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## Tumour cell invasion: an emerging role for basal epithelial cell extrusion

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### Abstract

Metastasis is the leading cause of cancer-related deaths, but it is unclear how cancer cells escape their primary sites in epithelia and disseminate to other sites in the body. One emerging possibility is that transformed epithelial cells could invade the underlying tissue by a process called cell extrusion, which epithelia use to remove cells without disrupting their barrier function. Typically, during normal cell turnover, live cells extrude apically from the epithelium into the lumen and later die by anoikis; however, several oncogenic mutations shift cell extrusion basally, towards the tissue that the epithelium encases. Tumour cells with high levels of survival and motility signals could use basal extrusion to escape from the tissue and migrate to other sites within the body.

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A crucial primary step for cancer metastasis is invasion, but we know very little about the mechanisms that govern it. As metastasis is the main reason that patients succumb to cancer, understanding the mechanisms that initiate metastasis will be crucial for targeting aggressive tumours. Because it has been difficult to directly follow tumour cell invasion from the epithelia, where most human cancers arise, we do not yet have a clear picture of the mechanisms that drive this process. In considering how tumour cells invade, it is helpful to understand how normal epithelia function and behave. Epithelia form a selective and protective barrier for all of the tissues that they encase. The polarized epithelium contains an apical surface that faces the lumen (external environment) and a basal surface that faces the basement membrane. Epithelia are the first line of defence against pathogens and toxins and, therefore, the cells that constitute epithelia are exposed to potential damage. As a result, many epithelia constantly turn over by cell division and death. We found that to maintain homeostatic epithelial cell numbers, when epithelia become too crowded owing to cell division elsewhere in the layer, some cells extrude and later die<sup>1</sup>. By extruding, cells that are destined for death are seamlessly ejected from the monolayer by concerted contraction of the cells that surround them<sup>2</sup>. Typically, because these cells extrude apically, they detach from the matrix and its associated survival signals, and die by anoikis. However, because metastatic tumour cells can, in some cases, override anoikis by upregulating survival signalling<sup>3,4</sup>, we propose that extrusion could enable them to escape the epithelium.

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#### Competing interests statement

The authors declare no competing interests.

Normally, epithelia extrude cells apically into the lumen, which would function to remove any transformed cells, thereby essentially suppressing tumorigenesis. Intriguingly, we have found that oncogenic signalling can alter normal apical extrusion and cause cells to instead extrude basally under the epithelium. In this way, basal extrusion could enable transformed cells that are refractory to cell death to invade the underlying stroma. In this Opinion article, we discuss how misregulation of extrusion and normal epithelial survival mechanisms could enable tumours to initiate metastasis by subverting a process that normally triggers epithelial cell death.

## Mechanisms of epithelial cell extrusion

Dying cells could pose a threat to the tight barrier that epithelia form, but they do not. Instead, epithelial cells that are destined to die are extruded by contraction of an actin and myosin ring in the surrounding cells, which squeeze cells out of the epithelium while closing the potential gap that could have formed from the exit of the cells (FIG. 1). All of the epithelia that have been observed, across animals from *Drosophila melanogaster*, zebrafish, mice and humans, extrude epithelial cells through what seems to be a highly conserved mechanism<sup>1,2,5-8</sup>. For a cell to extrude, it produces and secretes the bioactive lipid sphingosine-1-phosphate (S1P), which then binds to S1P receptor 2 (S1P2; also known as S1PR2; a G protein-coupled receptor) in the surrounding cells<sup>9</sup> to contract an intercellular actomyosin band that squeezes the cell out of the epithelium<sup>2</sup>. Additionally, we and others have observed that the cell being extruded also contracts, thereby aiding in its removal<sup>10</sup>. Epithelia extrude either live cells during homeostasis or dying cells in response to apoptotic stimuli<sup>1,2,6,11</sup>. We have found that epithelia maintain cell number homeostasis by extruding live cells once cells at a given site become up to 1.6-fold to 1.8-fold more crowded<sup>1</sup>. Crowding-induced extrusion occurs through activation of the mechanosensitive ion channel, PIEZO1, which presumably triggers calcium currents<sup>12</sup> to activate S1P-dependent extrusion of live cells, which later die by anoikis. Apoptotic stimuli, such as chemotherapies, toxins or pathogens that trigger cell death can also activate cell extrusion through the S1P-S1P2 pathway<sup>9</sup>, possibly in response to caspase activation<sup>11</sup>. Thus, both during natural cell turnover and following the induction of apoptosis, the S1P-S1P2 pathway activates cells to extrude in a manner that ensures no gaps form in the epithelia as cells are expelled.

