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Early mechanical selection of cell extrusion and extrusion signaling in cancer

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Abstract

Epithelial cells use the process of extrusion to promote cell death while preserving a tight barrier. To extrude, a cell and its neighbors contract actin and myosin circumferentially and basolaterally to seamlessly squeeze it out of the epithelium. Recent research highlights how early apical pulsatile contractions within the extruding cell might orchestrate contraction in three dimensions so that a cell extrudes out apically. Along with apical constrictions, studies of ion channels and mathematical modeling reveal how differential contraction between cells helps select specific cells to extrude. In addition, several studies have offered new insights into pathways that use extrusion to eliminate transformed cells or cause an aberrant form of extrusion that promotes cell invasion.

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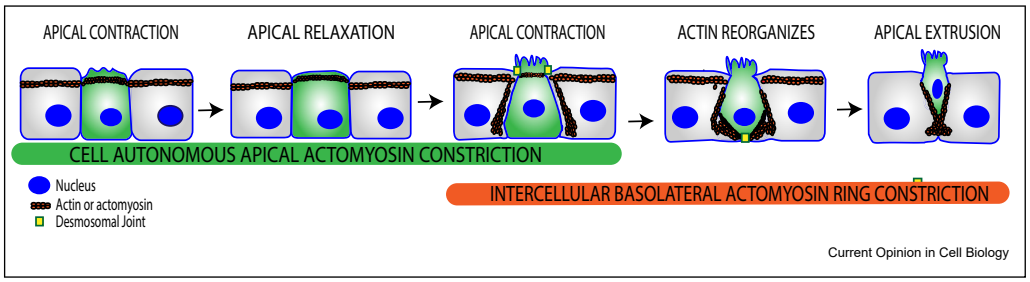
Epithelial cells provide a tight barrier to organs while turning over by proliferation and death. Epithelial cell extrusion preserves this barrier by seamlessly ejecting the cells before they die. To extrude, the lipid sphingosine-1-phosphate (S1P) binds the S1P receptor 2 ($S1P_2$) at the basolateral interface [1], which signals Rho to form an intercellular actomyosin ring within the extruding cell and its neighbors [1,2]. The ring contracts circumferentially and basally to expel the cell apically and replace it with surrounding cells [2]. Although apoptotic cells extrude, most epithelial cells extrude while alive, which later die by anoikis from excess crowding [3].

Early mechanical selection of cell extrusion

Crowding or apoptotic stimuli induce live or apoptotic cell extrusion, respectively; however, only a fraction of cells within the epithelium extrude. What designates one cell to extrude over its neighbors has remained a long-standing question in the field. Several reports suggest that differential tensions between cells may mechanically select which cells extrude [4–8]. As seen previously for apical cell extrusion (ACE) of cultured epithelial cells and basal cell extrusion (BCE) during *Drosophila* development [9–12], Atieh et al. [4**] found that apoptotic cells contract apically before contracting circumferentially and basally to extrude apically from a zebrafish epidermis (Figure 1). By inducing apoptosis throughout the zebrafish epidermis, Atieh et al. found that although many cells experience early apical pulsatile contractions, those that pulsed longest extrude. Similar to Kuipers et al. [9], they found that the early pulsatile contractions require actin and myosin, but not caspases or S1P, signals critical for extrusion. Inducing apoptosis reduced elasticity and tension throughout the monolayer, yet inhibiting S1P restored native tissue stiffness. From this, the authors surmised that reducing cell–cell tensions could promote pulsatile contractions throughout the monolayer, which then might select weaker cells for extrusion.

