Hypoxia response pathway in border cell migration

Inna Djagaeva and Sergey Doronkin*
Department of Anatomy and Neurobiology; University of Tennessee Health Science Center; Memphis, TN USA

Cell invasion and metastasis mark the most lethal phase of cancer, but little is known about the key molecular events that initiate this crucial turning point. Low oxygen, or hypoxia, is thought to be one trigger for metastasis. Hypoxic conditions within the tumor mass are thought to activate signaling pathways that stimulate invasiveness of cancer cells spreading the disease. However, the molecular basis of this process is not well understood. A recent study used Drosophila ovarian border cell migration to model the type of cell migration that occurs in tumors in response to oxygen deprivation through the activation of the hypoxia response pathway (Doronkin et al. Oncogene. 2009). This model organism approach revealed a highly sophisticated mechanism of control of cell migration that is regulated by multiple genetic inputs tied to the hypoxic response. Genetic manipulations with the components of the HIF-1 (hypoxia-inducible factor 1) pathway were able to either inhibit or block the migration of border cells or cause unprecedented acceleration of their migration. The HIF-1-mediated transcriptional cascade appears to be the major regulator of border cell locomotion. Based on the similarity of the fly and human HIF-1 pathways, this model organism study might lead to improvements in understanding hypoxia-induced metastasizing of human cancers. This article discusses new findings in the context of their relevance to cancer metastasis and speculates on the potential regulatory mechanisms and future research directions.

Introduction

The ability of tumor cells to metastasize to other locations in the body is the underlying cause of fatality in most cancers, but how tumor cells acquire this characteristic capability remains unclear. One trigger for metastasis is thought to be low oxygen tensions. Aggressive tumors rapidly surpass the capacity of the nearest blood vessel, leaving pockets of cells in a condition known as hypoxia, or low oxygen. Oxygen starvation causes the cells to migrate to distant sites and colonize organs where nutrients and space are less limiting. In fact, tumors that contain large regions of hypoxia are more likely to metastasize.1

Hypoxia stresses cells and activates survival mechanisms, including changes in gene expression, many of which result from the evolutionarily conserved mechanism involving the transcription factor HIF-1 (hypoxia-inducible factor 1). The HIF-1 transcription factor contains two subcomponents, HIF-1α (Sima in Drosophila) and HIF-1β (Tango in Drosophila). Cells adjust to hypoxia by inappropriate stabilization of HIF-1α/Sima, which is highly unstable in normal oxygen conditions. HIF-1α binds to the constitutively expressed HIF-1β to create an active transcription factor, launching the global hypoxic expression program. HIF-1-driven transcription promotes a variety of functions for cell survival and is thought to be important in tumor migration.2

Cell migration can generally be divided into two forms: single-cell migration and collective cell migration. Guided
migration of individual cells can be studied fairly easily and in simple environments of cultured cells. Therefore, single-cell migration is relatively well characterized. On the contrary, coordinated guided migration of cell groups remains poorly understood, despite its direct relevance to organogenesis, wound healing, and a key role in cancer metastasis. Perhaps the major obstacle in such studies is the lack of an adequate experimental model. An obvious disadvantage of studies of collective cell locomotion, in particular hypoxia-induced, is that they have been carried out primarily ex vivo in cultured cells, isolated from the comprehensive signaling networks that underlie guided cell migration in vivo.

Border cells in the Drosophila ovary are a group of eight to ten epithelial cells that become invasive and eventually perform a well-defined and guided migration during egg chamber development. Resulting from the spectacular work mostly from the Montell lab and Rorth lab, border cell migration emerges as a highly pliable model for studying epithelial to mesenchymal transition and directional collective cell migration. One of the major benefits of this model is that cells migrate in natural physiological conditions during actual living development.

Using border cell migration as a model for hypoxia-induced metastasizing, it has been recently detected that cell invasive-ness is under the sophisticated dose-dependent control of the HIF-1-dependent hypoxia response pathway.

**Short- and Long-Term Hypoxia Distinctly Affects Border Cell Migration**

One of the most interesting and puzzling findings from recent studies of border cell migration in low oxygen was a connection between the severity of hypoxia and the efficiency of cell migration. In particular, border cell migration was differentially regulated by the duration of oxygen starvation: short-term hypoxia (1% O2, 4 h) acted to inhibit cell migration while long-term hypoxia (1% O2, 8 h) often stimulated cell migration. The well-established mechanism of stabilization of HIF-1α/Sima when oxygen is needed suggests that, upon initiation of hypoxia, levels of Sima increase in the border cells. This result, along with the results on ectopic HIF-1α/Sima expression, indicated that the delays in cell migration seen in short-term hypoxia involved elevated levels of HIF-1α/Sima.