## Apical versus basal extrusion

Normally, epithelia extrude cells apically into the lumen, but in some situations, cells can also extrude basally into the underlying tissue. The direction in which a cell extrudes depends on where the actomyosin band contracts in neighbouring cells<sup>13</sup>. To extrude a cell apically, contraction occurs towards the base of the cell, whereas to extrude it basally, contraction occurs at the apex (FIG. 1). The direction in which a cell extrudes can have important consequences for the fate of the cell, especially when live, transformed cells are extruded. Apical extrusion eliminates cells with upregulated survival signalling through the lumen (FIG. 1). For example, in the intestine, apical extrusion would remove putative tumour cells into the waste canal. Similarly, transient mosaic expression of oncogenic *HRAS*<sup>V12</sup> or *v-src* transforms cells and causes them to self-segregate away from the wild-type epithelium in a process that is similar to but different from extrusion, which essentially

removes them<sup>14,15</sup>. In mammary or prostate glands, apical extrusion could lead to carcinoma *in situ* — a tumour type with good prognosis in which cells accumulate in the luminal space and are generally non-invasive<sup>16,17</sup>. However, basal extrusion preserves live cells within the organ (FIG. 1). During development, basal extrusion could enable cells to dedifferentiate from the epithelium and then differentiate into new cell types, as during neuroblast delamination in *D. melanogaster*<sup>18</sup>. For tumours, basal extrusion could enable transformed cells to invade the tissue that the epithelium encases to initiate metastasis. Intriguingly, oncogenic mutations can subvert the normal extrusion pathway, shifting the direction of extrusion from apical to basal, and this suggests a link between basal extrusion and invasiveness. A basally extruded cell could either divide and accumulate beneath the epithelium or invade, depending on its ability to cross the basement membrane by either degrading or invading the underlying matrix<sup>19</sup>. Many tumours express various matrix metallo proteinases, which suggests that matrix degradation may be intrinsic to transformation, enabling basally extruded transformed cells to transit through the basement membrane. However, *in vivo* studies have suggested that cancer cells can breach the basement membrane without degrading it, by extending invadopodia that squeeze through gaps in the matrix and push it apart<sup>20,21</sup>. Determining whether basally extruded cells can breach the basement membrane and how they do so will be important goals for future *in vivo* studies.

Apical extrusion seems to require at least two activities: S1P–S1P2 signalling and microtubule dynamics. Microtubules reorient to the basolateral interfaces of both the extruding and neighbouring cells to localize RHO guanine nucleotide exchange factor 1 (ARHGEF1; also known as p115RHOGEF) and thereby activate RHO-mediated actomyosin contraction under the extruding cell, driving it out apically<sup>13</sup> (FIG. 2a). Disruption of microtubule dynamics shifts extrusion basally<sup>13</sup>. Although microtubules reorient in both the extruding cell and its neighbours, cell-autonomous knockdown of a crucial microtubule regulator, adenomatous polyposis coli (APC), suggests that the direction in which a cell extrudes requires dynamic microtubules only within the extruding cell<sup>22</sup>. Because the S1P–S1P2–RHO pathway controls only apical but not basal extrusion<sup>23</sup>, one possibility is that, in the extruding cell, microtubules target S1P to restrict contraction and membrane recycling basolaterally, where it is needed to drive apical extrusion (FIG. 2a). When any machinery that controls apical extrusion is aberrant, cell-autonomous contraction of cortical actin and myosin at existing apical junctions could enable a cell to extrude basally. Recent studies show that cell-autonomous apical contraction precedes the basolateral contraction in the neighbouring cells, and this suggests that loss of basolateral contraction would naturally lead to basal extrusion<sup>10</sup>. However, how complete apical contraction is controlled is still unknown, as are other signals and mechanisms that might collaborate to control apical extrusion.

