Localized Lysosome Exocytosis Helps Breach Tissue Barriers

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Cell invasion across basement membrane barriers is important in both normal development and cancer metastasis. In this issue of *Developmental Cell*, Naegeli et al. (2017) identify a mechanism for breaching basement membranes. Localized lysosome exocytosis fuels generation of large, invasive cellular protrusions that expand tiny basement membrane openings.

Most animal organs and tissues are encased by basement membranes (BMs). BMs are dense, thin layers of extracellular matrix that serve as protective barriers and mark tissue boundaries. During certain developmental events and cancer metastasis, cells breach BM barriers in a two-stage process to establish contact with, or transmigrate into, neighboring tissues. At a first BM breaching stage, cells form invadosomes (e.g., invadopodia) that use proteases to degrade BM components and generate small openings (Eddy et al., 2017). However, it is unclear how cells accomplish the second major stage of greatly expanding the initially small BM openings to permit transmigration. In this issue of *Developmental Cell*, Naegeli et al. (2017) report an unexpected mechanism for expanding BM openings through localized lysosome exocytosis during *C. elegans* anchor cell invasion, using an impressive series of quantitative live imaging and genetic perturbation experiments (Figure 1A).

The two major stages of BM breaching can fulfill distinct functions. For example, BM breaching during mammalian organ development is restricted to the first stage, in which numerous microscopic openings are generated at growing epithelial tips to render BMs more distensible for tissue expansion while maintaining tissue integrity (Figure 1B; Harunaga et al., 2014). Epithelial cells can extend protrusions through these openings to expand these openings or contact neighboring cells for potential epithelial-mesenchymal signaling (Figure 1B). In other contexts, such as cancer metastasis, trophoblast implantation, and *C. elegans* anchor cell invasion, BM breaching proceeds to the second major stage, with substantial enlargement of small BM openings for cells to build inter-tissue connections and/or to transmigrate between tissues.

During *C. elegans* anchor cell invasion, the anchor cell breaches two juxtaposed BMs to build a passage connecting uterine and vulval tissues for passing fertilized eggs. This highly stereotypic process is ideal for characterizing BM breaching because of its amenability to genetic perturbation and live imaging (Hagedorn and Sherwood, 2011). Previous studies using this model revealed mechanisms of BM breaching, including guidance by netrin (UNC-6) signaling (Ziel et al., 2009), formation of multiple invadopodia for generating tiny holes in the BM (Hagedorn et al., 2013), non-proteolytic enlargement of the BM opening through BM sliding (Ihara et al., 2011), and a role for hemicentin (Morrissey et al., 2014). Interestingly, netrin signaling is not required for generating invadopodia and small BM breaches but is required for the subsequent formation of a large invasive protrusion during anchor cell invasion (Hagedorn et al., 2013).

Naegeli et al. (2017) searched for the source of plasma membrane required to build this netrin-dependent protrusion. After ruling out other membrane sources using fluorescence reporters for endoplasmic reticulum, Golgi apparatus, and endosomes, the authors discovered that lysosomal polarization toward the BM-contact site precedes invasive protrusion formation. Reducing lysosome numbers by uterine-specific knockdown of *ppk-3* strongly perturbed both invasive protrusion formation and anchor cell invasion, suggesting that lysosomes contribute the membrane needed to expand the invasive protrusion. They then demonstrated that netrin-DCC receptor interaction was crucial by genetic disruption and by successfully redirecting polarity toward ectopically expressed membrane-tethered netrin in a netrin null mutant. Thus, localized lysosome exocytosis directed by netrin-DCC signaling drives invasive protrusion formation in the anchor cell.

To identify the molecular machinery controlling localized lysosome exocytosis, Naegeli et al. (2017) used the powerful *C. elegans* RNAi toolkit to screen 116 genes encoding proteins involved in membrane trafficking, looking for perturbations in anchor cell invasion. This screen identified 22 candidate genes. Of these, they demonstrated by uterine-specific RNAi or genetic ablation that both SNAP-29 and the exocyst complex are important for invasive protrusion expansion, suggesting that they mediate localized lysosome exocytosis at the expanding protrusion.

