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Epithelial Cell Extrusion: pathways and pathologies

Swapna Aravind Gudipaty and Jody Rosenblatt*

Department of Oncological Sciences, Huntsman Cancer Institute, University Of Utah, 2000 Circle of Hope Drive, Salt Lake City, UT 84112, USA

Abstract

To remove dying or unwanted cells from an epithelium while preserving the barrier function of the layer, epithelia use a unique process called cell extrusion. To extrude, the cell fated to die emits the lipid Sphingosine 1 Phosphate (S1P), which binds the G-protein-coupled receptor Sphingosine 1 Phosphate receptor 2 (S1P₂) in the neighboring cells that activates Rho-mediated contraction of an actomyosin ring circumferentially and basally. This contraction acts to squeeze the cell out apically while drawing together neighboring cells and preventing any gaps to the epithelial barrier. Epithelia can extrude out cells targeted to die by apoptotic stimuli to repair the barrier in the face of death or extrude live cells to promote cell death when epithelial cells become too crowded. Indeed, because epithelial cells naturally turn over by cell death and division at some of the highest rates in the body, epithelia depend on crowding-induced live cell extrusion to preserve constant cell numbers. If extrusion is defective, epithelial cells rapidly lose contact inhibition and form masses. Additionally, because epithelia act as the first line of defense in innate immunity, preservation of this barrier is critical for preventing pathogens from invading the body. Given its role in controlling constant cell numbers and maintaining barrier function, a number of different pathologies can result when extrusion is disrupted. Here, we review mechanisms and signaling pathways that control epithelial extrusion and discuss how defects in these mechanisms can lead to multiple diseases. We also discuss tactics pathogens have devised to hijack the extrusion process to infect and colonize epithelia.

Keywords

Epithelial extrusion; Sphingosine-1-phosphate; Sphingosine-1-phosphate receptor 2; Piezo1; Rho; Microtubules

1. Introduction

1.1 Apical epithelial cell extrusion

Epithelia made of one or two layers of cells form dynamic barriers for the organs they encase. Maintaining the integrity of epithelia is central to prevent inflammation,

*Corresponding Author: Jody.Rosenblatt@hci.utah.edu.

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inappropriate growth factor signaling, and invasion by pathogens. Epithelial cell turnover by cell death (apoptosis) and division occurs at some of the highest rates within the body, potentially because they are under a constant barrage of toxins and pathogens. It may be due to this high rate of turnover that epithelia are the sites where ~90% of cancers arise, should cell death or division become misregulated. Cell death could potentially create gaps within the epithelium that compromise barrier function. However, all epithelia maintain a barrier, even when up to half of the cells are induced to die, by using a process termed cell extrusion that simultaneously removes dying cells while repairing any gaps that could have formed from the cell's exit [1]. Importantly, this extrusion process actually drives cell death during normal homeostatic turnover. When cells become too crowded, they activate some cells to extrude while they are still alive. Once they lose contact with the epithelium and underlying matrix, they die by anoikis, or apoptosis due solely to loss of survival signaling. Cell extrusion is critical for promoting cell death in epithelia: disrupting it causes epithelial masses to form at crowded regions where cells should have extruded (**Figure 1**) [2]. Additionally, inhibition of epithelial cell extrusion blocks cell death even in response to harsh apoptotic stimuli, suggesting that extrusion is necessary for promoting both natural and induced epithelial cell death by countering the survival signaling associated with cell attachment to the underlying matrix and other cells. Because of this, disruption of extrusion appears to be an important driver in a class of aggressive tumors that are resistant to chemotherapy [3].

1.1.1 Apical extrusion requires Sphingosine-1-Phosphate signaling through the S1P₂ receptor

Apical extrusion is a multistep process that begins by the cell autonomous contraction of the extruding cell followed by the formation and contraction of an actin ring in the cells neighboring the extruding cell [1,4]. To signal extrusion, the cell produces and secretes the bioactive lipid Sphingosine-1-phosphate (S1P) which binds to a G-protein coupled receptor, S1P receptor 2 (S1P₂), in the neighboring cells [5]. S1P₂ is a G11/12-type G-protein coupled receptor that activates Rho, a small GTPase, via the p115 RhoGEF to trigger actin and myosin recruitment and contraction at basolateral intracellular junctions [6]. The actin/myosin ring contracts circumferentially and downward along the basolateral surface of the extruding and neighboring cells to squeeze the extruding cell out while reforming intercellular junctions between the new neighbors [1,6,7]. Disruption of S1P, S1P₂, p115 RhoGEF, actin, or myosin will block extrusion and produce holes in epithelial barrier where cells die (Figure 2, Apical Extrusion) [5,6].

