Am J Transl Res 2019;11(9):5301-5312
www.ajtr.org /ISSN:1943-8141/AJTR0098753

Review Article

An emerging tumor invasion mechanism about the collective cell migration

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Received June 20, 2019; Accepted September 1, 2019; Epub September 15, 2019; Published September 30, 2019

Abstract: Traditionally, the metastasis has been detected in the late stage of the cancer, which mostly leads to death. The classical opinion about tumor metastasis is that tumor cell migration begins with the single tumor cell and goes through a series of complicated procedures, and lastly arrives and survives at distant tissues and organs. However, emerging studies have found a new migration mechanism called collective cell migration in many cancers. The collective cell migration could move as clusters with the tight cell-cell junction in the tumor microenvironments, toward the traction established by the leader cells. In addition, the collective cell migration has been shown to have higher invasive capacity and higher resistance to the clinical treatments than the single tumor cell migration. Interestingly, the collective clusters of tumor cells have been detected in the early stage of the cancer patient, which has led to the understanding of the significance of early cancer screenings. Here, we reviewed the major principles and guidance of the collective cell migration mechanisms, and the specific manifestations in the different tumors such as breast cancer and lung cancer.

Keywords: Invasion, migration, metastasis, collective cell migration

Introduction

Metastases are responsible for advanced cancers, which are usually connected with the worse clinical outcomes. The whole process is complex, multi-step but inefficient, including the escape of primary cancer cells into the circulatory system, and finally colonization and proliferation in the distant organs [1]. The traditional opinions demonstrated that the cancer invasion began with the single colonial growth tumor cell from the primary tumor, which has comprised the foundation of the tumor transmission models, such as epithelial-mesenchymal transition (EMT) and migratory cancer stem cells [2] (**Figure 1**). However, there are emerging evidences found in many types of cancers, like breast cancer, lung cancer, and mesenchymal tumors, whose metastases can also be seeded as large, cohesive cohorts of cells clustered into adjacent tissues. The first report of collective cell clusters was in 1950s, which found that the blood sample of cancer patient contained both individual and collective tumor cells [3]. Some studies have also been reported that the tumor clusters could travel more efficiently [1], and circulating tumor cells (CTC) clusters had significance of clinical outcomes [1]. Recent studies have suggested that collective cell migration revealed worse clinical outcomes than single cells.

Collective cell migration is a fundamental process which is a coordinated movement of group cells that maintain connected via cell-cell junctions [4-6]. This process has often been observed in the epithelial regeneration and the formation, and reshaping of large tissue structures during the embryonic development period, such as angiogenic sprouting and neural crest cell streaming [4, 7, 8]. Nowadays collective cell migration has been reported to connect with cancer migration, and even metastases [9, 10]. Collective cell migration is a comprehensive achievement of a variety of processes, such as collective polarization, mechanical coupling, and cytoskeletal kinetics. It majorly followed the guidance of cell-intrinsic multicellular organization, the leader-follower cell behavior, and integration of extracellular signal

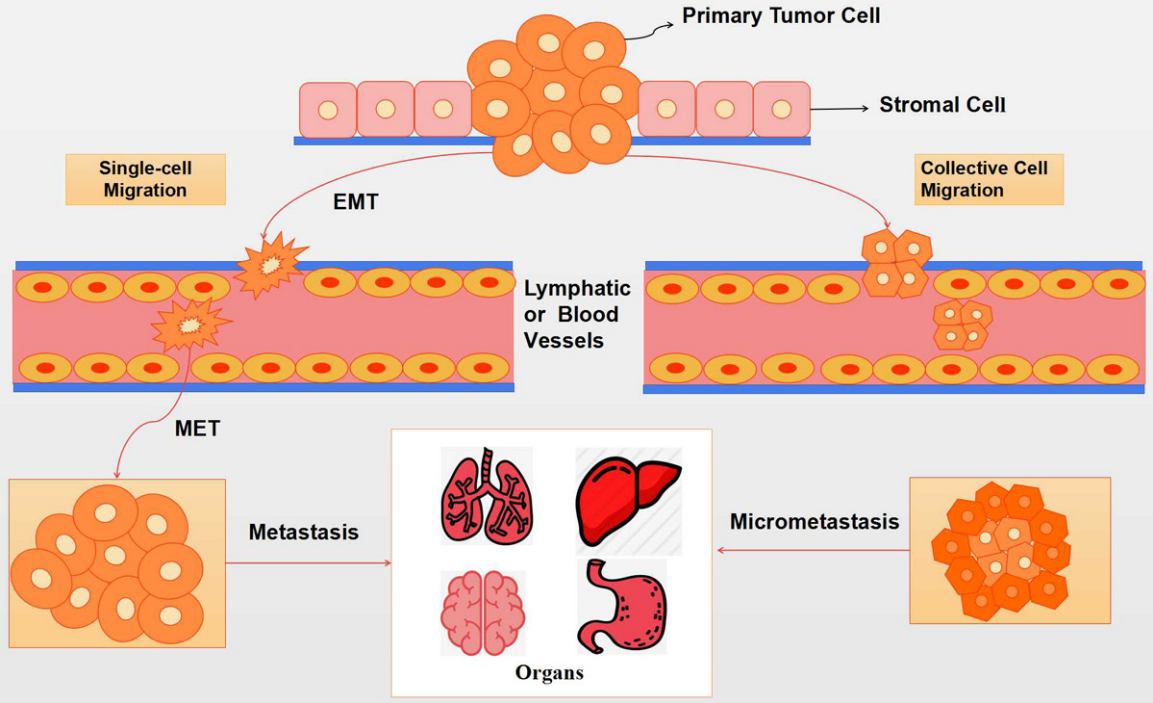

Figure 1. The invasion mechanism of single cancer cell and collective cancer cell. The single cell invasion begins with a single tumor cell from the primary tumor site and undertakes the EMT program to lose some epithelial characteristics, and then comes into the circulatory system, lastly arrives at the distant tissues and organs after experiencing the MET program which gains the epithelial characteristics again. The collective cell invasion begins with a clusters of cells of the primary tumor while does not need completed EMT program that remains some epithelial characteristics and locates at the secondary organs as a whole units.

guidance cues. The knowledge of collective cell migration in tumors has had great significance on the treatment of cancer. In this review we illuminated some common equipments and ways for studying collective cell migration, and discussed the emerging principles and guidance mechanisms of collective cell migration, and how it particularly performed in common tumors.

