REVIEW ARTICLE

Journal homepage
https://fpmpeb.journals.ekb.eg

Vol. 1, ISSUE 2 No 3/2024

Differential Analyses of Leader and Follower Cancer Cells

Ashraf M. Omar[1]*, Amira A. Abdellatef[2]

[1] Medical Biotechnology Department, Institute of Genetic Engineering and Biotechnology, City of Scientific Research and Technological Applications, Alexandria, Egypt.

[2] Protein Research Department, Institute of Genetic Engineering and Biotechnology, City of Scientific Research and Technological Applications, Alexandria, Egypt.

*Corresponding author: Ashraf M. Omar; ashraf.omar88@gmail.com

Abstract

Metastasis is a hallmark of cancer that is responsible for the greatest number of cancer-related deaths (~90%). For the metastasis to take place, the primary tumor mass engineers migratory cells that can invade adjacent tissues and circulate to distant organs where cancer cells have sufficient nutrients to grow and form new colonies. The migrating cell group typically consists of "LEADER" cells at the front, which navigate the path lead collective invasive packs, and "FOLLOWER" cells that trail behind and support the movement. Both leader and follower cells communicate and cooperate to achieve collective cancer migration and invasion. Recent studies showed that the elimination of a leader cell disrupts collective cell migration. However, The mechanisms controlling the synchronized migration of leader and follower cells are still unresolved. Therefore, the genetic and metabolic traits of leader and follower cells, enabling them to navigate their way into secondary nutrient-rich sites and escape the immune system surveillance, need further elucidation. Interestingly, the differential Multi-omic analyses of cancer leader and follower cells are an emerging area of research within the study of tumor heterogeneity and metastasis. This review highlights the recent findings about leader/follower cancer cells focusing on their unique proteomic, metabolic, and transcriptomic signatures rendering them their unique involvement in cancer metastasis and invasion.

Keywords: Metastasis; Cell migration; Leader cells; Follower cells; Cancer metabolism

1. Introduction

Cancer cells in the tumor microenvironment suffer from hypoxia and nutrition starvation due to insufficient blood supply and the excessive energy demand of the intensively proliferating cancer cells [1]. To endure such harsh conditions, Tumor cells utilize three main strategies; angiogenesis, metabolic rewiring, and metastasis (Figure 1)[2]. The latter is a hallmark of cancer that is responsible for approximately 90% of cancer-related deaths [3]. The high mortality rate linked to cancer metastasis is because surgical intervention becomes challenging once the tumor has spread to distant organs [4]. Moreover, metastatic cancer cells often develop resistance to chemotherapy, targeted therapy, and radiation [5-8]. This resistance arises from genetic mutations, epigenetic changes, or the presence of inherently resistant cancer stem cells [9-11]. Furthermore, once cancer spreads to vital organs (e.g., lungs, liver, brain, bones), it disrupts their normal function, leading to life-threatening complications [12].

These factors have spurred researchers to target cancer metastasis pathways to delay, prevent, or treat cancer metastasis [13]. However, to date, cancer metastasis is poorly understood. For the metastasis to take place, the primary tumor mass engineers migratory cells capable of invading adjacent tissues and circulating to distant organs where cancer cells may have sufficient nutrients to grow and establish new colonies [14]. This process involves cancer cells leaving their primary site, circulating the bloodstream, surviving pressure in blood vessels, adapting to new cellular environments at secondary sites, and evading immune system attacks [12] Little is known about what triggers the primary tumor mass to engineer the migratory cells. Moreover, the genetic and metabolic traits of the cancer migratory cells, enabling them to navigate their way into secondary sites and escape the immune system surveillance, need further elucidation [14, 15]. The secondary sites should also be prepared to host the migratory cells [premetastatic niche (PMN)] is still unresolved [16].

During metastasis, cancer cell migration occurs as a collective process, meaning that groups of cancer cells move together as a cohesive unit rather than individually

[17,18]. This collective migration is essential for cancer invasion and the establishment of metastases in distant organs [17, 18].