Microtubules play a critical role in both the dying cell and neighboring cells to target RhoGEF p115-dependent actomyosin contraction along the basal surface so that cells are pushed out apically [6]. Although microtubules reorient in both the dying and neighboring cells, mosaic knockdown of the microtubule regulator adenomatous polyposis coli (APC) suggests that microtubule dynamics are most essential in the extruding cell for controlling the direction of extrusion [8]. The fact that microtubule dynamics are critical in the extruding cell suggests that they may be important for localizing the S1P lipid to the basolateral surface of the extruding cell to restrict S1P₂ activation of Rho-mediated contraction to the basolateral surface. Disruption of microtubules randomizes rather than

completely reverses the direction of extrusion, suggesting that other factors such as cell polarity, matrix, and cell thickness may also contribute to the direction a cell can extrude.

Both apoptotic and live cell extrusion from epithelial overcrowding require the same S1P-S1P₂-Rho signaling pathway. In contrast to dying cell extrusion, which requires an apoptotic stimulus to generate S1P, crowding-induced live cell extrusion requires the stretch-activated channel Piezo-1 (Figure 1B) [2,9]. Live extruded cells resulting from a crowding stimulus die after expulsion from the rest of the epithelium due to anoikis, or death from loss of survival signaling. Morpholino-based knockdown of Piezo-1 or blocking the stretch activated signaling by gadolinium in developing zebrafish embryos prevents extrusion and death and forms cell masses at sites where cells would have extruded, demonstrating the critical role that Piezo-1 plays in controlling extrusion and maintaining constant cell numbers. Because Piezo-1 accumulates within the cytoplasm of crowded epithelial cells whereas S1P accumulates only in cells before they extrude, Piezo-1 is likely upstream S1P and Rho signaling, but how Piezo-1 activates S1P synthesis is still unknown [2].

Interestingly, compression, junctional tension, and contractility can also stimulate delamination, or basal extrusion, in the *Drosophila* notum through a different signaling pathway. Although it is not clear what pathway drives compression-based delamination in *Drosophila*, the pressure derives from cell growth rather than cell division. Because JNK activation is also required, however, compression in *Drosophila* may be activating cell death concomitantly with extrusion, as JNK is a known regulator of apoptosis in *Drosophila* [10]. Moreover, activation of cell death signals appears to be conserved during other developmental delaminations in *Drosophila*, as basal extrusion or delamination of amnioserosa cells during dorsal closure requires caspase activation [2,11,12]. Linking basal extrusion to apoptotic signaling in *Drosophila* may help ensure that cells die following extrusion into the underlying tissue. Vertebrates may instead rely on cells dying by anoikis post-extrusion, since apically extruded cells have no survival signaling in the lumen. Basal extrusion of live cells can lead to significantly altered outcomes, as discussed below.

Although it is not clear why in a given crowded region, some cells are more likely to extrude than others, one possibility is that regulation of cell adhesion to the matrix may be an important factor [3]. De-adhesion of extruding cells to the matrix may be a rate-limiting step in extrusion, based on two observations. Cells frequently extrude in convex regions of tissue where cells would have least contact with the matrix [2]. Additionally, the cell-adhesion signaling protein focal adhesion kinase (FAK), which plays a critical role in matrix-dependent cell survival [13], is typically inactivated in late stages of extrusion to promote cell death. In cases where extrusion is blocked and subsequent cell death is blocked, addition of FAK inhibitors are sufficient to rescue apoptosis to levels seen when extrusion is functional, suggesting that de-adhesion of the extruding cell and inactivation of FAK are critical limiting steps in extrusion-dependent apoptosis. Additionally, FAK inhibitor addition can shed off cell masses in zebrafish that result from defective S1P₂-dependent extrusion [3]. Because of this, FAK inhibitors may work in clinical trials for cancers that are chemotherapeutically resistant. The roles of FAK in cancer may also be more widespread, as its activity is upregulated in a variety of epithelial-based cancers. In these cases, inhibition of

FAK may be more effective for targeting cancer than traditional chemotherapies that do not interfere with intrinsic high survival signaling [14,15].

