins: bidirectional, allosteric signaling machines. *Cell* **110**, 673–687.
- Kalluri R (2009) EMT: when epithelial cells decide to become mesenchymal-like cells. *J Clin Invest* **119**, 1417–1419.
- Kats-Ugurlu G, Roodink I, de Weijert M, Tiemessen D, Maass C, Verrijp K, van der Laak J, de Waal R, Mulders P, Oosterwijk E *et al.* (2009) Circulating tumour tissue fragments in patients with pulmonary metastasis of clear cell renal cell carcinoma. *J Pathol* **219**, 287–293.
- Kedrin D, Gligorijevic B, Wyckoff J, Verkhusha VV, Condeelis J, Segall JE and van Rheenen J (2008) Intravital imaging of metastatic behavior through a mammary imaging window. *Nat Methods* **5**, 1019–1021.
- Kenny HA, Kaur S, Coussens LM and Lengyel E (2008) The initial steps of ovarian cancer cell metastasis are mediated by MMP-2 cleavage of vitronectin and fibronectin. *J Clin Invest* **118**, 1367–1379.
- Khalil AA and Friedl P (2010) Determinants of leader cells in collective cell migration. *Integr Biol (Camb)* **2**, 568–574.
- Khoja L, Shenjere P, Hodgson C, Hodgetts J, Clack G, Hughes A, Lorigan P and Dive C (2014) Prevalence and heterogeneity of circulating tumour cells in metastatic cutaneous melanoma. *Melanoma Res* **24**, 40–46.
- Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng J, Nakano T, Okawa K *et al.* (1996) Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science* **273**, 245–248.
- Kitzing TM, Wang Y, Pertz O, Copeland JW and Grosse R (2010) Formin-like 2 drives amoeboid invasive cell motility downstream of RhoC. *Oncogene* **29**, 2441–2448.
- Kiyokawa E, Hashimoto Y, Kobayashi S, Sugimura H, Kurata T and Matsuda M (1998) Activation of Rac1 by a Crk SH3-binding protein, DOCK180. *Genes Dev* **12**, 3331–3336.
- Kurniawan NA, Chaudhuri PK and Lim CT (2016) Mechanobiology of cell migration in the context of dynamic two-way cell-matrix interactions. *J Biomech* **49**, 1355–1368.
- Lammerding J, Fong LG, Ji JY, Reue K, Stewart CL, Young SG and Lee RT (2006) Lamins A and C but not lamin B1 regulate nuclear mechanics. *J Biol Chem* **281**, 25768–25780.
- Lammerding J, Schulze PC, Takahashi T, Kozlov S, Sullivan T, Kamm RD, Stewart CL and Lee RT (2004) Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. *J Clin Invest* **113**, 370–378.
- Langridge PD and Kay RR (2006) Blebbing of Dictyostelium cells in response to chemoattractant. *Exp Cell Res* **312**, 2009–2017.
- Laser-Azogui A, Diamant-Levi T, Israeli S, Roytman Y and Tsarfay I (2014) Met-induced membrane blebbing leads to amoeboid cell motility and invasion. *Oncogene* **33**, 1788–1798.
- Lauffenburger DA and Horwitz AF (1996) Cell migration: a physically integrated molecular process. *Cell* **84**, 359–369.
- Law AL, Vehlow A, Kotini M, Dodgson L, Soong D, Theveneau E, Bodo C, Taylor E, Navarro C, Perera U *et al.* (2013) Lamellipodin and the Scar/WAVE complex cooperate to promote cell migration in vivo. *J Cell Biol* **203**, 673–689.
- Lee HS, Lim CJ, Puzon-McLaughlin W, Shattil SJ and Ginsberg MH (2009) RIAM activates integrins by linking talin to ras GTPase membrane-targeting sequences. *J Biol Chem* **284**, 5119–5127.
- Leighton J, Kalla RL, Turner JM and Fennell RH Jr (1960) Pathogenesis of tumor invasion. II. Aggregate replication. *Cancer Res* **20**, 575–586.
- Leung T, Chen XQ, Tan I, Manser E and Lim L (1998) Myotonic dystrophy kinase-related Cdc42-binding kinase acts as a Cdc42 effector in promoting cytoskeletal reorganization. *Mol Cell Biol* **18**, 130–140.