Major principles and guidance of the collective cell migration mechanisms

Collective cell migration by cell-cell junction

Collective cell migration can move as a unit with the help of the cell-cell junction coupled to dynamic actin cytoskeleton. Cell-cell junction keeps cellular adhesion, polarization, and senses and integrates external guidance signals, then further passes mechanical signal processing and forces transmission within the migrating clusters in the whole movement [11, 13]. Like in embryonic development, cell-cell junction may be the production of the complementary adhesion systems [14]. The ingredients of cell-cell junction in the collectively migrating cells involve adhesion receptor and cytoskeletal adaptor systems, including desmosomal proteins, gap junctions, tight junction constituents and integration between immunoglobulin family members (**Table 2**) [8]. Most of them participate in the entire process of signal transmission mediated by cell-cell junction, such as PI3K/AKT, focal adhesion kinase (FAK) and Rho GTPases [10, 15, 16]. The adhesions take alterations in surface expression and cytoskeletal coupling under the control of upstreaming signaling, then generate various cell-cell junction [17]. And adhesion receptors can help to stabilize the junction between cells. Thus, the adhesion-positive cell-cell junction can be seen as a strong marker of the collective invasion [18]. Besides, the mechano-transducing bridge was made by these cell junctions in the nearby cells, which underlies organizations on the actin cytoskeleton [19]. Cell-cell junctions connect the actin cytoskeleton of multiple cell bodies, which establish the basis for conformity of the forces of individual cells

Table 1. Instruments and ways to research collective cell migration mechanism

Instruments or ways	Field	Function	References
Time-lapse microscopy	Intracellular mechanism	Group dynamics, molecular process evaluation, interaction between cells and cells and substrates and son on	[81]
Intravital imaging	Intracellular mechanism	Monitor of speed, location and type of invasion	[82]
Mouse models	Breast cancer and other cancers	Tumor development process, tumor microenviroments representation	[83-85]
3D matrix-based cell culture	Epithelial cancers, melanoma and other cancers	Tumor development process, tumor microenviroments representation	[9, 13]
Lineage analysis	CTC clusters	To define CTC clusters metastasis arisen from polyclonal origin	[1]
Gene ontology analysis	CTC clusters	To prove the survival advantage of clusters in circulation	[77]
Histological studies	Collective cell invasion	The morphology of collective cells and the extent of the invasion	[4]

Table 2. Adhesion systems and their roles in cell-cell junction

Adhesion systems	Location and roles	Relevant regulation proteins and signaling pathways	References
Adherens junctions (AJs).	Cell-cell junctions of epithelial and endothelial tissues Connect the actin cytoskeleton of adjacent cells	Rho family GTPases: Cdc42 in Par6/aPKC and CIP4 (Cdc42-interacting protein 4); activator of AJs: Rac guanine nucleotide exchange factor (GEF) TIAM1, and Nectin and Nectin-like proteins	[86-88]
Tight junctions (TJs).	In plasma membranes of adjacent cells Create a barrier as restricting diffusion as paracellular gates	Composed transmembrane proteins: claudin, occludin, tricellulin, marveld3, junctional adhesion molecules (JAMs), ZO1, ZO2, ZO3 Rho GTPase signaling: RhoA, Cdc42, Rac	[89-92]
Gap junctions (GJs).	Intercellular membrane channels Form a tight connection between adjacent cells contributing to cell-cell adhesion	Cx43, N-cadherin, cytoskeletal proteins such as microfilaments and microtubules	[93-95]
IgCAMs	Iimmunoglobulin-like cell-adhesion molecules Mediate adaptive cell-cell interactions	Proteins at the cell membrane: growth-factor receptors, integrins, cadherins and intracellular proteins: effectors of signal transduction pathways and cytoskeletal proteins	[96, 97]
Slit/Robo	Roundabout receptors (Robo) and their Slit ligand Control actin cytoskeletal dynamics by interacting with different signaling molecules	Netrin, GTPase activating proteins (GAPs), RhoA, Cdc42, Rac	[36]
Ephrin/Eph receptor.	Tyr kinase receptors and ephrins Control actin cytoskeletal dynamics in short-distance cell-cell signaling	Tyr kinase adaptor protein 1 (Nck1), Nck2, Vav2, Vav3, Src, ephexins RhoA, Cdc42, Rac	[98]
Integrins.	Transmembrane proteins that connect the cytoskeleton with ECM Important transducers of mechanical forces	F-actin, FAK, Src, ERK (extracellular signal-regulated kinase), JNK (c-Jun N-terminal kinase)	[99, 100]

Table 3. The proteins and signaling pathways in collective cell migration

Cell guidance/environment	The guidance and functions	Proteins and signaling pathways	References
Cell-cell junction	Connect the actin cytoskeleton of multiple cells with adhesions Keep cellular polarization and force transmission within the whole clusters	Rho GTPase signaling: RhoA, Cdc42, Rac PI3K/AKT/FAK/ERK	[10, 15, 16, 88]
Leader-follower polarization	Integrate signals of various environments and regulate the actomyosin contractility Connect with substrates and cells around via actin cytoskeleton	MAPK, ERK, FAK, Rac Rho/Rock signaling N-WASP, LIM Kinases 1 and 2, Notch1-Dll4	[6, 34, 41, 46, 50]
CTC cells	Express a hybrid epithelial-mesenchymal (hybrid E/M) phenotype during the metastasis progression	OCT4, NANOG, SOX2, and SIN3A Na+/K+ATPase	[57]
Breast cancer	Maintain the leader-follower polarization of $K14^+$ cells in MMTV-PyMT and TNBC models	YAP signaling, ITGA3, ITGA5, Rho signaling, POSTIN and TNC, DOCK10, ITGA11, DAB2, PDFGRA, VASN and PPAP2B	[50, 61, 63, 64]
Lung cancer	Promote collective cell migration and metastasis in lung cancers	LPP, vimentin	[79, 80]

and the leader-follower polarity in the whole group [20-22]. Cell-cell junctions can stabilize mechanical connections, however, cytoskeletal connections and adhesion sites are dynamic and constantly changing to adapt to the microenvironment during the migration [23]. What's more, the dynamic collective cell function deeply depends on different period cell-cell junctions, from hours to days or even weeks [24]. The critical cell-cell junction medicating the direction and speed of collective cell movements is in few minutes via unstable and short-lived adhesions sensing [25]. There are a series of cell-cell junction mechanisms that have been involved in tumor collective cell invasion. Epithelial tumors metastasis majorly referred to E-cadherin and β-catenin positive cell-cell junctions which can mediate AJs and cell-cell interactions in tumor cells [26]. EMT can reprogramme cell-cell junctions to enhance the ability of invasion and metastasis of epithelial cancers, which includes weakening or even dissolving cell-cell adhesions within the tumor cells, cleaving cadherins via up-regulateing the expression of stromal proteases, deregulating integrin adhesion systems, and turning Rho-mediated actomyosin contractility from cell-cell junctions toward cell-matrix interactions [27-29]. Thus, the adaptability of cell-cell coping program strategies makes cancer invasion and metastasis suitable in different tissue environments. The specific transmission to the different environments during the collective invasion process still remains to be further explored.