## Diverting extrusion basally

### Oncogenic signalling

Although there is no direct proof that basal extrusion drives tumour cell invasion, we have found that oncogenic mutations can manipulate apical extrusion, a process that normally

promotes cell death, into a process that could allow cells to escape into the stroma. Mutations that disrupt the tumour-suppressive function of APC or constitutively activate KRAS disrupt normal apical extrusion by disrupting either cytoskeletal dynamics or SIP signalling, respectively (FIG. 2b,c). *APC* is mutated or lost in >80% of colorectal cancers and is downregulated in some breast and prostate cancers<sup>24–26</sup>. We found that cells preferentially extruded basally when a truncated form of APC associated with tumour formation was expressed in either cell lines or in zebrafish epidermis<sup>22</sup>. The APC truncation mutant lacks the carboxyl terminus that binds to both microtubules and F-actin<sup>27–29</sup>. Because apical extrusion relies on coupling micro tubules to cortical actin to control where contraction occurs, loss of this domain disrupts this targeting, thereby driving extrusion basally. APC functions cell-autonomously to drive apical extrusion, as expression of the microtubule-binding domain of APC in the extruding cell alone is sufficient to rescue apical extrusion in colorectal cancer cell lines expressing the truncation mutant. This suggests that single cells accumulating sporadic *APC* mutations could extrude basally. This new function for APC in controlling the direction of extrusion could collaborate with its known function in driving uncontrolled proliferation through upregulated WNT signalling<sup>30,31</sup>, thereby enabling *APC*-mutant cells to both invade and proliferate.

Oncogenic *KRAS* mutations are crucial drivers of aggressive tumours, such as those of the pancreas, lung and colon<sup>32–34</sup>. When we expressed the oncogenic *KRAS*<sup>V12G</sup> mutation in MDCK (canine kidney epithelial cell) monolayers, they predominantly extruded basally in a cell-autonomous manner<sup>23</sup>. When grown in three-dimensional cysts surrounded by matrix, the basally extruded oncogenic *KRAS*-mutant cells proliferated into smaller cysts or migrated away as single cells. Similarly, constitutive mosaic expression of oncogenic *HRAS* in MCF-10A mammary epithelial cell cysts caused cells to either basally extrude or lead collective cell migration of the neighbouring wild-type cells within the cyst<sup>35</sup>. Although three-dimensional cultures are more representative of *in vivo* epithelia than monolayers, it is not clear how well the Matrigel that surrounds these cysts mimics the underlying matrix and stroma in real tissue. Therefore, the compelling behaviour of basally extruded cells from cysts will ultimately need to be assessed *in vivo* to determine whether basally extruded transformed cells can also escape beneath the epithelium in real tissue. We have found that unlike mutated APC, which disrupts microtubules, cells that express oncogenic *KRAS* degrade SIP and partially down-regulate SIP2, both of which are required for apical extrusion. SIP is degraded owing to high levels of autophagy, specifically in extruding *KRAS*-transformed cells. Disruption of autophagy (either genetically or chemically) rescued SIP accumulation and apical extrusion<sup>23</sup>. Because cells expressing oncogenic *KRAS* rely on autophagy for their increased survival<sup>36,37</sup>, current clinical trials are using chloroquine (an autophagy inhibitor) to target these cells. This treatment could also prevent tumour invasion by promoting apical extrusion<sup>38</sup>. Chloroquine treatment has already shown promising results in overcoming chemotherapy resistance in human HER2 (also known as ERBB2)-positive breast cancers that are also addicted to autophagy<sup>39</sup>. However, mouse models of genetically engineered pancreatic cancers that lack p53 have found that chloroquine treatment can actually exacerbate tumour growth<sup>40</sup>, suggesting some human tumours driven by *KRAS* mutants might also be chloroquine-resistant.