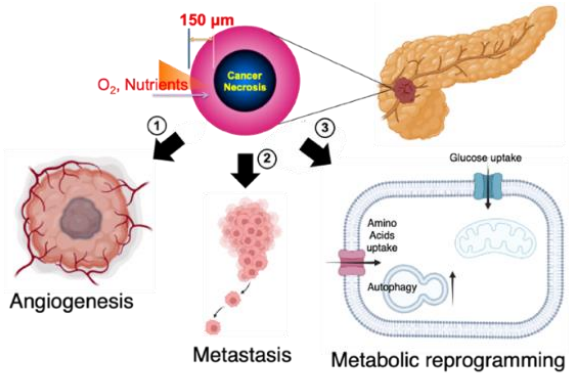

Fig. 1. Pancreatic cancer survival pathways

The migrating cell group typically consists of "LEADER" cells at the front, which navigate the path lead the invasive packs, and "FOLLOWER" cells that trail behind and support the movement (Figure 2) [18, 19]. Both leader and follower cells communicate and cooperate to achieve collective cancer migration and invasion [20]. Recent studies showed that eliminating leader cells disrupts collective migration [21]. The mechanisms controlling the synchronized migration of leader and follower cells are still unresolved. The differential metabolism of leader and follower cancer cells is an emerging area of research within tumor heterogeneity and metastasis.

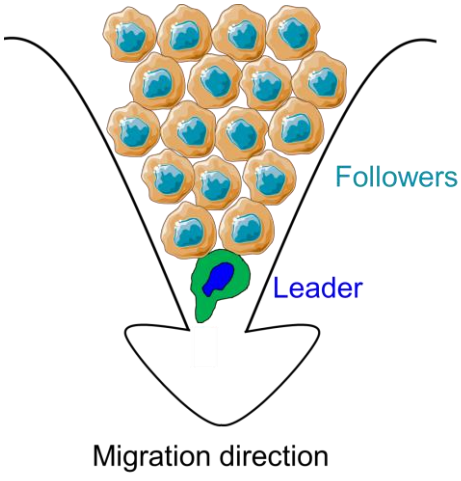

Figure 2. Leader and follower cancer cells

Herein, we highlight some of the recent findings about leader/follower cancer cells focusing on their unique proteomic, metabolic, and transcriptomic signatures rendering them their unique involvement in cancer metastasis and invasion.

2. Techniques used to define and possibly isolate leader from follower cells

Several techniques have been developed to isolate leader and follower cancer cells from the collectively migrating invasive cancer packs [20, 21, 24]. These techniques are aimed at identifying the phenotypic and functional differences between leader cells (which lead the migration) and follower cells (which follow behind). Spatiotemporal genomic and cellular analysis (SaGA) is an image-guided technique for the in situ selection, isolation, and expansion of leader and follower cells from collectively invading cancer cell packs [20, 22] This technique integrates 3D cell culture with fluorescence-activated cell sorting (FACS). For that purpose, spheroids were generated using cells expressing the Dendra2 fluorescent tag and were embedded in Matrigel where cells were allowed to migrate and invade. The selection of leader or follower cells was based on their position within the invasive pack. The exposure to a laser beam allowed the photoconversion and subsequent separation of cellular subpopulations using FACS (Figure 3). This technique was applied to several lung cancer cell lines [20, 22, 23]

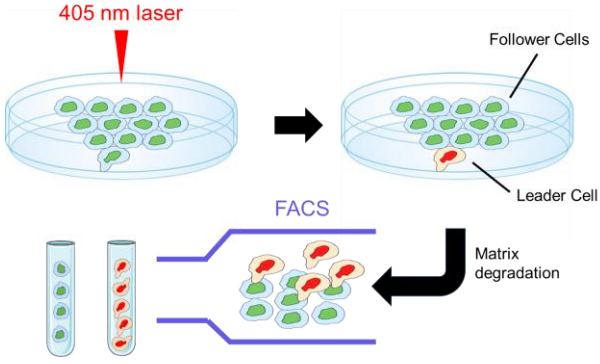

Figure 3. Isolation and purification of leader and follower cancer cells using SaGA technique

Furthermore, to isolate a leader cell during collective cell migration, a borosilicate glass pipette pulled using a micropipette puller, attached to a micromanipulator, was used to pierce the leader cell nucleus observed through phase-contrast microscopy [21].