The leader-follower behavior in collective cell migration

The guidance for individual tumor cell invasion has been widely understood, including chemical and physical guidance [9], which also works in the collective cell migration. What's more, the collective cell migration also needs to integrate guiding cellular signals for moving and maintaining the migration as a cohesive cell group. The process relates to several cell-intrinsic and extracellular mechanisms of guidance and polarity under the multicellular decisions [30]. The major mechanism refers to the leader-follower polarization, which is related to the coordination of two kinds of cells - leader and follower cells. In the front of the moving groups, there are usually localized leader cells which receive and integrate the signals, while the nearby cells with cell-cell junction, migrate to the direction given by the leader cells, calling follower cells [31, 32]. The leader cells maintain their polarity and invasion into tissue structures by acquiring their leading edge to the substrate supported by AJs signaling, such as actin-based structures, signal processing and special gene expression [33].

Like in mammalian sprouting vessels [33], the leader cells integrate the guidance of the extracellular inputs and downstream intracellular signals in the specific cell-type and tissue-context, such as mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK), FAK and Rho GTPases [6, 34-38]. The early activation of these pathways contributes to the intrinsic bipolarity in leader cells, which causes leader cell selection in cell subsets and inhibits the leader cell selection in adjacent cells, known as followers. Leader cells can regulate actin cytoskeleton polymerization, the actomyosin contractility and force transmission and then pass the force in the whole units to stabilize the focal adhesions with the substrates with the help of the activation of Rho GTPases in a case [39, 40]. Meanwhile the follower cells maintaining cell-cell junction could silence Rho/Rock signaling and reduce actomyosin contractility [41] (**Table 3**). The units of collective cell movements demonstrate leader cells that contact with nearby structures according to Rac-driven filopodal protrusions and substrate adhesions [36]. Moreover, the adhesion to substrates of the leader cells is mainly balanced by two kinds of forces: the integrin-based pulling force at the cell-cell junction of tumor cells, and the pushing force by the neighboring cells. The leader cells with mesenchymal characteristics grasp the direction and speed of migration by stretching actomyosin-mediated protrusions, degrading the surrounding microenvironments and resetting the extracellular matrix (ECM) [9]. The follower cells move with the cell-cell junction behind along the traction made by the leader cells and readjust the ECM [42].

Consequently, collective cell migration mainly relies on the leader-follower cell behavior besides the integrated mechanocoupling and guidance in migration transversion also contributes a lot. The actomyosin contractility has been found to exert as the central hub disposing coordinating mechanical sensing and

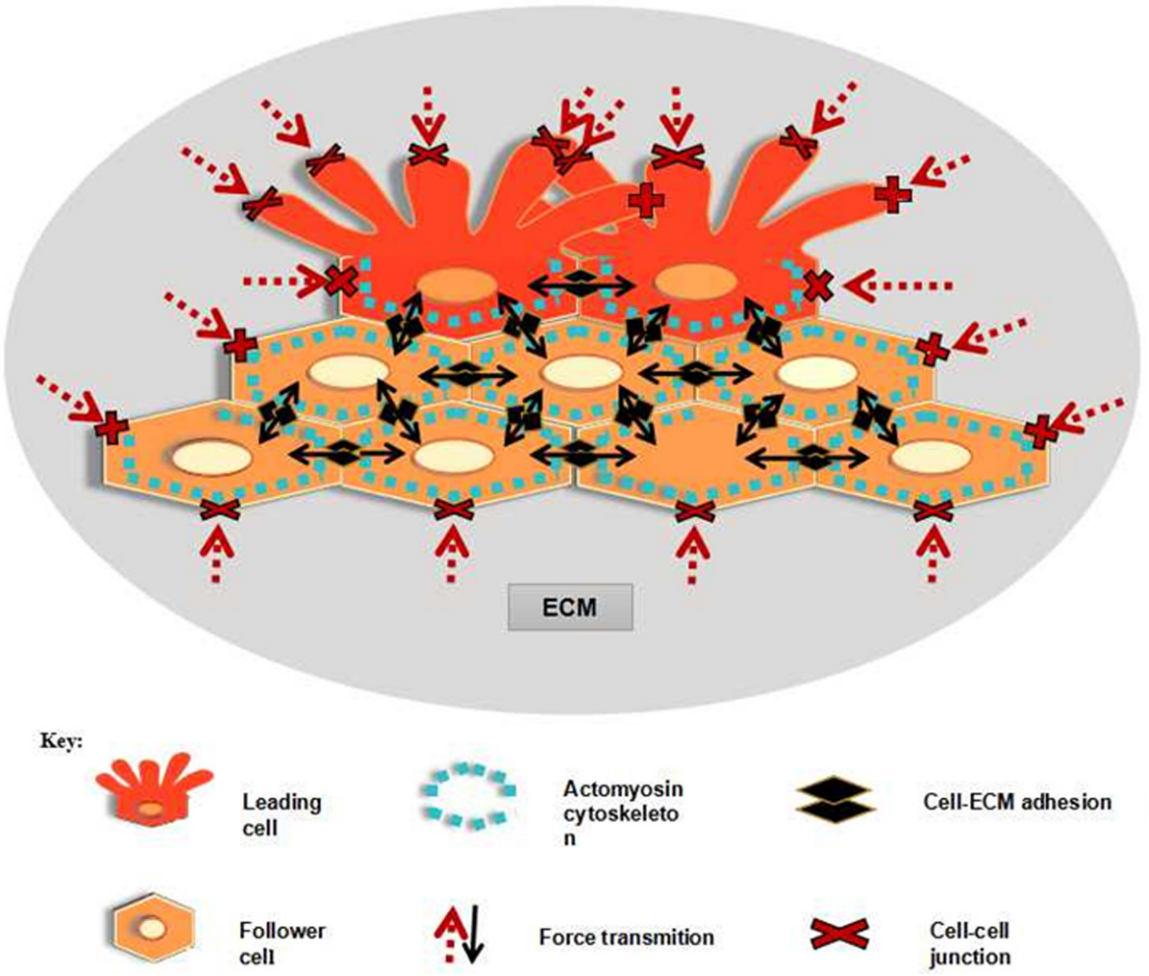

Figure 2. The guidance of leader-follower cell polarization in collective cell clusters. The leader cells could exert pulling force to the follower cells by establishing the cell-ECM adhesion and impose a pushing force to the ECM, and receive pushing force from the mitosis of rear cells. The actomyosin cytoskeleton plays an important role in the whole movements as the core of the force transmission from cells to the matrix and between cells. The collective cells move as a unit with the cell-cell junction that the actomyosin contractility level is low at the junctions, which makes the intracellular pressure which is important to the ECM remodelling.

mechanical transduction responses [43]. While collective tumor cells migrate in the tissue structures, cell adhesion molecules sense the changes in substrate, cytoskeletal rearrangements promoted by ECM and environmental sensing promoted by actomyosin contractility together contribute to signal polar activation and changes of gene expression (**Figure 2**), which is crucial for the translation of cytoskeletal forces into migration motion [13, 33, 44, 45].

