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Preview

An Arresting Story about Basement Membrane Invasion

Andrew J. Ewald[1,*]
[1]Departments of Cell Biology, Oncology, and Biomedical Engineering, School of Medicine, Johns Hopkins University, 855 N. Wolfe Street, Rangos 452, Baltimore, MD 21205, USA
*Correspondence: andrew.ewald@jhmi.edu
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In this issue of *Developmental Cell*, Matus et al. (2015) reveal that to invade past basement membrane, the *C. elegans* anchor cell must cease dividing before differentiating and expressing pro-invasion genes. This demonstration of invasion and proliferation as mutually incompatible cell states has implications for our understanding of cancer metastasis.

Epithelial tissues are fundamental building blocks of metazoan organs and are characterized by a basement membrane (alternately termed the basal lamina) that separates the epithelial cells from the surrounding connective tissue. The basement membrane serves as both a physical barrier and a rich source of molecular signals. Cells can acquire the ability to cross the basement membrane, a process termed invasion (Rowe and Weiss, 2008). In this issue of *Developmental Cell*, Matus et al. (2015) exploit the genetic tools and morphological simplicity of the *C. elegans* anchor cell system (Sherwood and Sternberg, 2003) to study the relationship between cell proliferation, differentiation state, and invasive behavior. They reveal that invasion requires cell-cycle arrest and that G1 arrest triggers changes in differentiation state and induces expression of mature invasion markers.

Invasion past basement membrane is a key part of diverse developmental and disease processes, including metastasis (Rowe and Weiss, 2008). For example, in breast cancer, invasion is specifically assessed by pathologists to distinguish benign from malignant disease (Polyak, 2010). Immune cells must also cross the basement membrane each time they exit or enter a blood vessel (Rowe and Weiss, 2008), and developmental remodeling of epithelial organs requires a restructuring of the basement membrane to enable tissue growth (Harunaga et al., 2014). A major barrier to the molecular analysis of invasion in mammalian systems is the large number of cells and cell types and the uncertain timing of initiation of invasion. The authors overcame this challenge by focusing on a geometrically simple example of developmental invasion in the worm *C. elegans*.

During *C. elegans* larval development, a single specialized uterine cell, named the anchor cell, is specified to cross the basement membrane and connect the uterine and vulval tissues. The anchor cell is non-proliferative and initiates invasion at a precise time and location, so defects in invasion can readily be assessed in genetic screens. Matus and colleagues (2015) utilized an RNAi screen to identify genes whose depletion resulted in excess anchor cells that failed to invade. These observations motivate the hypothesis that an invading cell might need to be in a post-mitotic, differentiated state. Beyond the specific interest in this model system, the relationship between proliferation and invasion is highly interesting in the context of cancer, as numerous studies have provided correlative evidence that the most invasive cancer cells are minimally or non-proliferative (Cheung et al., 2013; Gao et al., 2005). These data led to the "Go or Grow" hypothesis, which suggests that invasion and proliferation are spatiotemporally distinct states and that it is unlikely or impossible for a cancer cell to be optimized for both states at once (Hatzikirou et al., 2012). In this model, progression to malignancy can be viewed as a grow phase to form the primary tumor, followed by a transition to a go phase to spread to distant organs, and then a second transition back to the grow phase to develop large metastatic tumors.

A challenge to validating this model is that tumors contain many cells, and it is uncertain which are in the grow phase and which are in the go phase. Even if the cell state could be correctly identified, it would be difficult to isolate the molecular regulators of the go-grow transitions in complex tissues. A counter-model to "Go or Grow" is to instead view proliferation and migration as distinct cell behaviors that are subject to separate molecular control but that can be activated either separately or simultaneously. This concept of a highly motile cell that also excels at proliferation is at the core of the "migrating cancer stem cell" model (Brabletz et al., 2005). The great strength of the present work from Matus et al. (2015) is the extent to which the authors succeed in demonstrating that, at least for the anchor cell system, invasion and proliferation are mutually incompatible cell states. Furthermore, this study demonstrates that acquisition of a molecular toolkit for basement membrane invasion requires G1 arrest and a histone deactelyase-dependent reprogramming of cellular differentiation.

The authors began with a transcription factor screen in which only uterine cells are sensitive to the RNAi. The gene they focused on was *nhr-67/tlx*, as its depletion resulted in multiple non-invading anchor cells. The correlation between excess cells and a lack of invasion supported the idea that proliferation and invasion are mutually exclusive states and that the loss of *nhr-67* led to proliferation in the normally post-mitotic anchor cells. Consistent with this model, there was only one anchor cell in *nhr-67(pf88)* mutants at the time of specification, and laser ablation of this single anchor cell prevented the multiple-cell phenotype. Furthermore, single *nhr-67(pf88)* anchor cells were observed to divide in real-time

imaging, while normal anchor cells expressed markers consistent with G1 arrest. Through an elegant series of genetic experiments, the authors elucidated the molecular connections between *nhr-67* and cell-cycle arrest. They first demonstrated that the Cip/Kip family CDK inhibitor *cki-1* is upregulated in anchor cells, that the *cki-1* promoter contains multiple *nhr-67* binding sites, and that *cki-1* levels are reduced in worms following *nhr-67* depletion. Strikingly, exogenous expression of *cki-1* was sufficient to force G1 arrest and rescue anchor cell invasion in *nhr-67(pf88)* worms, while forced arrest in S or G2 was not. Taken together, these data reveal that NHR-67 promotes basement membrane invasion by directing an anchor cell to arrest in G1.

The authors then demonstrated that *nhr-67* depletion resulted in loss of pro-invasion genes, such as matrix metalloproteinases (MMPs) and actin regulators. Consistent with the idea that the acquisition of invasive behavior requires G1 arrest, *cki-1* expression rescued expression of these genes. The next question became: How is G1 arrest mechanistically coupled to the onset of invasive differentiation? With this question in mind, the authors reexamined data from a published whole-genome RNAi screen (Matus et al., 2010) and identified a histone deacetylase that was required for anchor cell invasion, *hda-1*. Importantly, loss of *nhr-67* resulted in reduction in *hda-1* levels, suggesting that *hda-1* functions downstream of *nhr-67*. Consistent with this concept, *hda-1*-depleted anchor cells were correctly specified and non-proliferative; they simply lacked expression of invasion effectors, such as MMPs. These data identify *hda-1* as a critical molecular connection between G1 arrest and the acquisition of the invasive phenotype.

The core concept that emerges from the present study is one of invasion as a distinct non-proliferative cellular differentiation state. In the anchor cell model system, entry into this invasive state requires both G1 arrest and histone deacetylase-dependent differentiation. The critical challenge now is to test the extent to which this molecular pathway is recapitulated during mammalian developmental and disease processes. If validated, genes regulating the reciprocal transitions between proliferation and invasion would be highlighted as critical therapeutic targets in cancer.

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