- Liu YJ, Le Berre M, Lautenschlaeger F, Maiuri P, Callan-Jones A, Heuze M, Takaki T, Voituriez R and Piel M (2015) Confinement and low adhesion induce fast

- amoeboid migration of slow mesenchymal cells. *Cell* **160**, 659–672.
- Lopez-Otin C and Matrisian LM (2007) Emerging roles of proteases in tumour suppression. *Nat Rev Cancer* **7**, 800–808.
- Lorentzen A, Bamber J, Sadok A, Elson-Schwab I and Marshall CJ (2011) An ezrin-rich, rigid uropod-like structure directs movement of amoeboid blebbing cells. *J Cell Sci* **124**, 1256–1267.
- Machesky LM, Mullins RD, Higgs HN, Kaiser DA, Blanchoin L, May RC, Hall ME and Pollard TD (1999) Scar, a WASp-related protein, activates nucleation of actin filaments by the Arp2/3 complex. *Proc Natl Acad Sci USA* **96**, 3739–3744.
- Madaule P, Eda M, Watanabe N, Fujisawa K, Matsuoka T, Bito H, Ishizaki T and Narumiya S (1998) Role of citron kinase as a target of the small GTPase Rho in cytokinesis. *Nature* **394**, 491–494.
- Madhavan M, Srinivas P, Abraham E, Ahmed I, Mathew A, Vijayalekshmi NR and Balaram P (2001) Cadherins as predictive markers of nodal metastasis in breast cancer. *Mod Pathol* **14**, 423–427.
- Maekawa M, Ishizaki T, Boku S, Watanabe N, Fujita A, Iwamatsu A, Obinata T, Ohashi K, Mizuno K and Narumiya S (1999) Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. *Science* **285**, 895–898.
- Manning CS, Hooper S and Sahai EA (2015) Intravital imaging of SRF and Notch signalling identifies a key role for EZH2 in invasive melanoma cells. *Oncogene* **34**, 4320–4332.
- Mantovani A, Allavena P, Sica A and Balkwill F (2008) Cancer-related inflammation. *Nature* **454**, 436–444.
- Martin-Villar E, Megias D, Castel S, Yurrita MM, Vilaro S and Quintanilla M (2006) Podoplanin binds ERM proteins to activate RhoA and promote epithelial-mesenchymal transition. *J Cell Sci* **119**, 4541–4553.
- Mason SD and Joyce JA (2011) Proteolytic networks in cancer. *Trends Cell Biol* **21**, 228–237.
- Massague J (2008) TGFbeta in cancer. *Cell* **134**, 215–230.
- Matsui T, Maeda M, Doi Y, Yonemura S, Amano M, Kaibuchi K, Tsukita S and Tsukita S (1998) Rho-kinase phosphorylates COOH-terminal threonines of ezrin/radixin/moesin (ERM) proteins and regulates their head-to-tail association. *J Cell Biol* **140**, 647–657.
- Medjkane S, Perez-Sanchez C, Gaggioli C, Sahai E and Treisman R (2009) Myocardin-related transcription factors and SRF are required for cytoskeletal dynamics and experimental metastasis. *Nat Cell Biol* **11**, 257–268.
- Mierke CT (2015) Physical view on migration modes. *Cell Adh Migr* **9**, 367–379.
- Mu D, Cambier S, Fjellbirkeland L, Baron JL, Munger JS, Kawakatsu H, Sheppard D, Broaddus VC and Nishimura SL (2002) The integrin alpha(v)beta8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF-beta1. *J Cell Biol* **157**, 493–507.
- Murrell M, Oakes PW, Lenz M and Gardel ML (2015) Forcing cells into shape: the mechanics of actomyosin contractility. *Nat Rev Mol Cell Biol* **16**, 486–498.
- Nabeshima K, Inoue T, Shimao Y, Kataoka H and Koono M (1999) Cohort migration of carcinoma cells: differentiated colorectal carcinoma cells move as coherent cell clusters or sheets. *Histol Histopathol* **14**, 1183–1197.
