Harnessing epithelial homeostatic mechanisms to fight cancer

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ABSTRACT **Cancer treatments have, in general, targeted the cancer cell itself. This approach has often been unsuccessful in the long term, especially for solid tumors. Even targeted therapies based on sequencing cancer genomes can be thwarted by genetic heterogeneity within tumors. Furthermore, genomic instability in cancer cells accelerates the generation of variants that are resistant to the treatment. Immunotherapies and anti-angiogenic treatments, which target the tumor-interacting and tumor-adjacent cells, have overcome some of these challenges, suggesting that other methods that target wild-type cells could be valuable in arresting tumor progression. Studies in *Drosophila* have uncovered mechanisms by which cells within an epithelium can react to neighboring cells that have genetic differences, resulting in the elimination of one population at the expense of another. Some of these mechanisms are now known to be conserved in mammals. The possibility of harnessing such mechanisms to empower normal epithelial cells to eliminate their precancerous neighbors before they develop into fully fledged cancers is an area of research that merits more attention.**

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INTRODUCTION

Most human cancers originate in epithelia as preneoplastic clones of mutant cells surrounded by wild-type cells (Figure 1, A and B). As cancers grow, there is a selection for additional mutations that promote growth, inhibit apoptosis, enable angiogenesis, and help evade immune surveillance (Figure 1C). Tumors are usually detected, either by physical examination or by a variety of diagnostic tools, only when they are sufficiently large. By that time, many have breached the underlying basement membrane and initiated the process of spreading to distant sites in the body (metastasis). Treatments that seek to kill tumor cells by exploiting a genetic weakness are often not fully effective, because advanced cancers are genetically heterogeneous. Moreover, tumor cells often have genetic lesions that increase the mutation rate. Thus, even when a tumor initially responds to a treatment, recurrence of a drug-resistant version is common and treatments can eventually become ineffectual.

Therapies that target wild-type cells in a tumor's environment, such as immunotherapies which subject the tumor to immune system attack (Ribas and Wolchok, 2018), or anti-angiogenic therapies which deprive a tumor of oxygen and nutrients (Jayson *et al.*, 2016), have shown promise in overcoming the challenges posed by tumors. Another potential way of treating cancers is to arrest their progression at a very early stage, before their genomes become unstable and before they become more genetically heterogeneous. Indeed, for colorectal cancers, the detection and excision of preneoplastic lesions by regular colonoscopy has been shown to reduce the incidence of advanced cancers (Citarda *et al.*, 2001). However, this type of approach based on regular screening is not easily applicable for the detection of tumors in many internal organs such as the pancreas; pancreatic cancers are almost invariably fatal unless detected at an early stage (David *et al.*, 2009). Novel systemic therapies that help eliminate early preneoplastic lesions could theoretically prevent a great number of life-threatening cancers.

Here we discuss homeostatic mechanisms that function in epithelial tissues that appear capable of eliminating clones of abnormal cells. Many of these mechanisms were discovered in studies of *Drosophila* imaginal discs, the larval precursors of adult tissues such as wings and eyes. Importantly, similar mechanisms have been shown to function in mammalian epithelia, suggesting that these

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Abbreviation used: MDCK , Madin-Darby canine kidney.

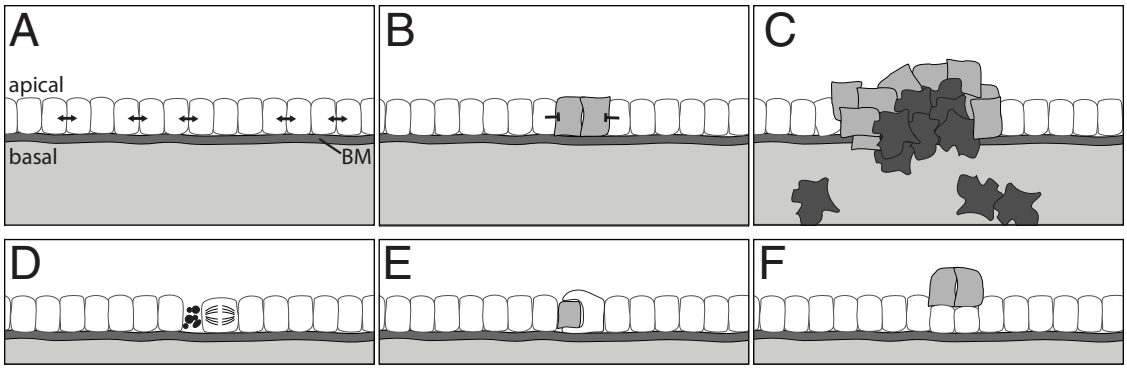

FIGURE 1: Epithelial homeostatic mechanisms can prevent tumorigenesis. (A) Under normal conditions, epithelial cells maintain tight physical contact with their neighbors. BM, basement membrane. (B) A preneoplastic clone (gray) arises when a single cell acquires a cancer-causing mutation and begins to proliferate. (C) If containment is unsuccessful, the clone proliferates and may acquire additional mutations leading to overgrowth and possibly invasion through the basement membrane and metastasis. (D–F) Neighboring cells (white) attempt to contain this proliferation by promoting the apoptosis of misspecified cells and replacing them through proliferation (D), by engulfing them (E), or by causing their extrusion from the epithelium (F).

homeostatic mechanisms might be evolutionarily ancient. Here we argue that a better understanding of epithelial homeostatic mechanisms, and the possibility of manipulating them, might lead to a novel therapeutic strategy: eliminating nascent preneoplastic lesions from epithelia before they become full-blown cancers.