However, cadherin-mediated cell-cell junctions at the mechanosensitive adhesions develop an antagonistic relationship with integrin-based adhesions that induce cell polarization and determine the migration direction by promoting cell contractility of the rear of end cells [46-48]. And the cadherin-mediated cell-cell junction also suppresses the formation of protrusion in leader cells and the combination of the integrins with the ECM by transversal RhoA-mediated contractility. Cadherin-mediated mechanical sensitive adhesions interact with the actomyosin network by the catenins like p120- and α- and β-catenin, and this interaction is vital for the maintenance of the cell groups [34]. The balance between the force of cadherin and integrin integrates the leader and follower cells moving migration. Furthermore, the actomyosin cytoskeletal needs to be continuously remodelled by the changes in transcriptional pro-

grams to pass different tissue structures [49]. For example, the leader cell plays its role via the Notch1-Dll4 lateral inhibition, while the Rho signaling regulates the rear cells [46]. Also, the expression of N-WASP participates in the formation of cellular protrusions extended by leader cells. Besides, the cellular protrusions need the help of LIM Kinases 1 and 2 to increase actin filament stability [50, 51]. Therefore, adhesion molecules regulations, dynamic changes of actomyosin cytoskeleton activities and specific gene expression programs in the leader-follower cells are fundamental in efficient collective cell migration.

Collective migration in CTC cells

The classic single tumor cell metastasis mechanism has been studied for some years, suggesting that cells need to undergo the EMT process to be invasive [52]. During the EMT, single tumor cells loose cell adhesion factors which are the characteristics of the epithelial cell and express classical mesenchymal markers induced by the tumor cell environment factors. When the single tumor cells reach the distant tissues, they will experience the MET program and reacquire the epithelial cell characteristics [53, 54]. However, the collective cell migration found in CTC clusters did not need to undergo the complete EMT process to accomplish the metastasis [55]. Tumor cells in CTC clusters could maintain epithelial gene expression and furthermore express a hybrid epithelial-mesenchymal (hybrid E/M) phenotype to finish the proliferation and metastasis [1, 56]. Moreover, in CTC clusters it has been found that stem-cell-related and proliferation-related genes are enriched and the transcription factors are hypomethylated in the binding site, such as OCT4, NANOG, SOX2, and SIN3A [57]. Interestingly, knockdown of the cell-cell junction or the Na+/K+ATPase inhibitors could turn CTC clusters into single CTC cells and lead to the DNA methylation remodelling at critical sites and further suppress the metastasis process, which provides potential clues to clinical treatments for the late stage of tumor diseases [57].

Specific manifestations of collective cell migration in the different tumors

Collective cell migration in breast cancer

There has been some common mechanisms for breast cancer invasion, including epithelial-mesenchymal transition (EMT), collective invasion, and the macrophage-tumor cell feedback loop [58]. Collective invasion has been reviewed as the frequently mechanism of breast cancer invasion, in which the leader cells expressed a basal epithelial gene program, including intermediate filament cytokeratin-14 (K14) and the nuclear transcription factor p63, rather than molecular EMT [26], and guided the rear cells to migrate toward the way established by the leader cells that maintain the cell-cell junction and the connection with the substrates in microenvironments. The studies for tumor invasion in breast cancers usually used multicolor lineage-tracing strategy in a classic mouse model of breast cancer-MMTV-PyMT [59], by which we could observe the major stages of metastasis such as collective invasion, locally clusters dissemination, CTC clusters and distant metastasis [60]. The $K14^+$ cells in MMTV-PyMT has been observed much more efficient than single seed cell in breast cancer invasion as they counted for 2% in the clusters but made >88% tumor collective invasion [26] and could enhance the survival of tumor clusters in distant tissues. $K14^+$ cells were enriched in local dissemination and CTC clusters connected with tumor spread, while $K14^-$ cells were predominated in large colonies and large metastasis associated with proliferation [60]. Compared to the $K14^-$ breast cancer cells, $k14^+$ cells in CTCs have been found to be enriched expression of 87 genes [61]. $K14^+$ cells have been shown to be required by collective cell invasion rather than single seed dissemination as the increased genes expression of cell-cell junction and the adhesion of cell-ECM [60]. Furthermore, in MMTV-PyMT model, the leader cell may acquire K14 expression according to the contraction of the tumor cell-matrix border rather than existed as fixed [26]. $K14^+$ cells could be induced by a stromal ECM which contains abundant fibrillar collagen and lacks collagen IV [26]. Meanwhile, collective cell invasion in basal-type breast cancer cells is limited because the tumor cells can only move through the paths patterned by fibroblasts in the ECM [62]. Therefore stromal fibroblasts play a role in breast tumor collective invasion as the leader cell by remodelling ECM, which determines the trails followed by the remaining tumor cells in the model of MMTV-PyMT and the activity of fibroblasts as regulated by YAP signaling [63]. The leading fibroblasts were regulated by ITGA3, ITGA5, and Rho signaling, while follower cells were dependent on

Cdc42 and MRCK [64]. Some studies reported that the $K14^+$ cells had some connections with the proteins required in metastasis transcribed by POSTIN and TNC encoding [61]. Similarly, collective migration could be eliminated by the hydrolysis of the MT1-MMP-mediated protein, which can express the leader cells creating the paths trailed by the follower tumor cells [65]. The intrinsic differences of tumor cell subpopulations could affect the migration movements of the collective invasion [65, 66]. In triple-negative breast cancer (TNBC) an epigenetically distinct subpopulation of breast tumor cells has been identified called "trailblazer" cells, which may be expressed by a pattern of genes and could be more invasive than the $K14^+$ breast tumor cells [50]. The cohort is comprised of 7 genes, including DOCK10, ITGA11, DAB2, PDFGRA, VASN and PPAP2B, which regulate the activation of collective invasion, and DOCK10 is required for tumor metastasis [50].

Cadherin has been found in most breast cancers as a crucial characteristic, and the level of membrane E-cadherin has played a dual role in promoting cancer invasion and metastasis [67]. A special collective migration mechanism has been explored in the 4T1 cells of a highly metastatic mouse breast carcinoma model, where tumor cells move expressing highly invasive phenotype remaining loosely inter-connected by tethers moderately mediated by E-cadherin [68] rather than extensive cell-cell junction similar to the other collective migration cells. Furthermore, knockdown of the E-cadherin would prevent the formation of tethers and switch the migration mode from collective to single-cell, and reduce the dissemination of 4T1 cells to the lung and others [69].

However, in the classic EMT program induced by the stromal cells, E-cadherin has usually been reported as down-regulated in the tumor migration cells with the loss of cell-cell adhesion and the phenotype of epithelial cells [70, 71]. Inflammatory breast cancer (IBC) has been a good model to explore the principles of collective cell dissemination, and is a highly aggressive subtype of breast cancer that mainly invades via CTC clusters [72]. CTC cells highly express E-cadherin which is the hallmark of epithelial traits, rather than go through EMT program during the metastasis process, which may have connections with the worse clinical outcomes compared to the non-IBC patients [61, 72, 73]. Together, the interaction between collective cell migration and single tumor cell migration regarding cancer metastasis requires further research.