- Nabeshima K, Inoue T, Shimao Y, Okada Y, Itoh Y, Seiki M and Koono M (2000) Front-cell-specific expression of membrane-type 1 matrix metalloproteinase and gelatinase A during cohort migration of colon carcinoma cells induced by hepatocyte growth factor/scatter factor. *Cancer Res* **60**, 3364–3369.
- Nakamura N, Oshiro N, Fukata Y, Amano M, Fukata M, Kuroda S, Matsuura Y, Leung T, Lim L and Kaibuchi K (2000) Phosphorylation of ERM proteins at filopodia induced by Cdc42. *Genes Cells* **5**, 571–581.
- Nalbant P, Hodgson L, Kravnov V, Touthkine A and Hahn KM (2004) Activation of endogenous Cdc42 visualized in living cells. *Science* **305**, 1615–1619.
- Nayal A, Webb DJ, Brown CM, Schaefer EM, Vicente-Manzanares M and Horwitz AR (2006) Paxillin phosphorylation at Ser273 localizes a GIT1-PIX-PAK complex and regulates adhesion and protrusion dynamics. *J Cell Biol* **173**, 587–589.
- Neuhaus B, Buhren S, Bock B, Alves F, Vogel WF and Kiefer F (2011) Migration inhibition of mammary epithelial cells by Syk is blocked in the presence of DDR1 receptors. *Cell Mol Life Sci* **68**, 3757–3770.
- Neuzillet C, Tijeras-Raballand A, Cohen R, Cros J, Faivre S, Raymond E and de Gramont A (2015) Targeting the TGFbeta pathway for cancer therapy. *Pharmacol Ther* **147**, 22–31.
- Ohashi K, Nagata K, Maekawa M, Ishizaki T, Narumiya S and Mizuno K (2000) Rho-associated kinase ROCK activates LIM-kinase 1 by phosphorylation at threonine 508 within the activation loop. *J Biol Chem* **275**, 3577–3582.
- Olson EN and Nordheim A (2010) Linking actin dynamics and gene transcription to drive cellular motile functions. *Nat Rev Mol Cell Biol* **11**, 353–365.
- Orgaz JL, Pandya P, Dalmeida R, Karagiannis P, Sanchez-Laorden B, Viros A, Albregues J, Nestle FO, Ridley AJ, Gaggioli C *et al.* (2014) Diverse matrix metalloproteinase functions regulate cancer amoeboid migration. *Nat Commun* **5**, 4255.
- Osmani N, Peglion F, Chavrier P and Etienne-Manneville S (2010) Cdc42 localization and cell polarity depend on membrane traffic. *J Cell Biol* **191**, 1261–1269.
- Otomo T, Tomchick DR, Otomo C, Panchal SC, Machius M and Rosen MK (2005) Structural basis of actin

- filament nucleation and processive capping by a formin homology 2 domain. *Nature* **433**, 488–494.
- Overall CM and Kleinfeld O (2006) Towards third generation matrix metalloproteinase inhibitors for cancer therapy. *Br J Cancer* **94**, 941–946.
- Overall CM and Lopez-Otin C (2002) Strategies for MMP inhibition in cancer: innovations for the post-trial era. *Nat Rev Cancer* **2**, 657–672.
- Page DL and Anderson TJ (1987) Diagnostic Histopathology of the Breast. Churchill-Livingstone, Edinburgh, UK.
- Palamidessi A, Frittoli E, Garre M, Faretta M, Mione M, Testa I, Diaspro A, Lanzetti L, Scita G and Di Fiore PP (2008) Endocytic trafficking of Rac is required for the spatial restriction of signaling in cell migration. *Cell* **134**, 135–147.
- Pankova K, Rosel D, Novotny M and Brabek J (2010) The molecular mechanisms of transition between mesenchymal and amoeboid invasiveness in tumor cells. *Cell Mol Life Sci* **67**, 63–71.
- Parri M, Taddei ML, Bianchini F, Calorini L and Chiarugi P (2009) EphA2 reexpression prompts invasion of melanoma cells shifting from mesenchymal to amoeboid-like motility style. *Cancer Res* **69**, 2072–2081.