Additionally, 3D microfluidic devices that replicate tumor microenvironments have been developed to distinctly identify leader and follower cells via dynamic real-time imaging.[24] In these devices, primary, heterogeneous tumor organoids isolated from mouse tumor tissues are placed inside the device after mixing with Collagen I to simulate the extracellular matrix. The organoids are then allowed to grow and imaged at fixed intervals to investigate leader cell development and directed collective migration [24].

3. Morphological differences between leader and follower cancer cells

Leader cells have been identified as enlarged cells with prominent actin fibers at the cellular leading end and have elevated β-actin mRNA levels. On the other hand, follower cells have smaller size and lower levels of β-actin mRNA [25]. The increased expression of β-actin mRNA in leader cells is linked to the formation of focal adhesions and lamellipodia. Leader cells with high β-actin mRNA levels exhibit large, distinct focal adhesions at the migrating front, while non-leader cells have fewer focal adhesions and lower β-actin mRNA levels [25].

Morphological studies of leader and follower cells from the invasion packs of H1299 human non-small cell lung carcinoma cells showed distinct differences [26]. Follower cells predominantly have mitochondria around the nucleus, whereas leader cells have mitochondria more frequently at the cell periphery. This peripheral mitochondrial distribution in leader cells indicates a higher energy demand at the leading edge. Increased pyruvate dehydrogenase (PDH) activity correlates with the mitochondrial localization at the cell periphery [26] While follower cells revert to the parental phenotype after a few generations, leader cells maintain their phenotype indefinitely [26].

4. Proteomic differences between leader and follower cancer cells

Naoya Yamaguchi and his team studied the collective migration of Madin-Darby Canine Kidney (MDCK) epithelial cells following a leader cell.[21] They found that the removal of a leader cell from the migrating cell pack with a micromanipulator stopped the cohesive migration of the remaining follower cells. The study revealed active Rac, integrin β1, and PI3K to be localized in the leading end of leader cells, but not in follower cells. Inhibiting Rac, integrin β1, and PI3K disrupted the collective migration. It was also discovered that Rac1, rather than integrin β1, mediated PI3K activation in leader cells.[21]

Cancer-associated fibroblasts (CAFs) have been identified as leader cells directing the collective tumor migration.[27] When spheroids of A431 human epidermoid carcinoma cells were co-cultured with CAFs, the CAFs reversed their polarity and migrated away from the spheroid, followed closely by A431 cells. CAFs remained in close contact with cancer cells in a leader/follower organization. This leader/follower relationship was maintained through direct physical interactions, with CAFs exerting a dragging force on the cancer cells. Furthermore, the co-cultured cells exhibit colocalization of E-cadherin, N-cadherin, β-catenin, and F-actin at their contact points. Knocking out E-cadherin in CAFs significantly reduced the leader cell population. Similarly, reducing N-cadherin expression in CAFs also decreased the number of leader cells, indicating that E-cadherin/N-cadherin junctions are essential for the leading effect provided by CAFs on A431 cells [27].

Cancer stem-like cells (CSCs) have been also reported to act as leader cells in the collective cancer invasion of MCF7 and MDAMB-231 breast cancer cells in a 3D invasion assay [28]. Studies have shown that when spheroids have CSCs and non-stem-like cancer cells (NSCCs) at a 1:1 ratio, CSCs are predominantly distributed at the outer layer of the spheroids. These CSCs, either $Nanog^{+}$ or $CD44^{+}CD24^{-}$, were primarily located in the leading position of the invading chain, driving the collective invasion. Moreover, the use of salinomycin, a selective inhibitor of $CD44^{+}CD24^{-}$ CSCs, reduced the peripheral distribution of CSCs on the cell spheroids and diminished the collective cell invasion. Most leader cells were found to simultaneously express E-cadherin, N-cadherin, and Nanog markers. whereas, most follower cells only expressed E-cadherin alone [28].