1.2 Basal epithelial extrusion

While vertebrate epithelia, observed in zebrafish, chick, mice, and humans, extrude cells apically into the lumen, when extrusion signaling is misregulated, cells can instead extrude basally underneath the epithelium (Figure 2, Basal Extrusion) [1, 2]. Although adult *Drosophila* gut epithelia extrude apically [11,16,17], most developing *Drosophila* epithelia extrude basally. Because the epithelia are polarized with their apical surfaces facing a luminal space and their basal surfaces facing the matrix and stroma, the direction a cell extrudes can be critical for determining its later fate. Cells that extrude apically into the lumen eventually die due to loss of survival signaling or anoikis. Transformed cells that upregulate survival signaling and override anoikis may not die after extruding apically, yet because the lumen is dead space, apical extrusion essentially still eliminates them, and, thereby, acts as a tumor suppressor. On the other hand, basal extrusion could allow these cells to invade the tissue the epithelium encases and migrate to other sites. Thus, basal extrusion could provide a potential mechanism for initiating tumor metastasis.

In vertebrates, basal extrusion may act as a default pathway when the signaling controlling apical extrusion becomes misregulated (Figure 2, Basal Extrusion). Normally, during apical extrusion, cell-autonomous actomyosin contraction of the extruding cell precedes the basolateral contraction that drives apical extrusion [4]. The S1P-S1P₂-Rho pathway only controls the basal contraction that controls apical extrusion; when it is disrupted, however, basal extrusion can still occur [6,18]. For this reason, oncogenic mutations in K-Ras or APC can subvert normal apical extrusion signaling resulting in basal extrusion and cell masses [19]. Inhibition of S1P₂ also increases basal extrusion rates and leads to the formation of large epithelial masses in both zebrafish epidermis and cultured epithelia [3]. Thus, oncogenic mutations that disrupt apical extrusion signaling could drive not only the formation of neoplastic masses but also promote tumor cell invasion and metastasis.

As extrusion is critical for controlling cell numbers and epithelial barrier function, it is not surprising that this process can become hijacked to drive a variety of different other epithelial-based pathologies. In this review, we discuss how misregulation of extrusion could trigger a variety of pathologies by subverting a process that normally triggers cell death. Some diseases directly result from misregulation of the S1P-S1P₂-Rho, pathway that controls apical extrusion, while others result from alterations in cell adhesions that affect extrusion laterally.

2. Pathologies resulting from defective extrusion

2.1 APC controls the direction of extrusion

Based on the finding that microtubules are necessary to control where actin and myosin II contract during apical extrusion (Figure 3A), Marshall *et al* examined the role of the tumor suppressor protein adenomatous polyposis coli (APC) [8]. P115 RhoGEF associates with microtubules to localize to the basolateral interface of the cell to locally activate actomyosin

contraction necessary for apical extrusion [6]. Since APC is a scaffold protein that regulates both microtubule dynamics and actin assembly, in addition to its better known role in regulating Wnt signaling, they investigated if APC mutations could affect the direction of extrusion [8]. Since microtubules are only faintly visible in the extruding cell, it was presumed that microtubules controlled actomyosin contraction in the cells neighboring an extruding cell [6]. However, the ability to knockdown and rescue APC protein function mosaically indicated that APC was necessary predominantly in the extruding cells, rather than their neighboring cells. Importantly, >80% of all colon cancers have APC mutations that delete its C-terminus, which contains EB1 and PDZ binding domains that regulate microtubules [20–22]. This truncation mutation drives cells to basally extrude in both cell culture and zebrafish epidermis. Thus, one tumor suppressor function of APC may be to ensure cells extrude apically to die instead of enabling basal extrusion and invasion. Expressing the microtubule-binding domain of APC in the extruding cell alone is sufficient to rescue apical extrusion in colon cancer cell lines with this APC mutation. Thus, while APC may co-ordinate microtubules with actin/myosin contraction in cells neighboring an extruding cell, these studies indicate a critical role for microtubule dynamics in the extruding cell (Figure 3B). This suggests that microtubules in the early extruding cell become highly dynamic, rather than depolymerizing completely, to reorient from an apical to basal network aligned perpendicular to the monolayer to one that could traffic S1P to the basolateral interface between the extruding cell and its neighbors. While it is not clear if S1P trafficking requires microtubules, S1P assembles into clear puncta along definitive tracks in early extruding cells, suggests that it may traffic to the basolateral surface along microtubules. Furthermore, the fact that APC mutations that drive colon cancer lack this microtubule regulation domain suggests that another important oncogenic activity may be diverting extrusion from apical to basal.