- Patsialou A, Bravo-Cordero JJ, Wang Y, Entenberg D, Liu H, Clarke M and Condeelis JS (2013) Intravital multiphoton imaging reveals multicellular streaming as a crucial component of in vivo cell migration in human breast tumors. *Intravital* **2**, e25294.
- Paul NR, Allen JL, Chapman A, Morlan-Mairal M, Zindy E, Jacquemet G, Fernandez del Ama L, Ferizovic N, Green DM, Howe JD *et al.* (2015) alpha5beta1 integrin recycling promotes Arp2/3-independent cancer cell invasion via the formin FHOD3. *J Cell Biol* **210**, 1013–1031.
- Paulus W, Baur I, Beutler AS and Reeves SA (1996) Diffuse brain invasion of glioma cells requires beta 1 integrins. *Lab Invest* **75**, 819–826.
- Petrie RJ, Gavara N, Chadwick RS and Yamada KM (2012) Nonpolarized signaling reveals two distinct modes of 3D cell migration. *J Cell Biol* **197**, 439–455.
- Petrie RJ, Koo H and Yamada KM (2014) Generation of compartmentalized pressure by a nuclear piston governs cell motility in a 3D matrix. *Science* **345**, 1062–1065.
- Pinner S and Sahai E (2008a) Imaging amoeboid cancer cell motility in vivo. *J Microsc* **231**, 441–445.
- Pinner S and Sahai E (2008b) PDK1 regulates cancer cell motility by antagonising inhibition of ROCK1 by RhoE. *Nat Cell Biol* **10**, 127–137.
- Pitts WC, Rojas VA, Gaffey MJ, Rouse RV, Esteban J, Frierson HF, Kempson RL and Weiss LM (1991) Carcinomas with metaplasia and sarcomas of the breast. *Am J Clin Pathol* **95**, 623–632.
- Polette M, Gilles C, de Bentzmann S, Gruenert D, Tournier JM and Birembaut P (1998) Association of fibroblastoid features with the invasive phenotype in human bronchial cancer cell lines. *Clin Exp Metastasis* **16**, 105–112.
- Poperechnaya A, Varlamova O, Lin PJ, Stull JT and Bresnick AR (2000) Localization and activity of myosin light chain kinase isoforms during the cell cycle. *J Cell Biol* **151**, 697–708.
- Prall F, Nizze H and Barten M (2005) Tumour budding as prognostic factor in stage I/II colorectal carcinoma. *Histopathology* **47**, 17–24.
- Premont RT, Perry SJ, Schmalzigaug R, Roseman JT, Xing Y and Claing A (2004) The GIT/PIX complex: an oligomeric assembly of GIT family ARF GTPase-activating proteins and PIX family Rac1/Cdc42 guanine nucleotide exchange factors. *Cell Signal* **16**, 1001–1011.
- Provenzano PP, Inman DR, Eliceiri KW, Trier SM and Keely PJ (2008) Contact guidance mediated three-dimensional cell migration is regulated by Rho/ROCK-dependent matrix reorganization. *Biophys J* **95**, 5374–5384.
- Raab M, Gentili M, de Belly H, Thiam HR, Vargas P, Jimenez AJ, Lautenschlaeger F, Voituriez R, Lennon-Dumenil AM, Manel N *et al.* (2016) ESCRT III repairs nuclear envelope ruptures during cell migration to limit DNA damage and cell death. *Science* **352**, 359–362.
- Ricart AD, Tolcher AW, Liu G, Holen K, Schwartz G, Albertini M, Weiss G, Yazji S, Ng C and Wilding G (2008) Volociximab, a chimeric monoclonal antibody that specifically binds alpha5beta1 integrin: a phase I, pharmacokinetic, and biological correlative study. *Clin Cancer Res* **14**, 7924–7929.
- Richardson BE and Lehmann R (2010) Mechanisms guiding primordial germ cell migration: strategies from different organisms. *Nat Rev Mol Cell Biol* **11**, 37–49.
- Ridley AJ (2015) Rho GTPase signalling in cell migration. *Curr Opin Cell Biol* **36**, 103–112.
- Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G, Parsons JT and Horwitz AR (2003) Cell migration: integrating signals from front to back. *Science* **302**, 1704–1709.