### Alternative mechanisms to divert extrusion basally

Apart from APC and KRAS, other factors that are typically associated with poor cancer prognosis could also shift the direction in which cells extrude from epithelia. Proteins controlling epithelial cell polarity, such as those in the scribble homologue (SCRIB)–lethal giant larvae homologue (LGL)–discs large homologue (DLG) and partitioning defective 6 homologue (PAR6)–PAR3–atypical protein kinase C (aPKC) complexes, are mutated in numerous carcinomas<sup>41</sup> and have been linked to their progression and metastasis<sup>42</sup>. Disruption of epithelial polarity could randomize the direction in which a cell extrudes, thereby increasing the incidence of basal extrusion (FIG. 3a). Another possibility is that activation of basal extrusion alone could occur without invoking other signals that activate apical extrusion (FIG. 3b). In several studies, basal extrusion seems to occur as a default pathway when apical extrusion fails<sup>10,23</sup>. Although it is not clear what controls basal extrusion, one candidate could be hyperactivation of RHO, which is associated with tumour cell migration<sup>43,44,20</sup>. It is plausible that simply activating RHO would cause cells to contract at the apical cell–cell junctions, where most actin and myosin II exists. In addition, comparatively weaker cell–cell or cell–matrix adhesions could cause some cells to preferentially detach (FIG. 3c). Studies of nanomechanical forces of single cells, both *in vitro* and from human breast cancer biopsies, revealed that metastatic breast tumour cells are less stiff than the rest of the tumour or surrounding normal tissue<sup>45</sup>, and this can substantially enhance their motility *in vitro*<sup>46</sup>. Similarly, reduced cell–cell adhesion by decreased expression of E-cadherin that is found in some metastatic tumours<sup>47</sup> could make these cells more susceptible to extrusion than others. Although reduced tension of a single cell within an epithelium under expansion forces could make that cell spread, under intrinsic crowding forces in regions of extrusion<sup>1</sup>, it would instead be more likely to extrude. Loss of E-cadherin and reduced cell stiffness have been linked to metastatic tumours, but it is unclear whether these factors drive cell extrusion or are a consequence of losing contact with the epithelium following extrusion. Therefore, further studies will need to identify what makes a cell susceptible to extrusion and whether factors that promote extrusion do so in a preferentially apical or basal manner.

### Basal extrusion and tumour invasion

We propose that tumour cells could exploit epithelial extrusion as a mechanism to initiate invasion into the underlying stroma. Current models for how cells escape the primary tumour to initiate metastasis fit into two broad categories: collective cell and single cell invasion<sup>21,48,49</sup>. The main difference between these modes of invasion is whether or not tumour cells maintain intracellular contacts as they migrate through the stroma and matrix (BOX 1). Histological sections showing streams of cells and single cells emanating from the tumour lend support to both types of motility<sup>21</sup>. In considering the hypothesis that tumour cells can invade using basal extrusion, it is important to establish the fate of transformed cells after they have extruded basally. When we transform MDCK cells with oncogenic KRAS, cells that extrude basally from cysts show two different behaviours: they either migrate away singly (FIG. 4a) or they proliferate, thereby forming a smaller cyst that is attached to the parent cyst<sup>23</sup> (FIG. 4b). Therefore, basal extrusion of transformed cells could enable cells to potentially migrate to other sites using either mode of invasion. It may be that

some cells are primed to lose E-cadherin after extrusion to become more mesenchymal-like or stem cell-like (FIG. 4a). Basal extrusion seems to drive cells to dedifferentiate during development, as is the case during neuroblast delamination from the neuroepithelium in *D. melanogaster* or blood stem cell budding from the endothelia of many vertebrates<sup>50,18</sup>. Similarly, in cancer, tumour-initiating cells may bud from the epithelium by basal extrusion, thereby increasing their ability to proliferate and survive in foreign sites. Alternatively, basally extruded cells may retain their E-cadherin, as suggested by the ability of extruded cells expressing oncogenic *KRAS* to form new intact cysts. As seen in MCF-10A cysts, an initial basally extruded cell with an *HRAS* mutation could lead the collective cell migration of other attached cells<sup>35</sup>. Cells that do not lose E-cadherin expression after basal extrusion may still be less differentiated, having more intrinsic ability to divide, but restricted to an epithelial rather than a mesenchymal or pluripotent cell fate. Given the right cues *in vivo*, these cells could undergo collective cell migration rather than simply divide (FIG. 4b). Although epithelia that are cultured in three dimensions behave more like epithelia *in vivo*, they lack the complex components of real tissue. Thus, future work will need to determine whether basal extrusion could drive either single or collective cell migration in an *in vivo* model system.

### Box 1

#### Current models of carcinoma invasion

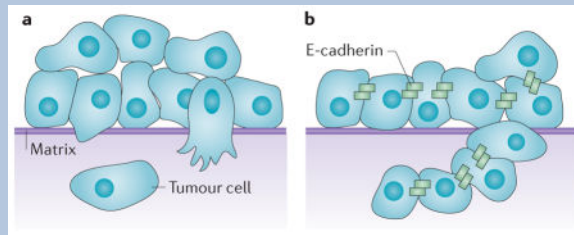
##### EMT: invasion of single tumour cells from a mass