2.2 K-RAS

Oncogenic K-Ras mutations drive many aggressive tumors, such as pancreatic, lung, gastric, and colorectal cancers [23–26]. While these activating mutations upregulate cell survival signaling and cell motility, critical for these tumor types, oncogenic K-Ras mutations can also drive basal extrusion (Figure 3C) [18]. Expression of the commonly occurring activating oncogenic mutation K-Ras^{V12} drives basal extrusion in a cell autonomous fashion. Unlike APC truncation, K-Ras^{V12} mutations do not affect microtubule orientation in the extruding or surrounding cells nor do they affect intrinsic cell polarity. Instead, K-Ras^{V12} extruding cells lack S1P, required for apical extrusion. The loss of S1P results not from lack of its synthesis but due to its degradation through high levels of autophagy. Several reports show that K-Ras^{V12} expression increases autophagy as a means of increasing cell survival [27,28]. However, as wild type extruding cells upregulate the LC3 autophagy marker, the combination of K-Ras^{V12} signaling and extrusion signaling may together promote high levels of autophagy that degrade S1P. Blocking autophagy in K-Ras^{V12} expressing cells by ATG knockdown or with small molecule inhibitors rescues both S1P expression and apical extrusion in these cells.

Importantly, K-Ras^{V12} cells grown in 3D cysts extrude cells basally that survive, migrate, and proliferate, suggesting they may use basal extrusion to invade the underlying tissue and

initiate metastasis [6,18,19]. By contrast, wild type MDCK cysts predominantly extrude cells apically, but the minor percentage that extrude basally die, despite extruding into matrigel, which should enable survival signaling. The high propensity of K-Ras^{V12} cells to basally extrude and upregulate survival signaling may also explain why tumors driven by this mutation are typically so deadly and metastatic. Interestingly, K-Ras^{V12} lesions are implicated in early stage pancreatic, colon, and lung cancer stages. Thus, the fact that a K-Ras^{V12} mutation is intrinsically invasive without other mutations suggests that invasiveness may be an early step in K-Ras^{V12}-driven cancers and that additional mutations, associated with later stages of these cancers, could enable better survival and growth of these disseminated cells. If so, these findings could suggest that development of new screening methods to identify early invasive cells that may better respond to chemotherapies.

Additionally, K-Ras^{V12} driven cancers such as pancreatic ductal adenocarcinoma (PDAC) have decreased levels of S1P₂ [18]. In PDAC cell lines, reduced S1P₂ expression leads to decreased apical extrusion, a significant increase in levels of basal extrusion, and formation of cell masses. Exogenous expression of S1P₂ is sufficient to rescue apical extrusion and dramatically reduce orthotopic pancreatic tumors and their metastases in a mouse model [3]. The loss of S1P₂ expression suggests that defective extrusion may contribute to PDAC development and highlights the role of K-Ras^{V12} in subverting the homeostatic S1P-S1P₂ apical extrusion process and promoting cell invasion and metastasis.

In contrast, Hogan *et al* identified a different behavior by inducibly expressing H-Ras^{V12} mosaically in an MDCK cultured epithelium; instead of basally extruding, they are eliminated apically by cell division-dependent dissociation of transformed cells from wild type cells. They have termed this 'Epithelial Defense Against Cancer (EDAC)' as a method that wild type cells use to rid themselves of transformed unproductive cells [29,30]. However, they also find a minority of H-Ras^{V12} cells are not eliminated apically and instead form protrusions underneath the surrounding cells to initiate basal invasion. H-Ras^{V12} cells here are eliminated apically by upregulating the tumor suppressor EPLIN1 that through enrichment of Caveolin-1 microdomains promotes Myosin II contraction. EPLIN and Cav-1 also trigger accumulation of filamin, an F-actin binding protein, that promotes vimentin and Rho-mediated contraction to exclude the cell apically [31,32]. Cells expressing H-Ras^{V12} require interaction with normal surrounding cells to cause their elimination apically. Together, these studies suggest a model where wild type cells have stronger adhesion to each other than to H-Ras^{V12} cells and by excluding contact with them cause their elimination. A recent study by Yamamoto *et al* suggests that normal cells surrounding H-Ras^{V12} cells require S1P₂-mediated Rho activation for EDAC. Yet, S1P production and signaling is not clearly required in either the normal or transformed cells and the mechanism for this process is still quite mysterious [33]. Since K-Ras^{V12} signaling downregulates S1P₂ expression, H-Ras^{V12} cell elimination may result from differences in S1P₂/Rho-mediated contractility and adherens junction maintenance between these two populations of cells.