- Rizki A, Weaver VM, Lee SY, Rozenberg GI, Chin K, Myers CA, Bascom JL, Mott JD, Semeiks JR, Grate LR *et al.* (2008) A human breast cell model of preinvasive to invasive transition. *Cancer Res* **68**, 1378–1387.
- Rorth P (2007) Collective guidance of collective cell migration. *Trends Cell Biol* **17**, 575–579.
- Roussos ET, Balsamo M, Alford SK, Wyckoff JB, Gligorijevic B, Wang Y, Pozzuto M, Stobezki R, Goswami S, Segall JE *et al.* (2011) Mena invasive (MenaINV) promotes multicellular streaming motility

- and transendothelial migration in a mouse model of breast cancer. *J Cell Sci* **124**, 2120–2131.
- Ruiz-Ontanon P, Orgaz JL, Aldaz B, Elosegui-Artola A, Martino J, Berciano MT, Montero JA, Grande L, Nogueira L, Diaz-Moralli S *et al.* (2013) Cellular plasticity confers migratory and invasive advantages to a population of glioblastoma-initiating cells that infiltrate peritumoral tissue. *Stem Cells* **31**, 1075–1085.
- Sadati M, Taheri Qazvini N, Krishnan R, Park CY and Fredberg JJ (2013) Collective migration and cell jamming. *Differentiation* **86**, 121–125.
- Sadok A, McCarthy A, Caldwell J, Collins I, Garrett MD, Yeo M, Hooper S, Sahai E, Kuemper S, Mardakheh FK *et al.* (2015) Rho kinase inhibitors block melanoma cell migration and inhibit metastasis. *Cancer Res* **75**, 2272–2284.
- Sahai E (2005) Mechanisms of cancer cell invasion. *Curr Opin Genet Dev* **15**, 87–96.
- Sahai E, Garcia-Medina R, Pouyssegur J and Vial E (2007) Smurf1 regulates tumor cell plasticity and motility through degradation of RhoA leading to localized inhibition of contractility. *J Cell Biol* **176**, 35–42.
- Sahai E and Marshall CJ (2003) Differing modes of tumour cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis. *Nat Cell Biol* **5**, 711–719.
- Saito K, Ozawa Y, Hibino K and Ohta Y (2012) FilGAP, a Rho/Rho-associated protein kinase-regulated GTPase-activating protein for Rac, controls tumor cell migration. *Mol Biol Cell* **23**, 4739–4750.
- Salbreux G, Charras G and Paluch E (2012) Actin cortex mechanics and cellular morphogenesis. *Trends Cell Biol* **22**, 536–545.
- Sanz-Moreno V, Gadea G, Ahn J, Paterson H, Marra P, Pinner S, Sahai E and Marshall CJ (2008) Rac activation and inactivation control plasticity of tumor cell movement. *Cell* **135**, 510–523.
- Sanz-Moreno V, Gaggioli C, Yeo M, Albregues J, Wallberg F, Viros A, Hooper S, Mitter R, Feral CC, Cook M *et al.* (2011) ROCK and JAK1 signaling cooperate to control actomyosin contractility in tumor cells and stroma. *Cancer Cell* **20**, 229–245.
- Sanz-Moreno V and Marshall CJ (2010) The plasticity of cytoskeletal dynamics underlying neoplastic cell migration. *Curr Opin Cell Biol* **22**, 690–696.
- Scholey JM, Taylor KA and Kendrick-Jones J (1980) Regulation of non-muscle myosin assembly by calmodulin-dependent light chain kinase. *Nature* **287**, 233–235.
- Scott RW and Olson MF (2007) LIM kinases: function, regulation and association with human disease. *J Mol Med (Berl)* **85**, 555–568.
- Seftor EA, Meltzer PS, Kirschmann DA, Pe'er J, Maniatis AJ, Trent JM, Folberg R and Hendrix MJ (2002) Molecular determinants of human uveal melanoma invasion and metastasis. *Clin Exp Metastasis* **19**, 233–246.
- Shiao SL and Coussens LM (2010) The tumor-immune microenvironment and response to radiation therapy. *J Mammary Gland Biol Neoplasia* **15**, 411–421.