Cuixia Yang and co-workers identified that in luminal breast carcinomas, cancer cells expressing high levels of CD44 ($CD44^{hi}$ cells) have high migration rates and lead the collective invasion, guiding other cancer cells expressing low levels of CD44 ($CD44^{lo}$ cells) that have lower migration rates and act as follower cells [29]. When CD44 was depleted, the migration of $CD44^{hi}$ leader cells was inhibited, whereas when the expression of CD44 was stimulated, the motility of $CD44^{lo}$ follower cells increased. $CD44^{lo}$ cells have the avility to convert into new $CD44^{hi}$ cells. Similarly, $CD44^{hi}$ leader cells could transition to $CD44^{lo}$ follower cells during the collective migration. The $CD44^{hi}$ leader cells exhibited higher expression of genes related to cell motility and extracellular matrix modification, such as MMP2, MMP9, Plau, and CLDN-1, -3, -11, and -7. Additionally, $CD44^{hi}$ leader cells expressed several mesenchymal markers not present in $CD44^{lo}$ follower cells. Moreover, $CD44^{hi}$ cells displayed a decrease in epithelial markers like CDH1 (E-cadherin), JUP (γ-catenin), KRT8, and KRT18, and an increase in mesenchymal markers like FN1 (fibronectin), VIM (vimentin), COX-2 (Ptgs2), and ZEB2 [29].

Invasion study of mouse tumor organoid invasion in a microfluidic system revealed that breast cancer leader cells expressing keratin 14 ($K14^{+}$ cells) could migrate to the leading edge in response to both biochemical signals (chemokine gradients) and biomechanical factors (interstitial fluid flow) [24]. When the CXC chemokine receptor (CXCR4) was modulated by its ligand, stromal cell-derived factor 1 (SDF1), cancer cells actively migrated towards and accumulated at the organoid's edge with the highest SDF1 concentration. Organoids depleted of CXCR4, as well as wild-type organoids treated with CXCR4 inhibitors, lost their ability to migrate directionally in response to SDF1 exposure. Furthermore, the discoidin domain receptor 2 (Ddr2) also contributed to the migration of breast cancer leader cells. Organoids from Ddr2-/- mice, as well as wild-type

organoids treated with Ddr2 inhibitors, were unable to migrate.[24]

Cathepsin B (CTSB) has been identified as a key factor in the defining leader cells in salivary adenoid cystic carcinoma (SACC) [30]. CTSB was significantly overexpressed at the invasive front of SACC compared to the central tumor area. In a three-dimensional spheroid invasion assay, CTSB expression was exclusively observed in leader cells. Reducing CTSB levels inhibited the migration and invasion capabilities of SACC-83 cells and disrupted the development of leader cells [30].

5. Metabolic differences between leader and follower cancer cells

MDA-MB-231 breast cancer cells was found to be able to switch between leader and follower cells roles during collective invasion, which reduces the lifespan of leader cells [31]. This role-switching is driven by the rapid depletion of energy reserves in leader cells, necessitating their transition with follower cells. Metabolic analysis showed that leader cells exhibit higher glucose uptake compared to follower cells and further increase glucose uptake when invasion becomes challenging. Additionally, glucose deprivation shortened the lifespan of leader cells, while boosting cellular energy levels through AMP-activated kinase (AMPK) activation extended it. Moreover, leader cells require their intracellular ATP/ADP ratio to surpass a certain threshold for effective invasion [31[