The apical elimination of groups of H-Ras^{V12} cells contrasts with the basal extrusion of single K-Ras^{V12} transformed cells [18]. Oncogenic K-Ras^{V12} cells extrude predominantly basally in a cell autonomous manner in both epithelial monolayers and 3D cysts in culture. The difference between cell behaviors in the different Ras studies may depend on the

isoform expressed and whether it is expressed acutely or constitutively. The vast majority of Ras-transformed tumors express a mutated K-Ras isoform, whereas only 10% of tumors express a mutated H-Ras isoform [34]. Potentially, H-Ras^{V12} cells may get eliminated by EDAC, whereas those expressing K-Ras^{V12} may contribute to tumor formation and progression. It is also likely that constitutive expression of either K-Ras or H-Ras may cause cells to extrude basally or migrate [18,35], whereas inducing acute expression of either Ras^{V12} isoform causes apical cell elimination. Since K-Ras^{V12} is expressed constitutively in carcinomas [36,37], any cells not eliminated by EDAC may result in carcinomas that cause loss of contact inhibition and basal extrusion.

2.3 Invasion by pathogens

For pathogens to successfully infect the body, they must first transit or invade epithelia, which act as the first line of defense for the gut and upper airways. A variety of enteropathogenic bacteria hijack extrusion to invade the gut epithelium. Understanding the mechanisms by which they do so may help reveal new information about how extrusion is normally regulated and could also help us find better ways to combat these infections. Of the pathogens studied so far, a number of strategies have arisen for how pathogens take over epithelia to gain access to the rest of the body.

Listeria monocytogenes (*Lm*) is an opportunistic bacterium, which colonizes the gut epithelium, and is responsible for a severe food-borne disease that can lead to infant mortality, miscarriage, and meningitis in immune-compromised individuals [38,39]. The gut epithelium undergoes rapid cell turnover with numerous cells extruding at the villus tip, which is the primary site of *Lm* invasion [40,41]. *Lm* invades at the junctions of extruding cells through the synergistic action of two bacterial surface proteins Internalin A (InlA) and Internalin B (InlB) [42–45]. *L. monocytogenes* infection through the gastrointestinal tract requires InlA, which binds the N-terminal extracellular domain of host E-cadherin, allowing *Lm* adhesion to the epithelium [46–48]. InlB binds to host c-Met, a basolaterally located receptor tyrosine kinase, to locally activate endocytosis of junctional protein complexes, including E-cadherin, which enables *Lm* entry into the host cell (Figure 4A) [45,49,50]. Both host receptors, E-cadherin and c-Met are not readily accessible to microorganisms on the apical or luminal side of the epithelium normally [51–53]. Strikingly, *Lm* takes advantages of extruding cells to gain access to both host proteins, which become exposed during the extrusion process [42]. During extrusion, multicellular junctions (MCJs) must seamlessly dissolve between the extruding cell and its neighboring cells and then reform once the cell has extruded as new neighbors are established. As junctions transiently de-adhere, they expose E-cadherin, which allows *Lm* binding through InlA followed by c-Met binding by InlB. Activation of c-Met increases the rate of clathrin-mediated endocytosis naturally occurring at MCJs accelerating the internalization of E-cadherin bound to *Lm* in the host cell [50,54]. Thus, by using a two-step mechanism, *Lm* targets dynamic junctional remodeling during epithelial cell extrusion to breach the intestinal barrier.