Collective cell migration in lung cancer

It has been reported that CTC clusters were detected in the blood of lung cancer patients, which have greater potential to metastasize and the clustered cells have more survival advantages than single CTC cells [74]. And the CTC clusters in SCLC (small cell lung cancer) has been shown a capability of being tumorospheres with loosely and irregular connection, which had resistance to radio chemotherapy and worse prognosis (**Figure 3**) [75, 76]. Also, the CTC clusters have been identified to have higher expression of IL6, BCL2, ERCC1, Ki-67 and IL-17 than single CTC cells, indicating that CTC clusters were highly invasive and connected to the worse clinical outcomes [77, 78]. Furthermore, during the early stage of the lung adenocarcinoma metastasis process, the tumor cells experience mostly epithelial-like collective invasion, and are surrounded by vim^+þ/$FSP1^+$ cancer-associated fibroblasts (CAF) which require vimentin to maintain heterotypic tumor cell-CAF interactions [79]. Additionally, LPP regulates the expression of matrix metalloproteinase 15 (MMP-15) by degrading N-cadherin in PC14PE6 cells to promote collective cell migration and metastasis in lung cancers [80]. Together, the emerging studies detailing the collective cell migration in lung cancers could contribute to clinical treatments.

Conclusion and outlook

The collective cell migration is an emerging mechanism distinguished from the classic single tumor cell metastasis, which does not need a complete EMT program and can move as a cohort unit in which cells maintain the cell-cell junction and express some epithelial characteristics. Also, collective cell migration has been reported to have greater potential for metastasis and dissemination and has higher therapeutic resistance compared to single cell migration. A summary of the instruments and typical methods used in collective cell migration, are shown in **Table 1**. There are some challenges remaining to be further studied. First, what are the intrinsic differences between different cell clusters in different cancer diseases? Second,

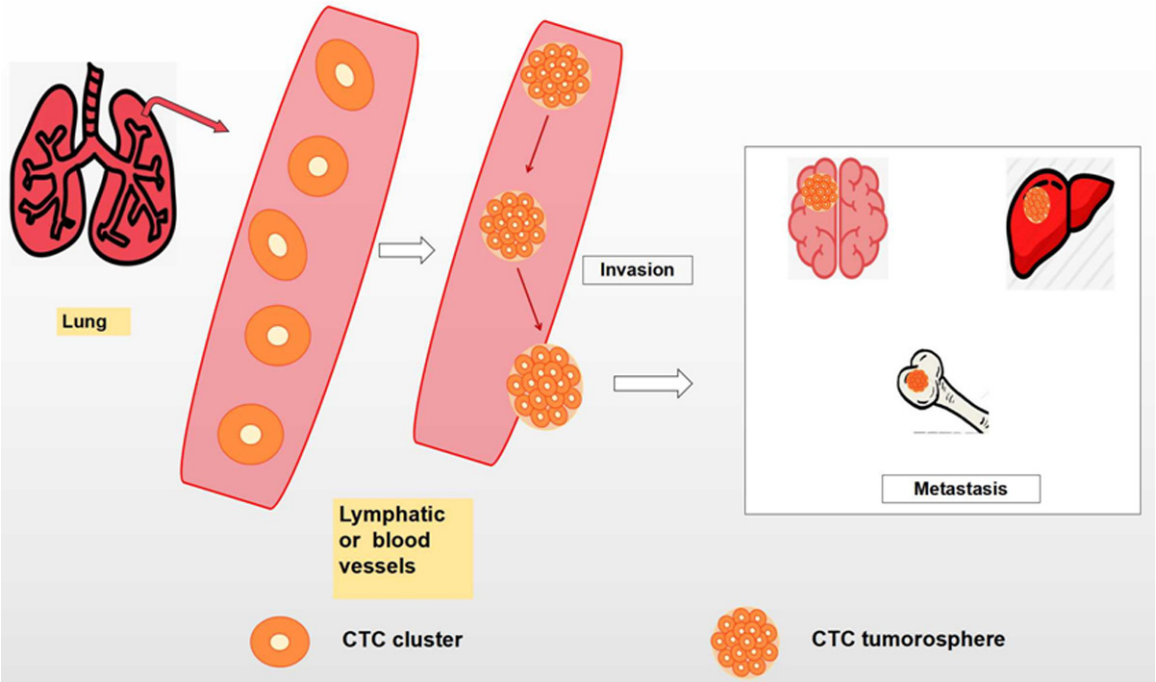

Figure 3. The invasion and metastasis mechanism of CTC clusters in SCLC. CTCs originate from the lung tumor and invade into the circulation. Small clusters of CTCs may become to tumorospheres. Then the whole tumorosphere extravasates from circulation into the distant organs as liver, bone and brain. Tumorospheres keep loosely and irregular connection and showed resistances to radiochemotherapy and worse clinical prognosis.

what are the specific mechanisms that change the single-cell migration to the collective cell migration? Third, how do the clusters adapt to the complicated environments during the metastasis process and finally survive in the distant organs? The discovery of collective cell migration has brought a new perspective to solving tumor disease. And there have been some FDA-approved medicines targeting collective clusters, which bring emerging clues to the terminal cancer treatments. Furthermore, screening of the collective cell migration could contribute to the detection of the early stage of tumor patients.

Acknowledgements

The work was supported by the National Natural Sciences Foundations of China (No: 81-773218, 81703009, 81802791, 81472773 and 81272566) and The Natural Sciences Foundations of Hunan Province (No: 2018-JJ3858 and 2017JJ3457).

Disclosure of conflict of interest

None.

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References

[1] Cheung KJ and Ewald AJ. A collective route to metastasis: seeding by tumor cell clusters. Science 2016; 352: 167-169.

[2] Hainaut P and Plymoth A. Targeting the hallmarks of cancer: towards a rational approach to next-generation cancer therapy. Curr Opin Oncol 2013; 25: 50-51.

[3] Talmadge JE and Fidler IJ. AACR centennial series: the biology of cancer metastasis: historical perspective. Cancer Res 2010; 70: 5649-5669.

[4] Friedl P, Locker J, Sahai E and Segall JE. Classifying collective cancer cell invasion. Nat Cell Biol 2012; 14: 777-783.

[5] Montell DJ. Morphogenetic cell movements: diversity from modular mechanical properties. Science 2008; 322: 1502-1505.

[6] Zegers MM and Friedl P. Rho GTPases in collective cell migration. Small GTPases 2014; 5: e28997.