Cytotoxicity screening of H1299 leader and follower cancer cells demonstrated that leader cells exhibited high resistance to most tested compounds compared to follower cells, except for certain antibiotics, including alexidine, chlorhexidine, and dequalinium.[26] The significant sensitivity of leader cells to the mitochondria-targeting agent alexidine implicated the crucial role of mitochondrial function in the survival of cancer leader cells. Alexidine-induced metabolic reprogramming in leader cells through S293 phosphorylation of PDH. On the other hand, H1299 follower cells had lower basal respiration and enhanced glucose uptake and lactate production, indicating increased glycolysis compared to leader cells.[26] Several intermediates in glycolysis, such as fructose-6-phosphate, fructose 1,6-bisphosphate, dihydroxyacetone phosphate, diphosphoglycerate, 3-phosphoglyceric acid, 2-phosphoglyceric acid, and phosphoenolpyruvate, were present in higher levels in follower cells. Additionally, the elevated levels of the pentose phosphate pathway (PPP) intermediate ribose-5-phosphate (R5P), along with the lower glucose-6-phosphate (G6P), suggested upregulated PPP activity in follower cells. Furthermore, H1299 follower cells expressed higher levels of glucose transporter 1 (GLUT1) compared to leader cells, supporting their increased glucose uptake and elevated glucose metabolism. Conversely, H1299 leader cells had higher oxidative phosphorylation (OXPHOS) than follower cells, as evidenced by their high sensitivity to the Complex I inhibitors piericidin and metformin [26].

Brian Cunniff and his team observed that mitochondria tend to move from to the cellular leading end (pseudopodia) in several cell types, including SKOV-3 human ovarian adenocarcinoma cells, human malignant mesothelioma, B16F10 mouse melanoma, HeLa cells, and REF52 rat embryo fibroblasts [32]. The metabolic profiles of SKOV-3 cell bodies and pseudopodia revealed that the former exhibited Warburg effect, characterized by the high glucose uptake and glycolysis [32]. SKOV-3 psueudopodia had ATP levels higehr than cell bodies, indicating that mitochondria are the ATP source in pseudopodia. Treating pseudopodia with the microtubule inhibitor nocodazole eliminated pseudopodial ATP, suggesting the release of mitochondria from leading edge structures. Moreover, exposing SKOV-3 cell bodies to the mitochondrial inhibitor oligomycin increased glycolytic flux and ATP production. Conversely, exposing SKOV-3 cell bodies to the glycolysis inhibitor 3-bromopyruvate suppressed glycolysis and ATP synthesis while increased mitochondrial respiration. In contrast, inhibiting glycolysis in pseudopodia did not affect mitochondrial respiration or ATP synthesis, whereas mitochondrial inhibition in the pseudopodia decreased ATP synthesis without affecting the glycolysis [32].

6. Transcriptomic differences between of leader and follower cancer cells

Transcriptomic analysis of H1299 leader and follower cancer subpopulations revealed the existence of 14 mutations that were selectively enriched in either leader or follower cells: six mutations were specific to leader cells, and eight to follower cells [23]. A notable leader-specific mutation was identified in Actin-related protein 3 (ARP3), known for promoting cellular migration by facilitating lamellipodia protrusion [23]. The overexpression of ARP3 is linked to the promotion of invasion, metastasis, and poor prognosis [33, 34] When a mutated ARP3 variant was introduced into ARP3-knockdown follower cells, their invasive ability increased.23 Additionally, lysine demethylase 5B (KDM5B) was identified as a site of leader cells' specific mutation [23]. KDM5B is involved in catalyzing the removal of di- and trimethylation from methylated histone H3, thereby regulating the invasive and migratory ability of cancer cells [35, 36]. The overexpression of wildtype KDM5B in leader cells suppressed their invasive behavior. Conversely, the overexpression of a mutant KDM5B enhanced the invasive ability of leader cells [23]. Transcriptome profiling of H1299 leader and follower cells also revealed vascular endothelial growth factor (VEGF) signaling transcripts to be increased in leader cells compared to follower cells [20]. Leader cells were observed to employ unconventional vasculogenesis signaling mechanisms, such as secreting VEGF to attract

follower cells and facilitate the formation of invasive cell chains [20]. In contrast, follower cells were found to enhance the growth of leader cells by increasing their mitotic efficiency [20]. This suggested a potential symbiotic relationship between leader and follower cancer cells within the collective invasion cohort [20].