The rapid turnover and extrusion of the gut epithelium would generally serve to prevent bacterial invasion by eliminating cells that are infected [55,56]. However, the pathogens *Shigella*, *Salmonella*, and enterohaemorrhagic *Escherichia coli* (EHEC) have devised ways

to inhibit extrusion to better enable infection by preventing cell detachment from the basement membrane [57,58]. *Shigella flexneri* (*Sf*) infection of the gut provides an excellent example of how pathogens inhibit normal epithelial turnover through extrusion by increasing cell adhesions (Figure 4B). *Sf* causes bacillary dysentery in humans by primarily invading colonic epithelial cells. To invade, *Sf* uses the type-III secretion system (T3SS) to deliver the OspE protein to host cells, which increases cell adhesion to the matrix and inhibits cell extrusion [59]. OspE, binds the host Integrin Linked Kinase (ILK) that links cell-adhesion receptors, integrins, and growth factors to the actin cytoskeleton to increase surface levels of β 1-integrin and reduce phosphorylation of FAK and paxillin, which together promote and stabilize focal adhesions [60–62]. Similarly, during *EHEC* infection, the OspE homolog EspO1-2, in addition to binding ILK, inhibits the guanine nucleotide exchange factor (GEF) EspM2 that activates RhoA to block extrusion of infected cells [63,64]. Interestingly, the OspE homologs in *Salmonella typhimurium*, *enteropathogenic E.coli* and *Citrobacter rodentium* also interact with host ILK and localize to FAs implying that pathogens use similar mechanisms to colonize epithelial cells [61]. Although these mechanisms may prevent extrusion, these pathogens could either activate or block apoptotic pathways during the initial stages of infection to make cells more susceptible to invasion [65].

Altering cell extrusion is not limited to bacterial pathogens but has recently been implicated in viral infection. Infection with respiratory syncytial virus (RSV) infects the airway epithelium of nearly all children but in rare cases can cause acute severe lower airway disease [66]. RSV infects the pseudostratified ciliated columnar epithelial cells of the human respiratory tract. In these severe cases, the airways become blocked with extruded cells that impede breathing. Here, the numerous epithelial cells loaded with virus extrude apically and accumulate in the intraluminal space, causing airway obstruction and inflammation. RSV-infected ciliated airway epithelial cells in culture undergo a morphological transition from columnar cells to cells with a rounded morphology and bulge out apically while retaining their membrane integrity. Since these cells demonstrate structural features similar to extrusion, it is not surprising that eventually some extrude from the epithelium [1]. Using RSV gene deletion mutants, Pickles and colleagues found that the RSV non-structural 2 (NS2) protein caused the ciliated cell rounding and increased cell shedding from RSV infection [67]. NS2 (along with the RSV protein NS1) suppresses the induction of IFN- α/β and has been shown to suppress the Interferon (IFN)-mediated host antiviral response [68,69]. In addition to NS2 protein, the RSV envelope fusion protein (F) may also contribute to the initiation of epithelial shedding, airway obstruction, and inflammation. RSV F-protein causes p53-dependent apoptosis in polarized epithelial cell culture leading to accelerated extrusion of apoptotic cells (Figure 4C) [70]. While epithelial cell shedding clears out RSV-infected cells to reduce viral titers, excessive cell shedding can block smaller bronchiolar airways, leading to a condition called bronchiolitis, which can be life threatening.

Despite the strategies used by microorganisms to colonize the host, epithelial-intrinsic mechanisms may provide general defense against pathogens. Ingested *Salmonella enterica* serovar *Typhimurium* (*Stm*) invades gut epithelia, and replicates within a unique cytoplasmic vacuole [71,72]. A substantial population also proliferates in the cytosol of these epithelial cells, creating a reservoir of infectious bacteria [73]. During early stages of *Stm* infection, host Neuronal Apoptosis Inhibitory Protein (NAIP) assembles with the Nod-like receptor

NLR4 to form the inflammasome complex. This complex recognizes the *Stm* type 3 secretion system to activate caspase-1 and secrete proinflammatory cytokines that promote extrusion of epithelial cells infected with *Stm* [74,75]. During later stages of infection, a subset of bacteria escape the cytoplasmic vacuole and hyper-replicate in the host epithelial cell cytoplasm [76]. At this stage, the *Salmonella* lipopolysaccharide activates caspase-11 and the NLRP3 inflammasome, leading to the extrusion of infected cells into the gut lumen [77–79]. The extruded cells eventually undergo inflammatory death and inflammasome-dependent secretion of cytokines such as IL-18 that lead to the mucosal inflammation, commonly seen during *Stm* infections [80]. It is still unclear why the host defense switches from NAIP/NLRP4 to caspase-11 mediated pathogen restriction during late stages of infection. Yet, both pathways increase gut epithelium turnover that is implicated in host defense against *Stm* [74,80].