- Shieh AC and Swartz MA (2011) Regulation of tumor invasion by interstitial fluid flow. *Phys Biol* **8**, 015012.
- Shtutman M, Levina E, Ohouo P, Baig M and Roninson IB (2006) Cell adhesion molecule L1 disrupts E-cadherin-containing adherens junctions and increases scattering and motility of MCF7 breast carcinoma cells. *Cancer Res* **66**, 11370–11380.
- Sood AK, Seftor EA, Fletcher MS, Gardner LM, Heidger PM, Buller RE, Seftor RE and Hendrix MJ (2001) Molecular determinants of ovarian cancer plasticity. *Am J Pathol* **158**, 1279–1288.
- Sounni NE, Dehne K, van Kempen L, Egeblad M, Affara NI, Cuevas I, Wiesen J, Junankar S, Korets L, Lee J *et al.* (2010) Stromal regulation of vessel stability by MMP14 and TGFbeta. *Dis Model Mech* **3**, 317–332.
- Starke J, Wehrle-Haller B and Friedl P (2014) Plasticity of the actin cytoskeleton in response to extracellular matrix nanostructure and dimensionality. *Biochem Soc Trans* **42**, 1356–1366.
- Sternlicht MD, Bissell MJ and Werb Z (2000) The matrix metalloproteinase stromelysin-1 acts as a natural mammary tumor promoter. *Oncogene* **19**, 1102–1113.
- Stroka KM, Jiang H, Chen SH, Tong Z, Wirtz D, Sun SX and Konstantopoulos K (2014) Water permeation drives tumor cell migration in confined microenvironments. *Cell* **157**, 611–623.
- Sumi T, Matsumoto K, Takai Y and Nakamura T (1999) Cofilin phosphorylation and actin cytoskeletal dynamics regulated by rho- and Cdc42-activated LIM-kinase 2. *J Cell Biol* **147**, 1519–1532.
- Tambe DT, Hardin CC, Angelini TE, Rajendran K, Park CY, Serra-Picamal X, Zhou EH, Zaman MH, Butler JP, Weitz DA *et al.* (2011) Collective cell guidance by cooperative intercellular forces. *Nat Mater* **10**, 469–475.
- Tan I, Ng CH, Lim L and Leung T (2001a) Phosphorylation of a novel myosin binding subunit of protein phosphatase 1 reveals a conserved mechanism in the regulation of actin cytoskeleton. *J Biol Chem* **276**, 21209–21216.
- Tan I, Seow KT, Lim L and Leung T (2001b) Intermolecular and intramolecular interactions regulate catalytic activity of myotonic dystrophy kinase-related Cdc42-binding kinase alpha. *Mol Cell Biol* **21**, 2767–2778.
- Thiery JP, Acloque H, Huang RY and Nieto MA (2009) Epithelial-mesenchymal transitions in development and disease. *Cell* **139**, 871–890.
- Thundimadathil J (2012) Cancer treatment using peptides: current therapies and future prospects. *J Amino Acids* **2012**, 967347.

- Tozluoglu M, Tournier AL, Jenkins RP, Hooper S, Bates PA and Sahai E (2013) Matrix geometry determines optimal cancer cell migration strategy and modulates response to interventions. *Nat Cell Biol* **15**, 751–762.
- Umamoto S, Bengur AR and Sellers JR (1989) Effect of multiple phosphorylations of smooth muscle and cytoplasmic myosins on movement in an in vitro motility assay. *J Biol Chem* **264**, 1431–1436.
- Vedula SR, Leong MC, Lai TL, Hersen P, Kabla AJ, Lim CT and Ladoux B (2012) Emerging modes of collective cell migration induced by geometrical constraints. *Proc Natl Acad Sci USA* **109**, 12974–12979.
- Vicente-Manzanares M, Ma X, Adelstein RS and Horwitz AR (2009) Non-muscle myosin II takes centre stage in cell adhesion and migration. *Nat Rev Mol Cell Biol* **10**, 778–790.
- Vogel WF, Abdulhussein R and Ford CE (2006) Sensing extracellular matrix: an update on discoidin domain receptor function. *Cell Signal* **18**, 1108–1116.