In a wound healing assay, leader cells near the wound edge exhibited elevated levels of Delta-like ligand 4 (Dll4) mRNA, while other cells either had decreased or normal Dll4 levels and acted as follower cells during migration [25]. Dll4, known as a Notch ligand originally discovered in arterial endothelium, dynamically controls the balance between tip cells and stalk cells in response to angiogenic stimulators, preventing excessive tip cell formation during angiogenesis [36]. Additionally, treatment with DAPT, an inhibitor of Notch receptor cleavage and signaling, reduced Notch1 mRNA and protein expression in leader cells, suggesting autoregulation of Notch1. Furthermore, leader cell numbers with overexpressed Dll4 levels was observed to rise at the boundary. Conversely, exposure to Jagged-1, another Notch ligand, overexpressed Notch1 levels and decreased the number of leader cells. Moreover, treatment with Dll4 short interfering RNA (siRNA) also decreased the number of leader cells [25].

7. Conclusion

In conclusion, the study of leader and follower cancer cell dynamics has illuminated intricate mechanisms underlying collective invasion and metastasis. Metabolomic analyses have highlighted distinct metabolic profiles between leader and follower cells, with leaders often exhibiting heightened glycolytic activity and followers showing enhanced oxidative phosphorylation. Proteomic investigations have identified key proteins such as actin-related protein 3 (ARP3) and lysine demethylase 5B (KDM5B), which play pivotal roles in promoting invasive behavior and regulating chromatin modifications, respectively. Transcriptomic studies have revealed significant differences in gene expression related to VEGF signaling, focal adhesion dynamics, and Notch pathway regulation, underscoring the complex interplay of signaling networks in driving collective cell migration.

Understanding these differential profiles has profound implications for therapeutic strategies aimed at disrupting collective invasion. Targeting specific metabolic vulnerabilities of leader cells, such as their reliance on glycolysis or mitochondrial function, holds promise for inhibiting their migratory and invasive capacities. Similarly, interventions targeting key proteins identified through proteomic studies, such as ARP3 and KDM5B, could provide new avenues for therapeutic intervention in metastatic disease.

Moving forward, integrating metabolomic, proteomic, and transcriptomic approaches will be crucial for deciphering the full spectrum of molecular mechanisms governing leader-follower interactions in cancer metastasis. Future research efforts should focus on elucidating how these molecular signatures evolve over the course of disease progression and how they can be exploited for developing more effective personalized therapies against metastatic cancer.