However, the situation appears to be more complex in long-term hypoxia, because this severe oxygen deprivation acted to stimulate border cell migration. Interestingly, stimulated migration was also detected in clusters with reduced HIF-1 activity. Does it mean that long-term hypoxia actually leads to reduced HIF-1 activity in border cells? If yes, then during border cell migration there are two distinct mechanisms of response to short- and long-term hypoxia. These important issues have to be clarified experimentally. Focusing on the mechanisms of accelerated border cell migration is especially important and may help understand how to inhibit or block cancer cell migration.

Relevant here, a discriminating control of HIF-1 regulation in response to short- and long-term hypoxia was reported in other systems. In a human lung epithelial cell line, HIF-1α protein levels were induced by acute hypoxia; however HIF-1α protein stimulation disappeared in prolonged hypoxia. Similar results were obtained in other studies in cells and mice. These results support the hypothesis that the HIF-1 pathway in border cells responds differently to short- and long-term hypoxia, thereby leading to distinct effects on migration.

These distinct mechanisms of hypoxia response, if they exist, could be operated by the specific feedback mechanisms, which are activated correspondingly to the duration of hypoxia. Alternatively, there could be an eventual uncontrollable reduction of the HIF-1-mediated effect, for example, due to a depletion of cellular resources. Oxygen shortage suppresses the metabolic rate to conserve energy and promote survival. Normally protein synthesis consumes 25–30% of the produced ATP, while gene transcription consumes only 1–5%. Therefore, it is not surprising that energy-consuming mRNA translation appears to be one of the most affected processes in hypoxia. Hypothetically, it may then reproduce a situation of the loss of HIF-1 and stimulate cell migration.

The distinct effect of the severity of hypoxia on border cell migration is intuitively understandable, if considered in the context of cancer metastasis. As tumors grow, the progressively increasing degree of hypoxia within the tumor is thought to promote metastasis. Apparently, whereas acute hypoxia stabilizes HIF-1 and activates the adaptation mechanisms in cellular metabolism, extensive oxygen starvation eventually diminishes the HIF-1 effect stimulating cell migration.

**Dualistic Nature of HIF-1 Function**

There is some degree of controversy in terms of defining the exact role of HIF-1 in tumor cell physiology. Independent of any specific mechanism, it has been demonstrated in multiple studies of cancer patients with a variety of tumor types that a high level of expression of HIF-1 subunits correlates with unfavorable prognosis and decreased survival. For example, increased expression of HIF-1α in human cancer cells has been shown to cause increased tumor growth, angiogenesis and metastasis. On the other hand, in both immunohistochemical analyses and animal studies, HIF-1α overexpression has been reported to be associated with increased patient survival. In some of these cases, the induction of downstream genes by HIF-1 may play an important role in promoting patient survival.

Most certainly, these controversial results represent a deficiency in the comprehensive understanding of the function of HIF-1, in particular during the process of metastasis.

This dualistic nature of HIF-1 function has started to get less controversial and confusing, at least in regulating border cell migration. The rather unexpected result that HIF-1 can either promote or repress cell migration, depending on the changes in the levels of HIF-1, has been demonstrated. Distinct migratory response of the border cells to different doses of HIF-1α/Sima revealed a nonlinear, or cyclic, character of HIF-1-dependent regulation, which might suggest that, in cancer cells, changing HIF-1 levels can be either deleterious or, quite
the opposite, stimulating for cell migration, depending on the particular levels of HIF-1.

HIF-1 regulates expression of over 100 genes that control the key aspects of tumor-igenesis, including invasion and metastasis, and many more genes are affected by downstream cascades. The specific responses in border cell migratory behavior to different doses of HIF-1 may suggest that different levels of HIF-1 contribute to proportional activation or repression of the different characteristic expression profiles. Because HIF-1-dependent genes can be inhibitors or activators of migration, it seems reasonable to hypothesize that resulting effects on migration would vary depending on the expression profile at a particular dose of HIF-1.