However, tissue-scale measurements could not directly test if individual cells contract to vie for space with respect to extrusion. A recent paper by Duszyc et al. investigated how differences in single cell tensions impact extrusion [5**]. Using methods to trigger apoptosis and modulate myosin contraction within single cells of zebrafish and cell culture epithelia, they found that cell–cell differential mechanical tensions prime cells for extrusion [5**]. Contraction within the cell mechanically signals Rho-mediated contraction in neighboring cell junctions through E-cadherin and myosin VI. Remarkably, although previous work shows that extrusion requires S1P [1], they found that the extruding cell itself need not supply it; instead, mechanically primed cells could extrude if S1P was added throughout the medium. Thus, together with the Atieh et al.'s article, this work suggests that differential contraction between cells may select a comparatively weaker cell for extrusion but requires S1P signaling to amplify sufficient cell contraction for extrusion. An important caveat of both studies is that they only

Figure 1



Early apical contraction in apoptotic extrusion. Apical contraction and relaxation occur in a cell before it extrudes [4**,9,20**]. Gagliardi et al. show that the apical ring contracts before forming the neighboring actomyosin ring [20**]. Thomas et al. show actomyosin accumulates at the desmosomal junctions before reorganizing into an intercellular actomyosin ring that contracts basally to extrude the cell out apically [21**].

investigate apoptosis-stimulated and not live cell extrusion, which is more common within our bodies (occurring at billions/hour) and essential for maintaining steady-state epithelial densities. Thus, future work will need to determine if differences in cell—cell tensions similarly select which cells undergo live cell extrusion. In addition, we know very little about what signals initiate the pre-extrusion early pulses.

Calcium and wave signaling induce extrusion

Several recent studies have investigated the roles of calcium and ERK waves within the monolayer. Crowding-induced live cell extrusion, occurring continuously to maintain steady state, requires calcium signaling via the stretch-activated calcium channel Piezo1 [3,13]. In addition, monolayers propagate calcium waves through epithelial cells that are necessary for actomyosin contraction, making them a prime candidate for initiating the early pulsatile contractions that probe tension variances within cells. Takeuchi et al. investigated the role of calcium waves in coordinating Ras-transformed ($HRAS^{G12V}$) and apoptotic extrusion [14**]. They found a cell fated for extrusion propagates calcium waves to neighboring cells through mechanosensitive TRPC1, gap junctions, and IP_3 receptors, which reorganize actin and myosin. Here, calcium waves and extrusion require TRPC1, rather than Piezo1 [3]. The use of this mechanosensitive calcium channel may reflect that any internal calcium source can trigger extrusion or that different calcium channels activate earlier extrusion steps.

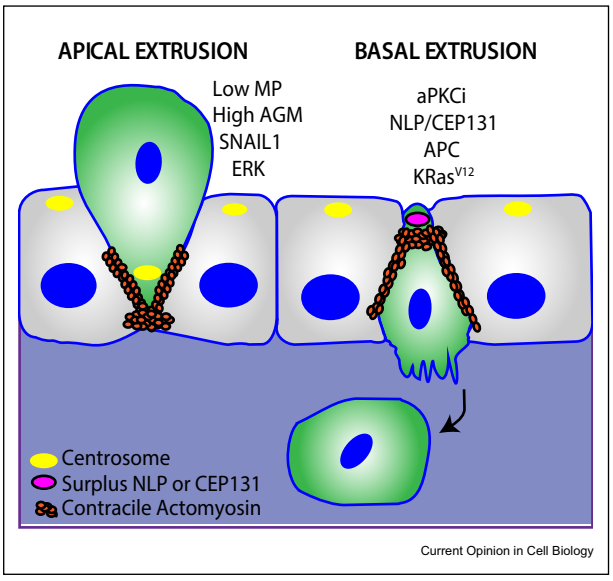
In addition to RAS, calcium mediates ERK activation through the MAP kinase pathway, which is essential for extrusion in MCF10A cells (Figure 2) [15**]. Using mosaically expressing $B\text{-}Raf^{V600E}$ cells in a wild-type background, Aikin et al. found that $B\text{-}Raf^{V600E}$-transformed cells exhibited sustained ERK signaling, whereas those surrounding them experienced pulsed ERK waves. Pulsatile ERK waves promoted cell survival and migration toward the oncogenic cells to help extrude them [15**]. Interestingly, differential ERK signaling led to opposing cell behaviors to coordinate transformed cell extrusion while bolstering survival and proliferation of the neighboring wild-type cells that replace it. Because calcium activates ERK, calcium waves likely initiate ERK waves. Future work will determine what activates the calcium patterns and if these steps play roles early selection of which cells extrude or the mechanics of extrusion itself.