8. References

1. Tao J, Yang G, Zhou W, Qiu J, Chen G, Luo W, et al. Targeting hypoxic tumor microenvironment in pancreatic cancer. J Hematol Oncol. 2021;14:1-25. https://doi.org/10.1186/s13045-020-01030-w
2. García-Caballero M, Sokol L, Cuypers A, Carmeliet P. Metabolic reprogramming in tumor endothelial cells. Int J Mol Sci. 2022;23:11052. https://doi.org/10.3390/ijms231911052
3. Guan X. Cancer metastases: challenges and opportunities. Acta Pharm Sin B. 2015;5:402-18. https://doi.org/10.1016/j.apsb.2015.07.005
4. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell. 2011;147:275-92. https://doi.org/10.1016/j.cell.2011.09.024
5. Boumahdi S, de Sauvage FJ. The great escape: tumour cell plasticity in resistance to targeted therapy. Nat Rev Drug Discov. 2020;19:39-56. https://doi.org/10.1038/s41573-019-0044-1
6. Chang L, Graham PH, Hao J, Bucci J, Cozzi PJ, Kearsley JH, et al. Emerging roles of radioresistance in prostate cancer metastasis and radiation therapy. Cancer Metastasis Rev. 2014;33:469-96. https://doi.org/10.1007/s10555-014-9493-5
7. Mal A, Bukhari AB, Singh RK, Kapoor A, Barai A, Deshpande I, et al. EpCAM-mediated cellular plasticity promotes radiation resistance and metastasis in breast cancer. Front Cell Dev Biol. 2021;8:597673. https://doi.org/10.3389/fcell.2020.597673
8. Xiang Y, Liu X, Wang Y, Zheng D, Meng Q, Jiang L, et al. Mechanisms of resistance to targeted therapy and immunotherapy in non-small cell lung cancer: promising strategies to overcoming challenges. Front Immunol. 2024;15:1366260. https://doi.org/10.3389/fimmu.2024.1366260
9. Chang JC. Cancer stem cells: Role in tumor growth, recurrence, metastasis, and treatment resistance. Medicine. 2016;95 https://doi.org/10.1097/MD.0000000000000476
10. Karami Fath M, Azargoonjahromi A, Kiani A, Jalalifar F, Osati P, Akbari Oryani M, et al. The role of epigenetic modifications in drug resistance and treatment of breast cancer. Cell Mol Biol Lett. 2022;27:52. https://doi.org/10.1186/s11658-022-00344-6
11. Bozic I, Nowak MA. Timing and heterogeneity of mutations associated with drug resistance in metastatic cancers. Proc Natl Acad Sci U S A. 2014;111:15964-8. https://doi.org/10.1073/pnas.1412075111
12. Nguyen DX, Bos PD, Massagué J. Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer. 2009;9:274-84. https://doi.org/10.1038/nrc2622
13. Ganesh K, Massague J. Targeting metastatic cancer. Nat Med. 2021;27:34-44. https://doi.org/10.1038/s41591-020-01195-4
14. Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. Nat Rev Cancer. 2009;9:239-52. https://doi.org/10.1038/nrc2618