2.4 Epithelial Barrier Function Defects

Extrusion is a key mechanism epithelia use to maintain homeostasis and prevent accumulation of excess cells [2,81]. In addition to S1P and the stretch-activated channel Piezo1, cell-cell adhesions may also regulate cell extrusion [29,82–84]. Mutations or reduction of key cell-cell adhesion molecules are known to cause a defect in cell extrusion (Figure 5) [85]. One model suggests that cell-cell adhesion can be altered through cleavage of junction proteins such as E-cadherin causing dynamic changes in adhesion between a compromised cell and its neighbors. In response to E-cadherin cleavage, the neighbors form contacts with new neighboring cells, possibly starting underneath the compromised extruding cell, which would also help promote its extrusion. Not surprisingly, monolayers that express low levels of E-cadherin have increased rates of extrusion [85]. Additionally, the elongation of cells surrounding a recently extruded cell in order to seal the gap also requires E-cadherin [84]. The loss of adhesion proteins in diseases such as Celiac and inflammatory bowel disease (IBD) emphasize their critical importance in maintaining barrier function but may also suggest aberrant regulation of extrusion in gut epithelia [86]. Sigmoid colon biopsies from patients with Crohn's disease (a form of IBD) show impaired tight junction (TJ) function and reduced expression of TJ proteins [87]. Although these diseases are typically considered immune diseases [88], an alternative explanation is that the primary defect lies in poor epithelial barrier function, potentially via extrusion defects. Epithelial defects within the digestive tract may be more apparent since these cells generally turn over by cell death and division at the highest rates in the body. In support of this idea, inducing apoptosis in these epithelia leads to focal epithelial lesions and barrier dysfunction [87,89].

Another example of a molecule involved in maintenance of cell adhesions is the mammalian LKB1, a serine-threonine kinase that is required for correct polarization of epithelial cells [90]. LKB1 is also a tumor suppressor protein that functions by phosphorylating several downstream kinases, including AMPK, MARK, and MELK (Fig-1) [91]. In *C. elegans*, during embryonic development, LKB1 activates Pig-1 and promotes a caspase independent cell extrusion process through cell detachment from the epithelium and endocytosis of cell-adhesion molecules [82]. In murine epithelia, LKB1 is required for regulating adherens junctions to prevent cell extrusion, as its loss leads to excess cell epithelial extrusion and disruption of epithelial integrity [92]. Alternatively, mammalian *lkb1* mutations cause Peutz-

Jeghers syndrome, where patients have high numbers of gastrointestinal polyps with a greater risk for many cancers. A proposed mechanism for polyp formation in these patients is defects in epithelial cell extrusion with increased proliferation rates [82,93–95].

In addition to defects in cell adhesion molecules, epithelial barrier lesions could arise from excessive extrusion. For example, asthma is an inflammatory disease of the bronchial airways, but it has been unclear what triggers inflammation. One possibility is that during an asthma attack, the constriction of bronchioles could cause so much crowding of airway epithelia that it could cause hyper-extrusion and destruction of the epithelia barrier [96–98]. The resulting loss of epithelial barrier may trigger increased inflammation and susceptibility to infection typically seen in patients after an asthma attack [99–101]. Future studies will define what roles, if any, extrusion plays in the pathology of diseases such as asthma and IBD, which could provide new strategies for treatments.

3. CONCLUSION

Prior to the discovery of extrusion and its underlying mechanism, a majority of studies on cell homeostasis were performed in response to apoptotic stimuli, *in vitro*, and without an understanding of how these processes occur normally in epithelial monolayers. Emerging experimental evidence unraveling mechanistic details of the extrusion process is establishing its role in many different pathologies and diseases. Defects in the S1P-S1P₂ pathway that control apical extrusion underscores its importance in tumor suppression. How different pathogens promote or inhibit extrusion not only helps us understand pathogen/host responses but also reveals new information about mechanisms controlling extrusion. Additionally, excessive or defective extrusion can disrupt epithelial barrier function and may be the primary defect in a number of inflammatory diseases. As all the signals identified that regulate apical extrusion have defined new roles for extrusion in different epithelial-based diseases, further identification of extrusion signals will likely reveal how extrusion misregulation can drive other diseases and, potentially, new treatments.