HIF-1 and Guidance of Migration

Cell migrations within the multicellular organism are usually guided. Cancer cells are thought to use preexisting host mechanisms that direct cell migration during normal physiological processes. In particular, cancer cells use chemotactic interactions for homing to preferential metastatic sites. Moreover, these interactions can dictate the rate of metastasis. Cancer cells do not migrate to expected sites of metastasis that no longer express the corresponding chemokine ligands and/or receptors. Drosophila border cells are also receptive to substrate microenvironment composition and normally employ a similar guidance mechanism. Migration of border cell is guided by two receptor tyrosine kinases (RTKs), PDGF- and VEGF-related receptor (PVR) and epidermal growth factor receptor (EGFR). Both receptors act redundantly during posterior migration toward the oocyte. If both PVR and EGFR are simultaneously disturbed, guidance fails and the cells do not reach their destination. Given the well-known, strong connection among hypoxia, the HIF-1 pathway, and, for example, VEGF, it could be expected that hypoxia regulates the rate of border cell migration by altering the guidance of migration. However, this hypothesis contradicts previous reports, at least in the case of accelerated migration. Induction and stimulation of border cell migration appears to be separable from reading the guidance, and guidance does not stimulate border cell motility. Thus, accelerated migration of border cells in long-term hypoxia occurs independent of the guidance.

In addition, the hypoxia response seems not to affect the ability of border cells to follow the guidance, because neither hypoxia nor genetic manipulations with the components of the hypoxia response pathway changed the vector of border cell migration. Although with different efficiency, cell clusters were always migrating toward the oocyte.

HIF-1 and Invasive Cell Transformation

During the process of metastasizing, the initially noninvasive cancer cells are thought to undergo transformation to become invasive. It has been suggested from work in other systems that the HIF-1 pathway is engaged in stimulating migration of initially nonmigratory cells. The mechanism of this HIF-1-mediated invasive transition is not clear.

The biology of increased motility of border cells involves reducing the levels of HIF-1, indirectly suggesting that reducing HIF-1 activity could be also associated with the mechanism of invasive cell transition. This hypothesis places HIF-1 in a position of a transcriptional switch to change the migratory state in cells in response to hypoxia. Invasive cell transition results in changes in cell behavior: induction of dynamic cellular extensions and release from prior attachments. Therefore, the mechanism of transition should involve changes in the regulation of cell-cell adhesion. For example, less tight contacts between cells might benefit cellular motility and vice versa. Indeed, one of the most characteristic changes during epithelial to mesenchymal transition is reduction in E-cadherin levels. A decreased accumulation of the key cell adhesion molecule E-cadherin in border cells with reduced HIF-1 function can be part of the potential mechanism of HIF-1-mediated invasive cell transition.

In border cells, E-cadherin expression and distribution are regulated via function of slow border cells (sIbo). The sIbo gene is absolutely required for border cell migration and encodes Drosophila CCAAT/enhancer binding protein (C/EBP), a basic region/leucine zipper transcriptional activator. Members of the C/EBP family have been shown to control the proliferation and differentiation of a variety of tissues and, of special notice, to transform normal mammary epithelial cells and induce epithelial to mesenchymal transition in culture. In border cells, E-cadherin is thought to be one of the critical targets for Slbo-dependent transcription: levels of E-cadherin were reduced in sIbo-mutants and elevated after overexpression of Slbo. In turn, expression of Slbo was altered in the sIma-mutant cells suggesting that function of Slbo is under the control of HIF-1. Thus, it appears that E-cadherin is regulated in border cells by the HIF-1/Slbo cascade. Intriguingly, C/EBP and HIF-1 pathways intersect in cancer and, moreover, hypoxia has been shown to regulate C/EBP in cancer cells. Therefore, potential involvement of the HIF-1/Slbo(C/EBP)/E-cadherin mechanism in regulation of cell invasiveness seems to be an interesting possibility.

Choosing the Leading Cell Within the Cluster

In border cells, formation of the E-cadherin-rich long cellular extensions from the leading cell clusters is clearly an essential first step in every round of migration through a “grapple and pull” mechanism. The mechanism of choosing the leading cell in the cluster is not known. However, it appears that the HIF-1 transcription factor plays a role here, too. Consistent detection of sima- or tango-mutant cells in the leading position in the cluster suggests that reducing HIF-1 levels is beneficial for obtaining the leading privilege in a group of collectively migrating cells. One obvious criterion for selecting the leading cell could be invasive capacity, so that only the most motile cell would lead a cell cluster. Reduction of HIF-1 function fits this criterion: the presence of sima- or tango-mutant cells in the leading position stimulated migration...
of the entire cluster. HIF-1-dependent regulation of E-cadherin-based cell adhesion could be one of the key components that define the leading cell fate.