In addition to calcium, other ion channels play critical roles in cell volume and death [16]. Necrosis occurs when cells swell to the point that they lyse [17]. In contrast, apoptotic volume decrease (AVD), characterized by cell shrinkage [17], occurs in response to activation of potassium and/or chloride channels. As AVD typically occurs up to hours before caspase activation [18] and potassium channel activation is critical within seconds of apoptotic stimuli for eliciting apoptotic extrusion [2], AVD could initiate early pulsatile contractions before extrusion. Mathematical modeling suggests that cytoskeletal-dependent contraction can further drive ion and water flux to sustain early fluctuations in cell volume [19]. In this way, potassium channel-activated cell shrinkage could trigger early cell contractions that might collaborate with calcium and actomyosin to further potentiate early pulsatile contractions before extrusion.

Coordinating contraction in 3D

All ACEs require actomyosin contraction circumferentially and basally, yet what coordinates contraction in two directions has remained elusive. Gagliardi et al. demonstrated that early contractions assemble into an early intracellular apical actin ring (iAAR) requiring the myotonic dystrophy kinase—related CDC42-binding kinase-alpha (MRCKα) that then promotes basolateral contraction [20**]. They show that caspase-dependent MRCKα cleavage prompts myosin-dependent iAAR assembly in MCF10A epithelial cells (Figure 1). iAAR

Figure 2



Extrusion and cancer. Apical cell extrusion can eliminate transformed cells through sustained ERK signaling [15**] and by low mitochondria membrane potential (MP) and high aerobic glycolysis metabolism (AGM) [25–27]. Expression of a nonphosphorylatable allele of Snail can activate RhoA hypercontractility and cause both apical cell extrusion (ACE) and basal cell extrusion (BCE) [5**,6**]. BCE can direct cells beneath the epithelium to potentially invade. Overexpression of aPKCi [36*] or centrosome proteins NLP or CEP131 [33*] can drive BCE by activating actomyosin contraction apically.

contraction redistributes actin to promote basal actomyosin ring assembly, contraction, and extrusion (Figure 1). Experimentally inducing MRCKα cleavage, independent of caspases, is sufficient to form the iAAR and trigger extrusion [20**]. As live cell extrusion occurs independently of caspase activation, it will be interesting to determine if live cell extrusion requires MRCKα activation independent of protease cleavage.

Although iAAR pulsing redistributes actomyosin to the basalolateral surface during ACE, what relocates actomyosin basally is unclear. Thomas et al. discovered that desmosomal junctions (DJs) may coordinate contraction in two directions, assisting basal actomyosin redistribution. Here DJs first attach to the existing apical actomyosin ring, as it contracts circumferentially, and then detach to enable basolateral contraction (Figure 1) [21**]. Depletion of DJ components disrupts actomyosin contractility, junctional tension, and extrusion. As DJs can coordinate contraction in three dimensions to cause ACE, future research will determine what regulates DJ—actin interactions to orchestrate this swap.

In addition, control of actomyosin contractions in the cell may depend on cell geometry conformations. Mathematical models have exploited mechanical interactions and tensions between cells to induce extrusion [7**,8]. Okuda and Fujimoto modeled cells as polyhedrons to show cell—cell boundary movements [7**]. Increased packing densities and topology changes break cell boundary symmetries that can affect extrusion direction. Cell—cell boundaries in 3D are typically represented as perpendicular interfaces connecting apical to basal planes, yet these interfaces are typically angled. Their model shows that asymmetric forces alter cell—cell boundary angles that alter extrusion direction: pressure toward the apical side promotes BCE, whereas pressure at the basal side promotes ACE. The model also invokes the number of neighbors at the apical or basal surface and differences in cell—cell tension, contractile, or adhesion forces to also suggest that differential tensions select for cellular extrusion. Critically, Okuda and Fujimoto highlight how geometries of cell—cell packing may determine the *direction* in which a cell extrudes, an important consideration in cancer.