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Abbreviations

S1P	Sphingosine-1-phosphate
S1P₂	Sphingosine-1-phosphate receptor 2
APC	Adenomatous polyposis coli
FAK	Focal adhesion kinase
Lm	Listeria monocytogenes
Stm	Salmonella typhimurium
RSV	Respiratory syncytial virus

GEF	Guanine nucleotide exchange factor
NAIP	Neuronal Apoptosis Inhibitory Protein
NS2	RSV non-structural 2 protein
TJ	Tight junction
IFN	Interferon

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Figure 1. Extrusion removes cells from the epithelium while maintaining barrier function

A) Apoptotic stimulus activates basolateral actomyosin contraction to squeeze the dying cell apically out of the epithelial layer and preserve the barrier function. **B)** During normal epithelial turnover, mechanical crowding from cell proliferation activates the stretch-activated channel Piezo1 to trigger some live cells to extrude. Live extruded cells later die by anoikis, or apoptosis due to loss of matrix-dependent survival signaling. The S1P/S1P₂ signaling pathway is critical for both apoptotic and live cell extrusion.

Figure 2. Signaling mechanisms controlling apical and basal extrusion

During apical extrusion, the cell destined to die produces and exports the bioactive lipid Sphingosine-1-phosphate (S1P) which binds to a G-protein coupled receptor, S1P receptor 2 (S1P₂), in the neighboring cells (inset). S1P₂ activates Rho to trigger actin and myosin recruitment and contraction along the basolateral surface of the extruding and neighboring cells to squeeze the extruding cell out apically. During wild-type apical extrusion, both the extruding and the neighboring cells reorient their microtubules basolaterally. Microtubule reorientation in the extruding cell presumably targets S1P to the base of the cell. In the neighboring cells, microtubules target p115 Rho-GEF (see text) to activate actomyosin contraction basolaterally and initiate apical extrusion. Loss of S1P₂, disruption of

microtubule dynamics due to mutations in adenomatous polyposis coli (APC) or expression of oncogenic K-Ras (K-Ras^{V12}) blocks apical extrusion and drives extrusion basally.

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Figure 3. Loss of Adenomatous Polyposis Coli (APC) or S1P₂, and oncogenic K-Ras expression causes cells to extrude basally

A) During wild-type extrusion, microtubule reorientation and S1P/S1P₂ signaling promote apical extrusion. **B)** Mutations in APC disrupt microtubule dynamics and cause the cell to extrude basally in a cell-autonomous manner. **C)** Expression of oncogenic K-Ras (K-Ras^{V12}) blocks apical extrusion by degrading S1P through autophagy and downregulating S1P₂, thus driving extrusion basally.

Figure 4. Pathogens hijack the extrusion process to infect and colonize epithelia

A) *Listeria monocytogenes* (*Lm*) invade at sites of cell extrusion by attaching to epithelial junctions through the synergistic action of two bacterial surface proteins Internalin A (InlA) and Internalin B (InlB), which bind to host E-cadherin and c-met, respectively (Inset). Endocytosis of junctions is accelerated at sites of extrusion, assisting E-cadherin-bound *Lm* internalization into the host cell. **B)** *Shigella*, *Salmonella*, and enterohaemorrhagic *Escherichia coli* (EHEC) inhibit extrusion by delivering the protein OspE (or homologs) into host cells. OspE binds the host Integrin Linked Kinase (ILK), which promotes and stabilizes focal adhesions, increases cell adhesion to the matrix, and inhibits cell extrusion (inset). These pathogens can either activate or block apoptotic pathways during the initial stages of infection to make cells more susceptible to invasion. **C)** Respiratory Syncytial Virus (RSV) protein NS2 causes cell shedding by suppressing IFN-mediated host antiviral response. The RSV envelope fusion protein (F) (Inset) causes p53-dependent apoptosis in polarized epithelial cell and contributes epithelial shedding, airway obstruction, and inflammation culture leading to accelerated extrusion of apoptotic cells. While epithelial cell shedding clears out RSV-infected cells to reduce viral titers, excessive cell shedding leads to blocking of smaller bronchiolar airways and bronchiolitis.

Figure 5. Mutations or downregulation of cell-cell adhesion molecules may cause defects in cell extrusion

The loss of key adhesion proteins (such as E-cadherin) or those regulating cell-cell adhesions (such as Lkb1) can result in aberrant extrusion, disruption of epithelial integrity, and possibly diseases such as celiac and inflammatory bowel disease (IBD).