[7] Andrew DJ and Ewald AJ. Morphogenesis of epithelial tubes: insights into tube formation, elongation, and elaboration. Dev Biol 2010; 341: 34-55.
[8] Bui J, Conway DE, Heise RL and Weinberg SH. Mechanochemical coupling and junctional forces during collective cell migration. Biophys J 2019; 117: 170-183.
[9] Friedl P and Gilmour D. Collective cell migration in morphogenesis, regeneration and cancer. Nat Rev Mol Cell Biol 2009; 10: 445-457.
[10] Trepat X and Fredberg JJ. Plithotaxis and emergent dynamics in collective cellular migration. Trends Cell Biol 2011; 21: 638-646.
[11] Canel M, Serrels A, Miller D, Timpson P, Serrels B, Frame MC and Brunton VG. Quantitative in vivo imaging of the effects of inhibiting integrin signaling via Src and FAK on cancer cell movement: effects on E-cadherin dynamics. Cancer Res 2010; 70: 9413-9422.
[12] Haeger A, Krause M, Wolf K and Friedl P. Cell jamming: collective invasion of mesenchymal tumor cells imposed by tissue confinement. Biochim Biophys Acta 2014; 1840: 2386-2395.
[13] Hegerfeldt Y, Tusch M, Bröcker EB and Friedl P. Collective cell movement in primary melanoma explants: plasticity of cell-cell interaction, beta1-integrin function, and migration strategies. Cancer Res 2002; 62: 2125-2130.
[14] Ikari A, Atomi K, Takiguchi A, Yamazaki Y, Hayashi H, Hirakawa J and Sugatani J. Enhancement of cell-cell contact by claudin-4 in renal epithelial madin-darby canine kidney cells. J Cell Biochem 2012; 113: 499-507.
[15] Peglion F, Llense F and Etienne-Manneville S. Adherens junction treadmilling during collective migration. Nat Cell Biol 2014; 16: 639-651.
[16] Parri M and Chiarugi P. Rac and Rho GTPases in cancer cell motility control. Cell Commun Signal 2010; 8: 23.
[17] Friedl P and Mayor R. Tuning collective cell migration by cell-cell junction regulation. Cold Spring Harb Perspect Biol 2017; 9.
[18] Cavallaro U and Dejana E. Adhesion molecule signalling: not always a sticky business. Nat Rev Mol Cell Biol 2011; 12: 189-197.
[19] Maness PF and Schachner M. Neural recognition molecules of the immunoglobulin superfamily: signaling transducers of axon guidance and neuronal migration. Nat Neurosci 2007; 10: 19-26.
[20] Bazellières E, Conte V, Elosegui-Artola A, Serra-Picamal X, Bintanel-Morcillo M, Roca-Cusachs P, Muñoz JJ, Sales-Pardo M, Guimerà R and Trepat X. Control of cell-cell forces and collective cell dynamics by the intercellular adhesome. Nat Cell Biol 2015; 17: 409-420.
[21] Desai RA, Gao L, Raghavan S, Liu WF and Chen CS. Cell polarity triggered by cell-cell adhesion via E-cadherin. J Cell Sci 2009; 122: 905-911.
[22] Lawson DA, Bhakta NR, Kessenbrock K, Prummel KD, Yu Y, Takai K, Zhou A, Eyob H, Balakrishnan S, Wang CY, Yaswen P, Goga A and Werb Z. Single-cell analysis reveals a stem-cell program in human metastatic breast cancer cells. Nature 2015; 526: 131-135.
[23] Baum B and Georgiou M. Dynamics of adherens junctions in epithelial establishment, maintenance, and remodeling. J Cell Biol 2011; 192: 907-917.
[24] Shamir ER and Ewald AJ. Adhesion in mammary development: novel roles for E-cadherin in individual and collective cell migration. Curr Top Dev Biol 2015; 112: 353-382.
[25] Theveneau E and Mayor R. Collective cell migration of epithelial and mesenchymal cells. Cell Mol Life Sci 2013; 70: 3481-3492.
[26] Cheung KJ, Gabrielson E, Werb Z and Ewald AJ. Collective invasion in breast cancer requires a conserved basal epithelial program. Cell 2013; 155: 1639-1651.
[27] Kalluri R and Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009; 119: 1420-1428.
[28] Parvani JG, Galliher-Beckley AJ, Schiemann BJ and Schiemann WP. Targeted inactivation of β1 integrin induces β3 integrin switching, which drives breast cancer metastasis by TGF-β. Mol Biol Cell 2013; 24: 3449-3459.
[29] Truong HH, Xiong J, Ghotra VP, Nirmala E, Haazen L, Le Dévédec SE, Balcioğlu HE, He S, Snaar-Jagalska BE, Vreugdenhil E, Meerman JH, van de Water B and Danen EH. β1 integrin inhibition elicits a prometastatic switch through the TGFβ-miR-200-ZEB network in E-cadherin-positive triple-negative breast cancer. Sci Signal 2014; 7: ra15.
[30] Cai D, Chen SC, Prasad M, He L, Wang X, Choesmel-Cadamuro V, Sawyer JK, Danuser G and Montell DJ. Mechanical feedback through E-cadherin promotes direction sensing during collective cell migration. Cell 2014; 157: 1146-1159.
[31] Cortese B, Palamà IE, D'Amone S and Gigli G. Influence of electrotaxis on cell behaviour. Integr Biol (Camb) 2014; 6: 817-830.
[32] Rørth P. Whence directionality: guidance mechanisms in solitary and collective cell migration. Dev Cell 2011; 20: 9-18.
[33] Khalil AA and Friedl P. Determinants of leader cells in collective cell migration. Integr Biol (Camb) 2010; 2: 568-574.
[34] Riahi R, Sun J, Wang S, Long M, Zhang DD and Wong PK. Notch1-Dll4 signalling and mechanical force regulate leader cell formation during