It seems that generating small clones provided particularly beneficial conditions for cell migration due to rather gradual and mild reduction of the persisting pre-recombinant E-cadherin (as well as other molecules) in mutant cells. Perhaps only moderate reduction of E-cadherin levels can stimulate migration. Strong loss of E-cadherin is known to inhibit cell migration.

During border cell migration, leading cells are dynamically replaceable so that border cells take the leading position by rotation. The strong association between the sima- and tango-mutant cells and leading position suggests that normal rotation was affected and that these cells, having once taken the leading position, were not replaced. These findings imply that loss of HIF-1 benefits not only for getting, but also for maintaining, the leading status. This hypothesis needs further confirmation by live imaging. One potential reason for cells to be replaced by rotation is to prevent the leading cell from overexhausting its resources. On the other hand, the rotation of border cells may also represent a search for the most efficient cell. Regardless of the exact principle, the lack of replacement of the sima- or tango-mutant cells lets us hypothesize that this may not apply to a cell with an unusually high motile capacity (for example, HIF-1-deficient cells), so that the less motile cell cannot replace the more active one. Further research is needed to clarify this matter.

Role of the Leading Cell

The presence of the leading cell in clusters raises important questions regarding the general principles of collective cell migration and, in particular, the leadership duties of the leader cell. For example, would a more “active” leading cell cause accelerated cluster migration? Or will its efforts be compensated by the normal rate of release from prior attachments that occur at the “rear” side of the cluster? In other words, is there an intraclustering regulatory network, and, if yes, does the leading cell control this network?

The caravan is as fast as its slowest camel. Similarly, the resulting rate of collectively migrating groups of cells is defined by the speed of the slowest moving member of the cluster. Indeed, there are numerous reports that the presence of a mutant cell caused delayed migration of the mosaic cluster; in particular, even one slbo-mutant cell inhibited migration of the whole mosaic cluster. Therefore, it is of special interest that a single sima- or tango-mutant leading cell was capable of stimulating migration of the entire cluster.

This means that the nonmutant cells of the cell clusters did not block the increased migratory activities of the mutant leading cells. Instead, they migrated faster, following the leader. These findings suggest that, during border cell migration, changes in HIF-1 activity in the leading cell are imperative for behavior of the nonleading cells in the cluster. Therefore, the lagging cells correct their interactions with the surrounding substrates to release adhesion coordinately with the forward-bearing forces of the leading cell. In that manner, cells in the cluster are able to migrate together at a higher rate.

Understanding this phenomenon is important, because this type of migration may be directly relevant to metastasis. In particular, these results may help us to find a model where one cancer cell with the appropriate “pro-invasive” gene expression profile may initiate migration of a cluster of cells.

Concluding Remarks

The rapid advances in understanding the basic biology of hypoxia-induced cancer metastasis provide useful prognostic and therapeutic information and promise to find novel, potentially selective and effective therapies. However, multiple strategies and new unorthodox approaches should be taken to better understand how cancer metastasis can be prevented or controlled. Drosophila genetics, as always, offers the research world new knowledge and paradigms and opens new directions. Further analysis of the border cell model might reveal new details of mechanisms and factors that promote cell invasion and migration. There is a lot to be learned, and much work remains to be done at this point.

It is hoped that an in vivo experimental approach available in border cell migration, along with identifying the molecular mechanism, will bring new insight to the key aspects of hypoxia-related migratory behavior in a changing three-dimensional context. Given a high degree of the evolutionarily conservatism of the hypoxia response cascade, this would contribute to a better understanding of such critical, yet poorly understood, processes as the invasive cell transition, initiation and arrest of cell migration, and molecular programs that control migratory behavior during various migrating steps and transitions between them. The recently exposed nonlinear mechanism of HIF-1 control of cell migration raises important question about the specific transcriptional regulation of these processes. Hopefully, identifying the targets of HIF-1 transcriptional regulation in border cells will lead to improvements in the current concept of hypoxia-induced metastasis in human cancers and will build a molecular basis for the development of novel therapeutics.