Epithelial-to-mesenchymal transition (EMT) might provide a mechanism by which some single cells disseminate from an epithelial tumour mass<sup>47,67,68</sup> (see the figure, part **a**). During EMT, tumour cells downregulate E-cadherin (thereby weakening cell-cell adhesions) and migrate away as single cells. The loss of E-cadherin and activation of matrix-degrading proteases changes cell behaviour to be more mesenchymal, which allows these cells to adapt and survive in different parts of the body, independently of normal epithelial survival signals. EMT could promote the dedifferentiation of cells into stem cells in development and cancer<sup>67,69</sup>. Mesenchymal cells could then transdifferentiate to an epithelial phenotype in different sites within the body to promote metastatic outgrowth. Several types of carcinomas with poor prognosis express various inducers of EMT, such as snail homologue 1 (SNAI1) and SLUG (also known as SNAI2), TWIST, and zinc finger E-box-binding homeobox 1 (ZEB1) and ZEB2, which downregulate E-cadherin, and this supports the idea that tumours use EMT to initiate invasion.

##### Collective tumour cell invasion from a mass

Tumour cells have been seen emanating in streams from a tumour cell mass in what is termed collective cell migration<sup>21,48,70</sup> (see the figure, part **b**). Cells can migrate as a continuous mass that disseminates from the primary tumour or as smaller discontinuous cohorts of nearby cells. Unlike single cells that invade by EMT, these cells maintain cell-cell adhesions and are cohesive as they migrate. Migration of a cell front requires degradation of the matrix and the secretion of matrix-degrading proteases. Owing to histological evidence and the fact that most metastases retain E-cadherin, most metastatic

carcinomas are thought to migrate collectively. In addition, it is thought that metastatic cells may alter their ability to migrate singly or collectively, depending on the matrix and the tissue that they encounter.



An important point that distinguishes basal extrusion from the other models is that extrusion of cells can occur away from the main tumour mass (FIG. 4a,b). We have found that although oncogenic *KRAS* can drive cells to lose contact inhibition, the sites where cells basally extrude are not necessarily the same as the areas where masses form<sup>23</sup>. This could account for why pancreatic, colon and lung tumours that are driven by *KRAS* are typically metastatic with poor prognosis. The idea that tumours could metastasize independently of a primary tumour is alarming but not unheard of<sup>51,52</sup>. Indeed, molecular profiling of different tumour types suggests that some tumours develop with higher likelihoods of metastasizing than others<sup>53–56</sup>. Although transformed cells could invade at sites that are distinct from the primary mass using epithelial-to-mesenchymal transition (EMT) or collective cell migration, the invasive aspects in these models typically derive from general loss of epithelial organization and compressive forces that arise in surrounding tissue owing to growth of the primary tumour.

## Concluding remarks

It is not clear why some tumours metastasize, whereas others do not. Our understanding of tumour invasion is mitigated by our ability to capture the natural formation and invasion of a tumour from an epithelium *in vivo*. Two-photon confocal intravital imaging of mammary mouse tumours has provided unprecedented resolution of migrating tumour cells *in vivo*<sup>57–62</sup>. One caveat to this approach, however, is the necessary introduction of a wound to provide a window for microscope access to the tumour, which could result in signalling and inflammation that is not present in naturally occurring tumours<sup>63</sup>. Future studies using zebrafish could provide an excellent animal model to directly visualize tumours invading directly from epithelia, as embryos are transparent and can be readily imaged without the invasive techniques that are intrinsic to current tumour models<sup>64</sup>. The zebrafish epidermis provides an excellent model for the epithelial bilayer that encases lungs and mammary glands. We are developing tools to label, knock down and express genes in single cells, where we can directly follow cells dividing, migrating and invading from the epithelium *in situ* (G.M.S., A. V. Gardner, G. Eisenhoffer and J.R., unpublished observations). Additionally, a new method to test the metastatic potential of human tumours has been developed in zebrafish embryos<sup>65,66</sup>. In this model, within only 48 hours after injecting cancer cells into the yolk sacs of 2-day-old zebrafish embryos, the metastatic potential was found to mimic mouse xenografts that were observed over the course of 2 months<sup>54</sup>, and this zebrafish model allows tumour cell dissemination to be filmed live.

Once a tumour cell has gained access to the circulation, the extravasation, survival and establishment of micrometastases in a distal organ are thought to be surprisingly efficient<sup>4</sup>. Therefore, identifying whether tumour cells use basal extrusion to initiate invasion into the underlying stroma before entering the circulation is of crucial importance to our understanding of metastasis. Understanding the molecular and genetic profiles of cells that can extrude basally and survive might help us to define the metastatic potential of some tumours. Furthermore, identifying whether basal extrusion might be enhanced with zinc finger E-box-binding homeobox 1 (ZEB1), snail homologue 1 (SNAI1) and SLUG (also known as SNAI2), which drive EMT, or with matrix metalloproteinases, which are typically upregulated with collective cell migration, will be important to determine the relationship of extrusion to these previously defined invasion modes. New imaging methods that allow us to follow metastases from their initial local invasion to their colonization in distant organs will also be important for determining how cells invade and migrate and whether basal extrusion is a crucial step in this process. A better understanding of how different tumours invade will be essential for preventing their spread.