15. Tarragó-Celada J, Cascante M. Targeting the metabolic adaptation of metastatic cancer. Cancers (Basel). 2021;13:1641. https://doi.org/10.3390/cancers13071641
16. Wang H, Pan J, Barsky L, Jacob JC, Zheng Y, Gao C, et al. Characteristics of pre-metastatic niche: the landscape of molecular and cellular pathways. Mol Biomed. 2021;2:1-32. https://doi.org/10.1186/s43556-020-00022-z
17. Friedl P, Gilmour D. Collective cell migration in morphogenesis, regeneration and cancer. Nat Rev Mol Cell Biol. 2009;10:445-57 https://doi.org/10.1038/nrm2720
18. Wang X, Enomoto A, Asai N, Kato T, Takahashi M. Collective invasion of cancer: Perspectives from pathology and development. Pathol Int. 2016;66:183-92. https://doi.org/10.1111/pin.12391
19. Chen BJ, Wu JS, Tang YJ, Tang YL, Liang XH. What makes leader cells arise: Intrinsic properties and support from neighboring cells. J Cell Physiol. 2020;235:8983-95. https://doi.org/10.1002/jcp.29828
20. Konen J, Summerbell E, Dwivedi B, Galior K, Hou Y, Rusnak L, et al. Image-guided genomics of phenotypically heterogeneous populations reveals vascular signalling during symbiotic collective cancer invasion. Nat Commun. 2017;8:15078. https://doi.org/10.1038/ncomms15078
21. Yamaguchi N, Mizutani T, Kawabata K, Haga H. Leader cells regulate collective cell migration via Rac activation in the downstream signaling of integrin β1 and PI3K. Sci Rep. 2015;5:1-8. https://doi.org/10.1038/srep07656
22. Khatib TO, Amanso AM, Pedro B, Knippler CM, Summerbell ER, Zohbi NM, et al. A live-cell platform to isolate phenotypically defined subpopulations for spatial multi-omic profiling. bioRxiv. 2023. https://doi.org/10.1101/2023.02.28.530493
23. Zoeller EL, Pedro B, Konen J, Dwivedi B, Rupji M, Sundararaman N, et al. Genetic heterogeneity within collective invasion packs drives leader and follower cell phenotypes. J Cell Sci. 2019;132 https://doi.org/10.1242/jcs.231514
24. 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;79:1899-912. https://doi.org/10.1158/0008-5472.CAN-18-2828
25. Riahi R, Sun J, Wang S, Long M, Zhang DD, Wong PK. Notch1-Dll4 signalling and mechanical force regulate leader cell formation during collective cell migration. Nat Commun. 2015;6:6556. https://doi.org/10.1038/ncomms7556
26. Commander R, Wei C, Sharma A, Mouw J, Burton L, Summerbell E, et al. Subpopulation targeting of pyruvate dehydrogenase and GLUT1 decouples metabolic heterogeneity during collective cancer cell invasion. Nat Commun. 2020;11:1533. https://doi.org/10.1038/s41467-020-15219-7
27. Labernadie A, Kato T, Brugués A, Serra-Picamal X, Derzsi S, Arwert E, et al. A mechanically active heterotypic E-cadherin/N-cadherin adhesion enables fibroblasts to drive cancer cell invasion. Nat Cell Biol. 2017;19:224-37. https://doi.org/10.1038/ncb3478
28. Quan Q, Wang X, Lu C, Ma W, Wang Y, Xia G, et al. Cancer stem-like cells with hybrid epithelial/mesenchymal phenotype leading the collective invasion. Cancer Sci. 2020;111:467-76. https://doi.org/10.1111/cas.14285
29. Yang C, Cao M, Liu Y, He Y, Du Y, Zhang G, et al. Inducible formation of leader cells driven by CD44 switching gives rise to collective invasion and metastases in luminal breast carcinomas. Oncogene. 2019;38:7113-32. https://doi.org/10.1038/s41388-019-0899-y
30. Wu JS, Li ZF, Wang HF, Yu XH, Pang X, Wu JB, et al. Cathepsin B defines leader cells during the collective invasion of salivary adenoid cystic carcinoma. Int J Oncol. 2019;54:1233-44 https://doi.org/10.3892/ijo.2019.4722
31. Zhang J, Goliwas KF, Wang W, Taufalele PV, Bordeleau F, Reinhart-King CA. Energetic regulation of coordinated leader-follower dynamics during collective invasion of breast cancer cells. Proc Natl Acad Sci U S A. 2019;116:7867-72. https://doi.org/10.1073/pnas.1809964116
32. Cunniff B, McKenzie AJ, Heintz NH, Howe AK. AMPK activity regulates trafficking of mitochondria to the leading edge during cell migration and matrix invasion. Mol Biol Cell. 2016;27:2662-74. https://doi.org/10.1091/mbc.e16-05-0286
33. Lv J, Liu J, Xiao M, Xu H, Xu C, Zhang X, et al. ARP3 promotes tumor metastasis and predicts a poor prognosis in hepatocellular carcinoma. Pathol Res Pract. 2018;214:1356-61. https://doi.org/10.1016/j.prp.2018.05.028
34. Yang ZL, Miao X, Xiong L, Zou Q, Yuan Y, Li J, et al. CFL1 and Arp3 are biomarkers for metastasis and poor prognosis of squamous cell/adenosquamous carcinomas and adenocarcinomas of gallbladder. Cancer Invest. 2013;31:132-9. https://doi.org/10.3109/07357907.2012.756113
35. Yoo J, Kim GW, Jeon YH, Kim JY, Lee SW, Kwon SH. Drawing a line between histone demethylase KDM5A and KDM5B: their roles in development and tumorigenesis. Exp Mol Med. 2022;54:2107-17. https://doi.org/10.1038/s12276-022-00902-0
36. Dai B, Huang H, Guan F, Zhu G, Xiao Z, Mao B, et al. Histone demethylase KDM5A inhibits glioma cells migration and invasion by down regulating ZEB1. Biomed Pharmacother. 2018;99:72-80. https://doi.org/10.1016/j.biopha.2018.01.020