Invasion by Force: The *C. elegans* Anchor Cell Leads the Way

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How cells breach basement membrane barriers remains an area of active research. In this issue of *Developmental Cell*, Kelley et al. (2019) reveal that the *C. elegans* anchor cell uses physical force to breach basement membrane in the absence of matrix metalloproteases during its developmental invasion.

Mammalian development requires basement membrane (BM) breaching; however, mechanisms remain only partially understood. Early mouse embryos rely on physical forces to transition from a spherical to a cylindrical shape, and during this process, the anterior visceral endoderm (AVE) cells breach the BM at the distal tip of the embryo to invade upward into the visceral endoderm (VE) and form the distal visceral endoderm (DVE) (Matsuo and Hiramatsu, 2017). BM breaching at the distal site is triggered by mechanical forces generated between the embryo and maternal tissues after implantation (Matsuo and Hiramatsu, 2017). However, studying BM remodeling has remained challenging, in part, due to the difficulty of imaging or modeling the complex ultrastructure of the BM.

C. elegans provides a powerful model, as it has a well-characterized developmental program and is amenable to both genetics and imaging. The single gonadal anchor cell must breach a vulval BM to reach the vulval cells, and this event has served as a model for both normal and cancer cell invasion (Figure 1). Many of the same cytoskeletal and signaling proteins found in cancer cell invadopodia, including Arp2/3, Wasp, and Src proteins, are recruited to the site where the anchor cell breaches the BM (Lohmer et al., 2014). Matrix metalloproteases (MMPs) are major drivers of extracellular matrix (ECM) degradation during invasion especially in diseases such as cancer. However, it is difficult to investigate the role of MMPs in *in vivo* mouse models since there are about 23 MMPs, and they have complex and somewhat overlapping functions. The *C. elegans* anchor cell (AC) expresses only 6 MMP genes (*zmp-1-6*) from which zmp-1, 2, 3, 4, and 6 are important during AC invasion, and they are located either at the invadopodia (ZMP-1) or near the invading AC. In this issue of *Developmental Cell*, Kelley et al. (2019) deleted the five key MMPs and found to their surprise that even a quintuple knockout did not completely prevent crossing of the BM. The anchor cell instead formed an invasive protrusion that appears to breach BM through physical forces, shown to require branched actin networks produced by Arp2/3 in an accompanying study (Cáceres et al., 2018).

Interestingly, Kelley et al. observed that special energy requirements accompany BM breaching. The authors performed a genome-wide synergistic screen with the MMP-deficient animals to identify new genes and pathways that synergized with MMPs and promoted invasion. Of particular interest was the mitochondrial adenine nucleotide transporter ant-1.1. Knockdown of this gene caused a dramatic and unexpected block of invasion in animals lacking MMPs. Therefore, this showed a clear synergistic effect between MMPs and ANT-1.1 protein and suggested that mitochondria could produce sufficient energy in the absence of MMPs to promote invasion. Indeed, actin dynamics and myosin-based contractility produce force for breaching, requiring energy from ATP. Mitochondrial oxidative phosphorylation is the major cellular source of ATP, and although most cells are small enough that ATP can readily diffuse through the cytoplasm, cells recruit mitochondria to sites of active actin polymerization and protrusion (Cunniff et al., 2016; Schuler et al., 2017). Kelley et al. observed the recruitment of ANT-1.1 and mitochondria to sites of BM breach by the anchor cell, along with several key actin and adhesion proteins. Furthermore, by using an ATP biosensor, they found that ATP can be produced by mitochondria polarized to the site of invasion in order to supply energy for actin polymerization and thus creating forces/tension allowing the cell to crawl and invade the BM (Figure 1). In support of this observation, it was previously shown that lymphocytes can also polarize their mitochondria to sites of interaction during migration on inflamed endothelium (Morlino et al., 2014).

In adult mammals, immune cells probably spend the most energy remodeling and breaching BMs. They require to transit in and out of blood vessels and into tissues to access sites of infection or injury. Neutrophils readily crawl through blood vessel walls by first squeezing between endothelial cells and then crawling through the BM and along pericytes, cells that sit on the other side of the BM. Proebstl et al. found that pericytes undergo a shape change that widens weak points of venular BM to allow neutrophils to pass through (Proebstl et al., 2012). Pro-inflammatory cytokines TNF-α and IL1-β stimulated pericytes to increase mechanical force and open the channels (Proebstl et al., 2012). Macrophages may also traverse BM in a protease-independent fashion, and they are thought to promote an MMP-independent mechanism for cancer cells to travel through BM (Guiet et al., 2011).

In addition to immune cells, cancer-associated fibroblasts (CAFs) are key in remodeling of BM and promoting invasion. While CAFs can use proteases to help cancer cells invade, CAFs also invade through BM without the use of MMPs (Glentis et al., 2017). CAFs can protrude into pre-existing holes in the

BM and use mechanical forces of their actin cytoskeleton to widen the holes and promote invasion. This mechanism seems very analogous to the *C. elegans* anchor cell's mechanism of BM invasion, and it also demonstrates a synergy between cancer and stromal cells to cause tissue remodeling and BM breaching (Glentis et al., 2017).

It is clear from these numerous examples that normal and cancer cells have adaptable strategies to cross barriers such as BM. These include harnessing metabolic pathways to promote remodeling, pre-existing ''doors'' in BM for cells to crawl through, and active force generation via actin and myosin. Kelley et al. bring many of these mechanisms together in a beautiful system in *C. elegans* development, which forms a paradigm for how invasion can work in health and disease.

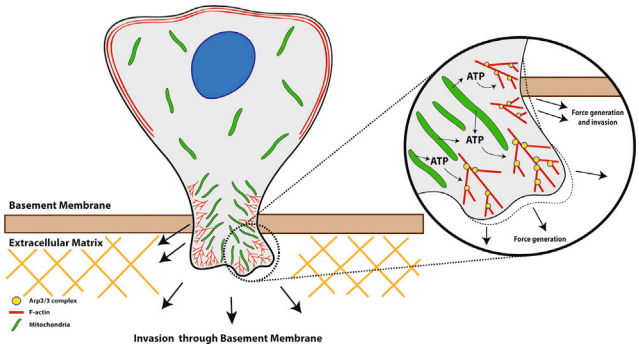

Figure 1. Invasion of a Cell through Basement Membrane Using Physical Forces

Cells can breach the basement membrane (BM) and invade through it, by generating mechanical forces using branched actin networks. Actin is polymerized at the leading edge of a cell, allowing it to generate force against the BM. Mitochondria are recruited at the protrusive edge of the cell during invasion in order to supply energy (ATP) needed for actin polymerization and efficient invasion. The *C. elegans* anchor cells serve as a model for invasive behavior of cancer cells.

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