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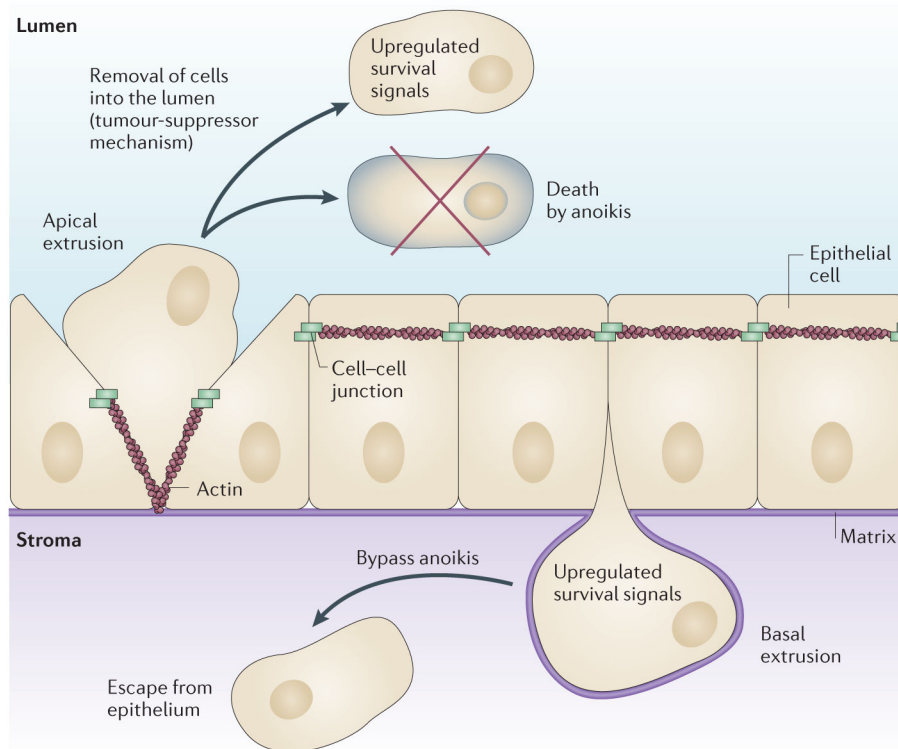
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