Naegeli et al. (2017) then demonstrated that the invasive protrusion is a specialized plasma membrane domain demarcated by a diffusion barrier around the protrusion neck established by dystroglycan-mediated cell-BM adhesion. Importantly, dystroglycan knockdown disrupted both the diffusion barrier and invasive protrusion, suggesting that the anchor cell uses this ring-shaped cell-BM adhesion as a scaffold to support outward invasive protrusion.

This impressive paper from Naegeli et al. (2017) unravels a key piece of the BM breaching puzzle by bringing lysosomes into the spotlight to explain the poorly characterized second stage following invadopodia-mediated generation of small breaches. A particularly crucial concept from this study is that lysosomes can provide a local membrane source for building

an invasive cellular structure. A new puzzle, however, is why this release of lysosomal contents does not shrink cell volume, which actually increases as the large protrusion expands (Figure 1A). An important future direction will be to test the authors' speculation that osmotic swelling drives the volume increase, which might even contribute mechanical force for expanding small BM openings.

Numerous studies of mammalian cancer cell invasion have focused on the first stage of BM breaching, especially on invadopodia as micro-invasive structures degrading matrix (Eddy et al., 2017). Only a few *ex vivo* studies and tumor tissue histology describe large invasive protrusions representing the second stage of BM breaching (e.g., Hotary et al., 2006), but they were not analyzed in detail, perhaps because similar mechanisms were assumed to drive both stages. However, this is clearly not the case during *C. elegans* anchor cell invasion, in which the second major stage (large invasive protrusion), but not the first stage (invadopodia), depends on netrin-DCC signaling and localized lysosome exocytosis (Hagedorn et al., 2013 and this work). Therefore, it will be important to determine whether cancer cells also rely on netrin-DCC signaling and localized lysosome exocytosis to accomplish the second stage of BM breaching.

Intriguingly, lysosome exocytosis would also release lysosomal enzymes extracellularly at the invasive protrusion, which could help degrade BM or other tissue components. Although lysosomal enzymes are only active at low pH, an acidic microenvironment is created by many tumor cells through ion exchangers such as NHE1 (Cardone et al., 2005). Whether locally secreted lysosomal enzymes facilitate BM breaching also deserves future examination.

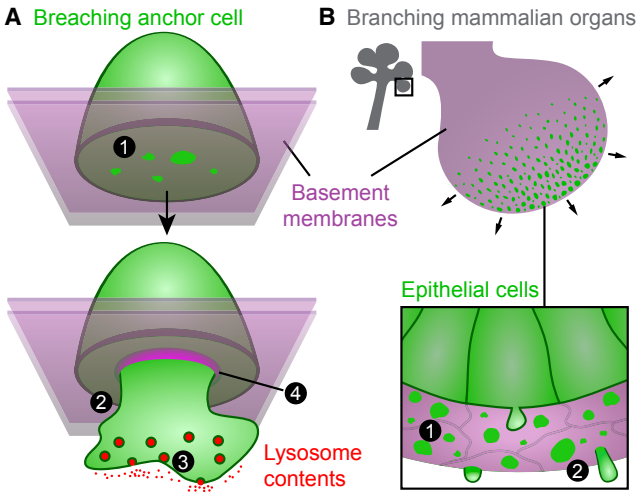

Figure 1. Basement Membrane Breaching in Two Developmental Contexts

(A) BM breaching during *C. elegans* anchor cell invasion occurs in two major stages. First, multiple invadopodia make tiny openings through two juxtaposed BMs (1). Second, a large invasive protrusion forms (2) through localized lysosome exocytosis (3), with combined degradation and circumferential pushing aside of BM components at the edges of the expanding breach in the BM (4). (B) BM breaching in mouse embryonic organs, including salivary gland, lung, and kidney, during branching morphogenesis is characterized by numerous small openings (1) at the tips of epithelial buds. Epithelial cells frequently extend small bleb-like protrusions through these openings (2) that may help expand the openings and even mediate contact-dependent signaling with the surrounding mesenchymal cells. Whether other steps discovered for *C. elegans* BM breaching occur in normal mammalian development or in cancer cell invasion through BMs will need to be determined in the future.

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