References

1. Chaudary N, Hill RP. Hypoxia and metastasis in breast cancer. Breast Dis 2006; 26:55-64.
2. Rankin EB, Giaccia AJ. The role of hypoxia-inducible factors in tumorigenesis. Cell Death Differ 2008; 15:678-85.
3. Servant G, Weiner OD, Herzmark P, Balla T, Sedat JW, Bourne HR. Polarization of chemotactic receptor signaling during neutrophil chemotaxis. Science 2000; 287:1037-40.
4. Parent CA, Devreotes PN. A cell's sense of direction. Science 1999; 284:765-70.
5. Friedl P, Hegerfeldt Y, Tusch M. Collective cell migration in morphogenesis and cancer. Int J Dev Biol 2004; 48:441-9.
6. Rorh P. Initiating and guiding migration: lessons from border cells. Trends Cell Biol 2002; 12:325-31.
7. Starz-Gaiano M, Montell DJ. Genes that drive invasion and migration in Drosophila. Curr Opin Genet Dev 2004; 14:86-91.
8. Rorh P. Collective cell migration. Annu Rev Cell Dev Biol 2009; 25:407-29.
9. Jang AC, Starz-Gaiano M, Montell DJ. Modeling migration and metastasis in Drosophila. J Mammary Gland Biol Neoplasia 2007; 12:103-14.
10. Prasad M, Montell DJ. Cellular and molecular mechanisms of border cell migration analyzed using time-lapse live-cell imaging. Dev Cell 2007; 12:997-1005.
11. Bianco A, Poukula M, Cliffe A, Mathieu J, Luque CM, Falga TA, Rorh P. Two distinct modes of guidance signalling during collective migration of border cells. Nature 2007; 448:362-5.
12. Desvignes S, Dajac L, Nagle ME, Reiter LT, Seagraves TN. Dose-dependent modulation of HIF-1alpha/simA controls the rate of cell migration and invasion in Drosophila ovari border cells. Oncogene 2009; 29:1123-34.
13. Uchida T, Rossignol F, Marthay MA, Mounier R, Courtte S, Clottes E, Clerici C. Prolonged hypoxia differentially regulates hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha expression in lung epithelial cells: implication of natural antipode HIF-1alpha. J Biol Chem 2004; 279:14871-8.

14. Yu AY, Frid MG, Shimoda LA, Wiener CM, Stenmark K, Semenza GL. Temporal, spatial and oxygen-regulated expression of hypoxia-inducible factor-1 in the lung. Am J Physiol 1998; 275:818-26.

15. Sato M, Tanaka T, Maeto T, Sando Y, Suga T, Maeto Y, et al. Inducible expression of endothelial PAS domain protein-1 by hypoxia in human lung adenocarcinoma A549 cells. Role of Scl family kinases-dependent pathway. Am J Respir Cell Mol Biol 2002; 26:127-34.

16. Stroka DM, Burkhardt T, Desbuquois I, Wenger RH, Neil DA, Bauer C, et al. HIF-1 is expressed in normoxic tissue and displays an organ-specific regulation under systemic hypoxia. FASEB J 2001; 15:2445-53.

17. Cameron CM, Harding P, Hu WS, Kaufman DS. Activation of hypoxic response in human embryonic stem cell-derived embryoid bodies. Exp Biol Med (Maywood) 2008; 233:1044-57.

18. Rolfe DF, Brown GC. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol Rev 1997; 77:731-58.

19. Semenza GL. Evaluation of HIF-1 inhibitors as anti-cancer agents. Drug Discov Today 2007; 12:853-9.

20. Li L, Lin X, Staver M, Shoomaker A, Semizarov D, Fesik SW, Shen Y. Evaluating hypoxia-inducible factor-1alpha as a cancer therapeutic target via inducible RNA interference in vivo. Cancer Res 2005; 65:7249-58.

21. Hiraga T, Kizaka-Kondoh S, Hirokawa K, Hiroaki H, Yoneda T. Hypoxia and hypoxia-inducible factor-1 expression enhance osteolytic bone metastases of breast cancer. Cancer Res 2007; 67:4157-63.

22. Liao D, Corde C, Seagroves TN, Johnson RS. Hypoxia-inducible factor-1alpha is a key regulator of metastasis in a transgenic model of cancer initiation and progression. Cancer Res 2007; 67:563-72.

23. Beasley NJ, Leek R, Alam M, Turley H, Cox GJ, Gatter K, et al. Hypoxia-inducible factors HIF-1alpha and HIF-2alpha in head and neck cancer: relationship to tumor biology and treatment outcome in surgically resected patients. Cancer Res 2002; 62:2495-7.