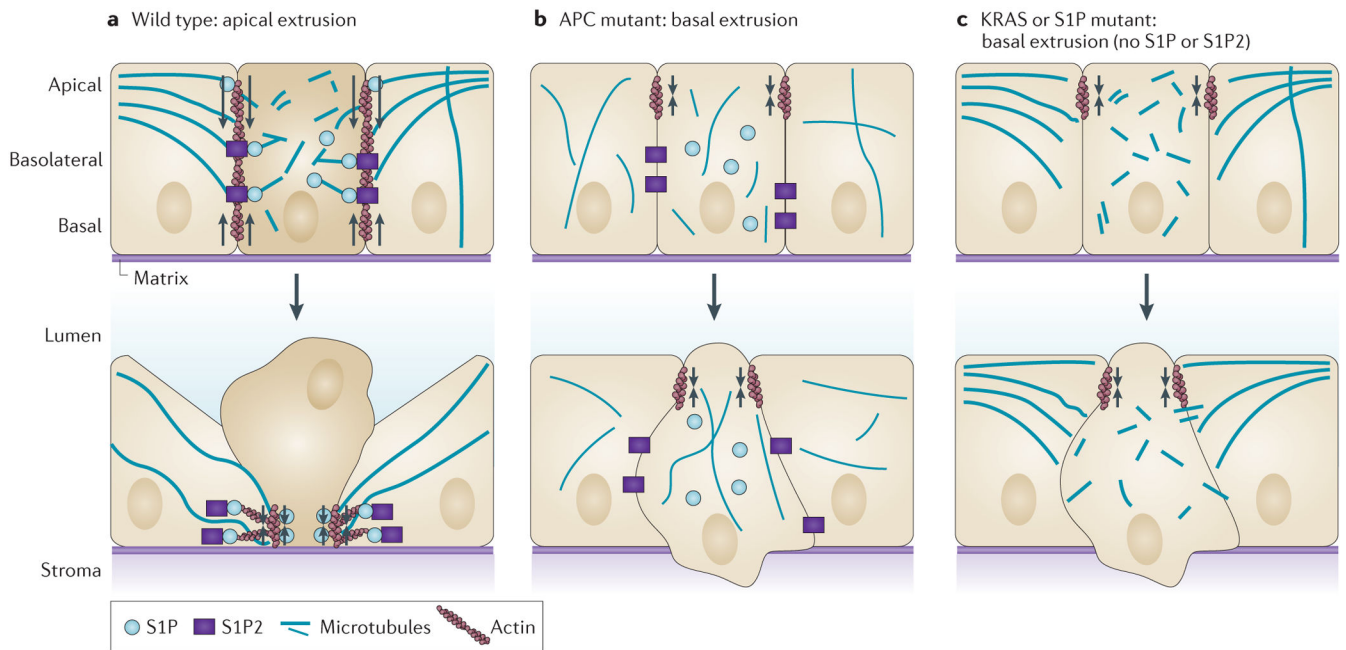
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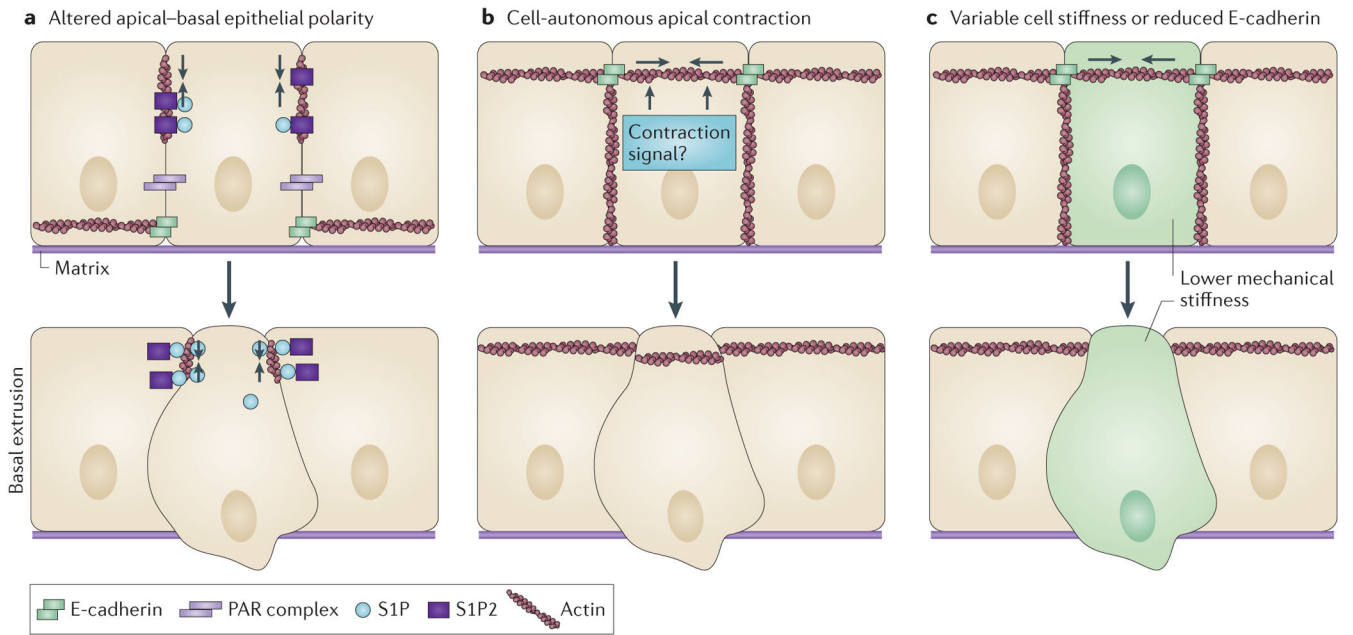