- Wang X, Enomoto A, Asai N, Kato T and Takahashi M (2016) Collective invasion of cancer: perspectives from pathology and development. *Pathol Int* **66**, 183–192.
- Wang F, Kovacs M, Hu A, Limouze J, Harvey EV and Sellers JR (2003) Kinetic mechanism of non-muscle myosin IIB: functional adaptations for tension generation and maintenance. *J Biol Chem* **278**, 27439–27448.
- Weigelin B, Bakker G and Friedl P (2012) Intravital third harmonic generation microscopy of collective melanoma cell invasion. *Intravital* **1**, 32–43.
- Wicki A, Lehembre F, Wick N, Hantusch B, Kerjaschki D and Christofori G (2006) Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton. *Cancer Cell* **9**, 261–272.
- Wilkinson S, Paterson HF and Marshall CJ (2005) Cdc42-MRCK and Rho-ROCK signalling cooperate in myosin phosphorylation and cell invasion. *Nat Cell Biol* **7**, 255–261.
- Willis R (1952) *The Spread of Tumors in the Human Body*. Butterworth, London.
- Wojciak-Stothard B and Leiper J (2008) Rho GTPases and hypoxia in pulmonary vascular endothelial cells. *Methods Enzymol* **439**, 267–283.
- Wolf K and Friedl P (2011) Extracellular matrix determinants of proteolytic and non-proteolytic cell migration. *Trends Cell Biol* **21**, 736–744.
- Wolf K, Mazo I, Leung H, Engelke K, von Andrian UH, Deryugina EI, Strongin AY, Bocker EB and Friedl P (2003) Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis. *J Cell Biol* **160**, 267–277.
- Wolf K, Te Lindert M, Krause M, Alexander S, Te Riet J, Willis AL, Hoffman RM, Figdor CG, Weiss SJ and Friedl P (2013) Physical limits of cell migration: control by ECM space and nuclear deformation and tuning by proteolysis and traction force. *J Cell Biol* **201**, 1069–1084.
- Wolf K, Wu YI, Liu Y, Geiger J, Tam E, Overall C, Stack MS and Friedl P (2007) Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion. *Nat Cell Biol* **9**, 893–904.
- Wong HS, Jaumouille V, Heit B, Doodnauth SA, Patel S, Huang YW, Grinstein S and Robinson LA (2014) Cytoskeletal confinement of CX3CL1 limits its susceptibility to proteolytic cleavage by ADAM10. *Mol Biol Cell* **25**, 3884–3899.
- Wyckoff JB, Pinner SE, Gschmeissner S, Condeelis JS and Sahai E (2006) ROCK- and myosin-dependent matrix deformation enables protease-independent tumor-cell invasion in vivo. *Curr Biol* **16**, 1515–1523.
- Wyckoff J, Wang W, Lin EY, Wang Y, Pixley F, Stanley ER, Graf T, Pollard JW, Segall J and Condeelis J (2004) A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. *Cancer Res* **64**, 7022–7029.
- Yamazaki D, Kurisu S and Takenawa T (2009) Involvement of Rac and Rho signaling in cancer cell motility in 3D substrates. *Oncogene* **28**, 1570–1583.
- Yang N, Higuchi O, Ohashi K, Nagata K, Wada A, Kangawa K, Nishida E and Mizuno K (1998) Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. *Nature* **393**, 809–812.
- Yano H, Mazaki Y, Kurokawa K, Hanks SK, Matsuda M and Sabe H (2004) Roles played by a subset of integrin signaling molecules in cadherin-based cell-cell adhesion. *J Cell Biol* **166**, 283–295.
- Yin Z, Sadok A, Sailem H, McCarthy A, Xia X, Li F, Garcia MA, Evans L, Barr AR, Perrimon N *et al.* (2013) A screen for morphological complexity identifies regulators of switch-like transitions between discrete cell shapes. *Nat Cell Biol* **15**, 860–871.
- Zoller M (2011) CD44: can a cancer-initiating cell profit from an abundantly expressed molecule? *Nat Rev Cancer* **11**, 254–267.
- Zucker S, Cao J and Chen WT (2000) Critical appraisal of the use of matrix metalloproteinase inhibitors in cancer treatment. *Oncogene* **19**, 6642–6650.