collective cell migration. Nat Commun 2015; 6: 6556.
[35] Chapnick DA and Liu X. Leader cell positioning drives wound-directed collective migration in TGFβ-stimulated epithelial sheets. Mol Biol Cell 2014; 25: 1586-1593.
[36] Yamaguchi N, Mizutani T, Kawabata K and Haga H. Leader cells regulate collective cell migration via Rac activation in the downstream signaling of integrin β1 and PI3K. Sci Rep 2015; 5: 7656.
[37] Zhang X, Moore SW, Iskratsch T and Sheetz MP. N-WASP-directed actin polymerization activates Cas phosphorylation and lamellipodium spreading. J Cell Sci 2014; 127: 1394-1405.
[38] Han J, Li L, Hu J, Yu L, Zheng Y, Guo J, Zheng X, Yi P and Zhou Y. Epidermal growth factor stimulates human trophoblast cell migration through Rho A and Rho C activation. Endocrinology 2010; 151: 1732-1742.
[39] Mayor R and Carmona-Fontaine C. Keeping in touch with contact inhibition of locomotion. Trends Cell Biol 2010; 20: 319-328.
[40] Reffay M, Parrini MC, Cochet-Escartin O, Ladoux B, Buguin A, Coscoy S, Amblard F, Camonis J and Silberzan P. Interplay of RhoA and mechanical forces in collective cell migration driven by leader cells. Nat Cell Biol 2014; 16: 217-223.
[41] Das T, Safferling K, Rausch S, Grabe N, Boehm H and Spatz JP. A molecular mechanotransduction pathway regulates collective migration of epithelial cells. Nat Cell Biol 2015; 17: 276-287.
[42] Sadati M, Taheri Qazvini N, Krishnan R, Park CY and Fredberg JJ. Collective migration and cell jamming. Differentiation 2013; 86: 121-125.
[43] Pandya P, Orgaz JL and Sanz-Moreno V. Actomyosin contractility and collective migration: may the force be with you. Curr Opin Cell Biol 2017; 48: 87-96.
[44] Friedl P and Wolf K. Tube travel: the role of proteases in individual and collective cancer cell invasion. Cancer Res 2008; 68: 7247-7249.
[45] Hidalgo-Carcedo C, Hooper S, Chaudhry SI, Williamson P, Harrington K, Leitinger B and Sahai E. 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 2011; 13: 49-58.
[46] Dupin I, Camand E and Etienne-Manneville S. Classical cadherins control nucleus and centrosome position and cell polarity. J Cell Biol 2009; 185: 779-786.
[47] Burute M and Thery M. Spatial segregation between cell-cell and cell-matrix adhesions. Curr Opin Cell Biol 2012; 24: 628-636.
[48] Etienne-Manneville S. Adherens junctions during cell migration. Subcell Biochem 2012; 60: 225-249.
[49] Plutoni C, Bazellieres E, Le Borgne-Rochet M, Comunale F, Brugues A, Séveno M, Planchon D, Thuault S, Morin N, Bodin S, Trepat X and Gauthier-Rouvière C. P-cadherin promotes collective cell migration via a Cdc42-mediated increase in mechanical forces. J Cell Biol 2016; 212: 199-217.
[50] Yu X, Zech T, McDonald L, Gonzalez EG, Li A, Macpherson I, Schwarz JP, Spence H, Futó K, Timpson P, Nixon C, Ma Y, Anton IM, Visegrády B, Insall RH, Oien K, Blyth K, Norman JC and Machesky LM. N-WASP coordinates the delivery and F-actin-mediated capture of MT1-MMP at invasive pseudopods. J Cell Biol 2012; 199: 527-544.
[51] Scott RW, Hooper S, Crighton D, Li A, König I, Munro J, Trivier E, Wickman G, Morin P, Croft DR, Dawson J, Machesky L, Anderson KI, Sahai EA and Olson MF. LIM kinases are required for invasive path generation by tumor and tumor-associated stromal cells. J Cell Biol 2010; 191: 169-185.
[52] Thiery JP, Acloque H, Huang RY and Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009; 139: 871-890.
[53] Puisieux A, Brabletz T and Caramel J. Oncogenic roles of EMT-inducing transcription factors. Nat Cell Biol 2014; 16: 488-494.
[54] De Craene B and Berx G. Regulatory networks defining EMT during cancer initiation and progression. Nat Rev Cancer 2013; 13: 97-110.
[55] Fischer KR, Durrans A, Lee S, Sheng J, Li F, Wong ST, Choi H, El Rayes T, Ryu S, Troeger J, Schwabe RF, Vahdat LT, Altorki NK, Mittal V and Gao D. Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. Nature 2015; 527: 472-476.
[56] Jolly MK, Boareto M, Huang B, Jia D, Lu M, Ben-Jacob E, Onuchic JN and Levine H. Implications of the hybrid epithelial/mesenchymal phenotype in metastasis. Front Oncol 2015; 5: 155.
[57] Gkountela S, Castro-Giner F, Szczerba BM, Vetter M, Landin J, Scherrer R, Krol I, Scheidmann MC, Beisel C, Stirnimann CU, Kurzeder C, Heinzelmann-Schwarz V, Rochlitz C, Weber WP and Aceto N. Circulating tumor cell clustering shapes DNA methylation to enable metastasis seeding. Cell 2019; 176: 98-112, e14.
[58] Cheung KJ and Ewald AJ. Illuminating breast cancer invasion: diverse roles for cell-cell interactions. Curr Opin Cell Biol 2014; 30: 99-111.
[59] Hwang PY, Brenot A, King AC, Longmore GD, George SC. Randomly distributed K14(+) breast tumor cells polarize to the leading edge

and guide collective migration in response to chemical and mechanical environmental cues. Cancer Res 2019; 79: 1899-1912.
[60] Cheung KJ, Padmanaban V, Silvestri V, Schipper K, Cohen JD, Fairchild AN, Gorin MA, Verdone JE, Pienta KJ, Bader JS and Ewald AJ. Polyclonal breast cancer metastases arise from collective dissemination of keratin 14-expressing tumor cell clusters. Proc Natl Acad Sci U S A 2016; 113: E854-863.
[61] Tripathi S, Jolly MK, Woodward WA, Levine H and Deem MW. Analysis of hierarchical organization in gene expression networks reveals underlying principles of collective tumor cell dissemination and metastatic aggressiveness of inflammatory breast cancer. Front Oncol 2018; 8: 244.
[62] Dang TT, Prechtl AM and Pearson GW. Breast cancer subtype-specific interactions with the microenvironment dictate mechanisms of invasion. Cancer Res 2011; 71: 6857-6866.
[63] Calvo F, Ege N, Grande-Garcia A, Hooper S, Jenkins RP, Chaudhry SI, Harrington K, Williamson P, Moeendarbary E, Charras G and Sahai E. Mechanotransduction and YAP-dependent matrix remodelling is required for the generation and maintenance of cancer-associated fibroblasts. Nat Cell Biol 2013; 15: 637-646.
[64] Gaggioli C, Hooper S, Hidalgo-Carcedo C, Grosse R, Marshall JF, Harrington K and Sahai E. Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. Nat Cell Biol 2007; 9: 1392-1400.
[65] Carey SP, Starchenko A, McGregor AL and Reinhart-King CA. Leading malignant cells initiate collective epithelial cell invasion in a three-dimensional heterotypic tumor spheroid model. Clin Exp Metastasis 2013; 30: 615-630.
[66] Wolf K, Wu YI, Liu Y, Geiger J, Tam E, Overall C, Stack MS and Friedl P. Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion. Nat Cell Biol 2007; 9: 893-904.
[67] Elisha Y, Kalchenko V, Kuznetsov Y and Geiger B. Dual role of E-cadherin in the regulation of invasive collective migration of mammary carcinoma cells. Sci Rep 2018; 8: 4986.
[68] Fernandez SV, Robertson FM, Pei J, Aburto-Chumpitaz L, Mu Z, Chu K, Alpaugh RK, Huang Y, Cao Y, Ye Z, Cai KQ, Boley KM, Klein-Szanto AJ, Devarajan K, Addya S and Cristofanilli M. Inflammatory breast cancer (IBC): clues for targeted therapies. Breast Cancer Res Treat 2013; 140: 23-33.
[69] Tao K, Fang M, Alroy J and Sahagian GG. Imagable 4T1 model for the study of late stage breast cancer. BMC Cancer 2008; 8: 228.
[70] Wendt MK, Taylor MA, Schiemann BJ and Schiemann WP. Down-regulation of epithelial cadherin is required to initiate metastatic outgrowth of breast cancer. Mol Biol Cell 2011; 22: 2423-2435.
[71] Hwang S, Zimmerman NP, Agle KA, Turner JR, Kumar SN and Dwinell MB. E-cadherin is critical for collective sheet migration and is regulated by the chemokine CXCL12 protein during restitution. J Biol Chem 2012; 287: 22227-22240.
[72] Jolly MK, Boareto M, Debeb BG, Aceto N, Farach-Carson MC, Woodward WA and Levine H. Inflammatory breast cancer: a model for investigating cluster-based dissemination. NPJ Breast Cancer 2017; 3: 21.
[73] Rodriguez FJ, Lewis-Tuffin LJ and Anastasiadis PZ. E-cadherin's dark side: possible role in tumor progression. Biochim Biophys Acta 2012; 1826: 23-31.
[74] Murlidhar V, Reddy RM, Fouladdel S, Zhao L, Ishikawa MK, Grabauskiene S, Zhang Z, Lin J, Chang AC, Carrott P, Lynch WR, Orringer MB, Kumar-Sinha C, Palanisamy N, Beer DG, Wicha MS, Ramnath N, Azizi E and Nagrath S. Poor prognosis indicated by venous circulating tumor cell clusters in early-stage lung cancers. Cancer Res 2017; 77: 5194-5206.
[75] Klameth L, Rath B, Hochmaier M, Moser D, Redl M, Mungenast F, Gelles K, Ulsperger E, Zeillinger R, Hamilton G. Small cell lung cancer: model of circulating tumor cell tumorospheres in chemoresistance. Sci Rep 2017; 7: 5337.
[76] Yu N, Zhou J, Cui F, Tang X. Circulating tumor cells in lung cancer: detection methods and clinical applications. Lung 2015; 193: 157-71.
[77] Guo Y, Xu F, Lu T, Duan Z and Zhang Z. Interleukin-6 signaling pathway in targeted therapy for cancer. Cancer Treat Rev 2012; 38: 904-910.
[78] Sarioglu AF, Aceto N, Kojic N, Donaldson MC, Zeinali M, Hamza B, Engstrom A, Zhu H, Sundaresan TK, Miyamoto DT, Luo X, Bardia A, Wittner BS, Ramaswamy S, Shioda T, Ting DT, Stott SL, Kapur R, Maheswaran S, Haber DA and Toner M. A microfluidic device for label-free, physical capture of circulating tumor cell clusters. Nat Methods 2015; 12: 685-691.
[79] Richardson AM, Havel LS, Koyen AE, Konen JM, Shupe J, Wiles WG 4th, Martin WD, Grossniklaus HE, Sica G, Gilbert-Ross M and Marcus AI. Vimentin is required for lung adenocarcinoma metastasis via heterotypic tumor cell-cancer-associated fibroblast interactions during collective invasion. Clin Cancer Res 2018; 24: 420-432.
[80] Kuriyama S, Yoshida M, Yano S, Aiba N, Kohno T, Minamiya Y, Goto A and Tanaka M. LPP inhib-