**Figure 1. The direction in which a cell extrudes has important consequences for its fate** Extrusion removes either live or dying epithelial cells in response to crowding during homeostasis or apoptotic stimuli, respectively. Typically, live cells extrude apically from the epithelium into the lumen by contracting an intercellular actomyosin band basolaterally to squeeze the cell out (left). Apically extruded cells generally die in the lumen by the loss of survival signals from the matrix — a process called anoikis (top). Tumour cells with upregulated survival signalling could still be eliminated through the lumen by apical extrusion, which could function like a tumour-suppressor mechanism (top). Less frequently, cells extrude basally by apical contraction (right), back into the tissue that the epithelium encases. Several oncogenic mutations disrupt apical extrusion, thereby driving cells to extrude basally instead. Because basally extruded cells with upregulated survival signals can bypass anoikis, this may provide a novel mechanism to enable oncogenic cells with upregulated survival signalling to initiate invasion (bottom).



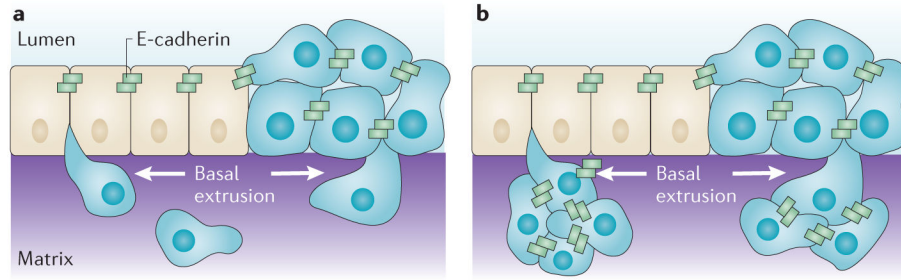
**Figure 2. Modes of diverting extrusion basally**

**a** | During wild-type apical extrusion, the cell that is destined for extrusion (dark beige), as well as its neighbouring cells (light beige), reorient their microtubules to the basolateral interface. Reorientation of microtubules in the cell destined for extrusion is required for apical extrusion and presumably restricts the biologically active lipid sphingosine-1-phosphate (S1P) to the basolateral surface, where it binds to S1P receptor 2 (S1P2) expressed in the neighbouring cells to trigger apical extrusion. Microtubule reorientation in the neighbouring cells might reinforce RHO-mediated actomyosin contraction (arrows) at the basolateral surface. **b** | Mutations in the tumour suppressor adenomatous polyposis coli (APC) that disrupt microtubule dynamics can function cell-autonomously in the extruding cell to drive extrusion basally. Cell-autonomous apical contraction of cortical actin and myosin (arrows) at apical epithelial cell junctions can extrude the cell basally into the basement membrane. **c** | Oncogenic KRAS disrupts apical extrusion by downregulating both S1P and S1P2. Autophagy degrades S1P, and both S1P puncta and apical extrusion can be rescued by disrupting autophagy. Without apical extrusion signalling, junctional apical actin and myosin contraction (arrows) results in basal extrusion.



**Figure 3. Alternative mechanisms to divert extrusion basally**

**a** | Improper localization or disruption of proteins controlling apical–basal epithelial cell polarity could increase basal extrusion by randomizing the direction in which a cell extrudes and disrupting apical extrusion. For example, E-cadherin and partitioning defective (PAR) protein complexes, which are crucial for localizing actin and myosin at apical contacts, could organize actin elsewhere if polarity is disrupted. **b** | Cell-autonomous apical contraction (arrows) alone could result in basal extrusion. Although it is not clear what activates contraction during basal extrusion, one candidate could simply be activation of RHO, which would cause actin and myosin — which are typically concentrated apically at adherens junctions — to contract at the top of the cell. **c** | Stiffness of tumour cells has been shown to have an important role during cell invasion. Under compressive forces, Cells that are less stiff (green), would be more primed to extrude than neighbouring, stiffer cells. Similarly, reduced E-cadherin levels, which are also associated with metastatic cells, would result in reduced attachment to surrounding cells, and these cells with lower E-cadherin levels might be more likely to become detached by extrusion. Although either of the above mechanisms could enable cells to extrude apically or basally, cells may be more likely to extrude basally, which seems to be the default direction in the absence of canonical extrusion signalling. S1P, sphingosine-1-phosphate; S1P2, S1P receptor 2.



**Figure 4. Model of single and collective cell-autonomous basal extrusion of tumour cells during tumour invasion**

Transformed cells (blue) could invade by basal extrusion, either when surrounded by wild-type cells (beige) or from homogeneously transformed neighbouring cells (blue). **a** | Basally extruded cells could downregulate E-cadherin once they lose contact with other epithelial cells and migrate away as single cells. **b** | Basally extruded cells could retain E-cadherin expression and proliferate and migrate together by collective cell migration.