Extrusion roles in cancer

Extrusion can suppress or promote cancer, depending on the direction the cell is ejected. Recent studies have added valuable information into how ACE can suppress tumor formation, whereas BCE can promote its spread.

Tumor suppression

Cell competition, a process in which physiologically fitter cells remove deviant cells, can extrude transformed cells, using a process termed epithelial defense against cancer (EDAC) [22,23]. Alternatively, cancer cells could spread by outcompeting wild-type cells. Tsuboi et al. found that oncogenic cells with higher proliferation rates can intercalate anisotropically into spaces left from wild-type cell extrusions [24]. Contrastingly, metabolic differences in Ras^{V12} cells compared with wild-type neighbors promote their extrusion [25—27]. Ras^{V12} cells have lower mitochondrial membrane potential and high aerobic glycolysis compared with neighboring wild-type cell that promotes their elimination (Figure 2) [25]. Further investigation on mitochondria membrane potentials, Sasaki et. al. found that restoring mitochondrial membrane potential in Ras^{V12} cells with a high-fat diet prevented their elimination from mouse small intestinal and pancreatic epithelia [26**]. Higher fatty acid oxidation metabolism mediated by acetyl-CoA decreased cell extrusion [26**], whereas overexpression of carnitine palmitoyltransferase 1A, a fatty acid oxidation gene, increased cell extrusion and survival by evading anoikis [28**]. This interesting line of work suggests that metabolic differences may alter EDAC and that diet could impact our ability to stave off cancers.

Promotion and invasion

BCE into the stroma could enable cell invasion and metastasis. Oncogenes can disrupt ACE signaling to instead drive aberrant BCE [29,30]. Interesting findings

suggest that Snail, a classic epithelial to mesenchymal (EMT) driver upregulated in cancer, may act simply by upregulating actomyosin contractility and extrusion (Fig. 2) [6**,31]. Expressing Snail^{6SA} in MCF7 cells (from human breast adenocarcinoma) increased RhoA expression and activation, causing hypercontractility and both apical and basal extrusion in a myosin-dependent manner. As GSK-3β cannot phosphorylate Snail^{6SA}, it does not transcriptionally downregulate E-cadherin [32] or upregulate classic EMT genes [6**]. In this way, activation of Snail could promote invasion of cells through BCE, independent of transcriptional changes in epithelial-specific genes. Interestingly, Snail^{6SA} only promoted extrusion when expressed mosaically, confirming other findings that differential cell–cell tensions promote extrusion.

Other findings highlight new proteins critical for deciding the direction a cell will extrude. Ninein-like protein (NLP) or centrosomal protein 131 kDa (CEP131) overexpression causes abnormal centrosome structures that contract actomyosin apically, driving BCE (Figure 2) [33*]. In both instances, adding S1P or $S1PR_2$ agonist can rescue ACE [29,30,33*], supporting the role of S1P as a tumor suppressor. Some cancers overexpress the polarity protein atypical protein kinase C iota (aPKCi) [34,35], which, similar to mutations in APC, KRAS^{V12}, NLP, and CEP131, promotes BCE (Figure 2). Villeneuve et al. found that aPKCi overexpression shifts vinculin from apical cell–cell junctions to basal ones, causing more contractility at the apex, which is needed for BCE and promoting basal protrusions that could help the invading cell migrate [36*].

New investigations on mechanics between cells, actin assembly, and ion channels have generated new insights into our understanding of the mechanisms that drive extrusion. They have underscored the importance of apical pulsatile constrictions and generating differential cell tensions in selecting cells that extrude and in coordinating contraction in three dimensions. Uncovering the early signals that trigger pulsatile constrictions and discovering if apical pulsing is a conserved mechanism for all types of extrusion remains open questions for the field. Clarifying this pathway may also reveal how DJs promote basolateral contraction, which is essential for ACE. Elucidating what regulates where actomyosin contracts during extrusion will be critical to understanding how it goes awry to promote invasion of neoplastic cells instead of eliminating them.

Conflict of interest statement

Nothing declared.

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** of outstanding interest

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