its collective cell migration during lung cancer dissemination. Oncogene 2016; 35: 952-964.
[81] Bouvard D, Pouwels J, De Franceschi N and Ivaska J. Integrin inactivators: balancing cellular functions in vitro and in vivo. Nat Rev Mol Cell Biol 2013; 14: 430-442.
[82] Dotan I, Allez M, Danese S, Keir M, Tole S and McBride J. The role of integrins in the pathogenesis of inflammatory bowel disease: approved and investigational anti-integrin therapies. Med Res Rev 2019; [Epub ahead of print].
[83] Kania A and Klein R. Mechanisms of ephrin-Eph signalling in development, physiology and disease. Nat Rev Mol Cell Biol 2016; 17: 240-256.
[84] Hivert B, Liu Z, Chuang CY, Doherty P and Sundaresan V. Robo1 and Robo2 are homophilic binding molecules that promote axonal growth. Mol Cell Neurosci 2002; 21: 534-545.
[85] Juliano RL. Signal transduction by cell adhesion receptors and the cytoskeleton: functions of integrins, cadherins, selectins, and immunoglobulin-superfamily members. Annu Rev Pharmacol Toxicol 2002; 42: 283-323.
[86] Cavallaro U and Christofori G. Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. Nat Rev Cancer 2004; 4: 118-132.
[87] Wei CJ, Xu X and Lo CW. Connexins and cell signaling in development and disease. Annu Rev Cell Dev Biol 2004; 20: 811-838.
[88] Xu X, Li WE, Huang GY, Meyer R, Chen T, Luo Y, Thomas MP, Radice GL and Lo CW. Modulation of mouse neural crest cell motility by N-cadherin and connexin 43 gap junctions. J Cell Biol 2001; 154: 217-230.
[89] Willecke K, Eiberger J, Degen J, Eckardt D, Romualdi A, Güldenagel M, Deutsch U and Söhl G. Structural and functional diversity of connexin genes in the mouse and human genome. Biol Chem 2002; 383: 725-737.
[90] Oda Y, Otani T, Ikenouchi J and Furuse M. Tricellulin regulates junctional tension of epithelial cells at tricellular contacts through Cdc42. J Cell Sci 2014; 127: 4201-4212.
[91] Van Itallie CM and Anderson JM. Architecture of tight junctions and principles of molecular composition. Semin Cell Dev Biol 2014; 36: 157-165.
[92] Luissint AC, Nusrat A and Parkos CA. JAM-related proteins in mucosal homeostasis and inflammation. Semin Immunopathol 2014; 36: 211-226.
[93] Zihni C, Mills C, Matter K and Balda MS. Tight junctions: from simple barriers to multifunctional molecular gates. Nat Rev Mol Cell Biol 2016; 17: 564-580.
[94] Mandai K, Rikitake Y, Mori M and Takai Y. Nectins and nectin-like molecules in development and disease. Curr Top Dev Biol 2015; 112: 197-231.
[95] Mertens AE, Rygiel TP, Olivo C, van der Kammen R and Collard JG. The Rac activator Tiam1 controls tight junction biogenesis in keratinocytes through binding to and activation of the Par polarity complex. J Cell Biol 2005; 170: 1029-1037.
[96] Harris TJ and Tepass U. Adherens junctions: from molecules to morphogenesis. Nat Rev Mol Cell Biol 2010; 11: 502-514.
[97] Shapiro L and Weis WI. Structure and biochemistry of cadherins and catenins. Cold Spring Harb Perspect Biol 2009; 1: a003053.
[98] Tambe DT, Hardin CC, Angelini TE, Rajendran K, Park CY, Serra-Picamal X, Zhou EH, Zaman MH, Butler JP, Weitz DA, Fredberg JJ and Trepat X. Collective cell guidance by cooperative intercellular forces. Nat Mater 2011; 10: 469-475.
[99] He L, Wang X, Tang HL and Montell DJ. Tissue elongation requires oscillating contractions of a basal actomyosin network. Nat Cell Biol 2010; 12: 1133-1142.
[100] Ewald AJ, Werb Z and Egeblad M. Dynamic, long-term in vivo imaging of tumor-stroma interactions in mouse models of breast cancer using spinning-disk confocal microscopy. Cold Spring Harb Protoc 2011; 2011: pdb.top97.