DISCOVERY OF EPITHELIAL HOMEOSTATIC MECHANISMS IN *DROSOPHILA*

Drosophila epithelial cells can compare their fitness levels with that of their neighbors and activate processes that culminate in the elimination of unfit cells. Ginés Morata and Pedro Ripoll demonstrated in 1975 that normal epithelial cells of the imaginal discs eliminate their slow-growing neighbors, a phenomenon they named "cell competition" (Morata and Ripoll, 1975). These investigators studied the properties of mutations known as *Minutes* that are now known to, in most cases, inactivate genes encoding ribosomal proteins. Cells that are heterozygous for a *Minute* mutation grow more slowly. Despite this handicap, *Drosophila* that are heterozygous for *Minute* mutations develop to become near-normal fertile adults, although their development takes much longer than wild-type flies. Morata and Ripoll (1975) devised an ingenious experiment where they generated patches of *Minute* heterozygous cells within wing-imaginal discs composed of wild-type cells. Instead of observing abnormally small patches of *Minute/+* tissue in adult flies, because of the expectation that these patches of cells would grow especially slowly, they observed that these patches were eliminated completely. Thus, *Minute/+* cells are viable provided the entire tissue is composed of them. However, when patches of *Minute/+* cells are generated in the midst of wild-type cells, they are eliminated.

While Morata and Ripoll (1975) correctly inferred the existence of cell competition from an examination of adult flies, many years passed before the process was actually visualized in imaginal discs. This was facilitated by methods to efficiently generate *Minute/+* clones in imaginal discs and to mark these clones, thus allowing the unambiguous identification of wild-type and *Minute/+* cells, especially at the clonal borders. In these experiments, it was observed that *Minute/+* cells at the edge of the clone (i.e., those that are adjacent to wild-type cells) undergo apoptosis (Moreno *et al.*, 2002). Thus, the *Minute/+* clone is progressively eliminated by apoptosis at its receding boundary where *Minute/+* cells are directly adjacent to wild-type cells (Figure 1D). Subsequent studies showed that cells driven to grow faster by increased levels of Myc are able to eliminate wild-type cells in their vicinity and have been dubbed "supercompetitors" (de la Cova *et al.*, 2004; Moreno and Basler, 2004). Similarly, clones of cells with enhanced Wg/Wnt (Vincent *et al.*, 2011) or JAK/STAT signaling (Rodrigues *et al.*, 2012), or decreased Hippo signaling (Tyler *et al.*, 2007), also outcompete wild-type cells. Since wild-type cells can be "winners" when they are adjacent to *Minute/+* cells and "losers" when they are near cells with more Myc, the fate of a cell in such a contest cannot be predicted by its intrinsic properties but rather by how it compares to its neighbors. Indeed, since many of the mutations that make cells supercompetitors affect genes that are frequently mutated in human cancers, it has been suggested that the ability of cancer cells to proliferate is augmented by their ability to kill their wild-type neighbors as has now been clearly demonstrated using tumor models in *Drosophila* (Eichenlaub *et al.*, 2016; Suijkerbuijk *et al.*, 2016).

We are unable to provide a comprehensive review on cell competition here, but instead direct readers to recent reviews covering competition in *Drosophila* (Baker, 2017; Nagata and Igaki, 2018) and vertebrates (Kon, 2018). Since the initial discovery of cell competition, classically defined as apoptosis of less fit cells, occurring at their border with more fit cells, a variety of similar homeostatic phenomena have been described in *Drosophila* imaginal discs that eliminate aberrant cells. Clones of cells with mutations in the apicobasal polarity regulators *scribble* and *lgl*, generated in the midst of wild-type cells, are eliminated, even though tissues composed entirely of homozygous mutant *scribble* or *lgl* cells show unconstrained growth. Clones of cells with mutations in *mahjong*, which encodes a protein that binds to Lgl, are also eliminated, though interestingly these clones do not have obvious defects in apicobasal polarity (Brumby, 2003; Menendez *et al.*, 2010; Tamori *et al.*, 2010). In addition, imaginal discs can eliminate misspecified cells, such as cells that express genes characteristic of a leg fate within a wing disk, often by basal extrusion of the entire clone (Adachi-Yamada and O'Connor, 2002; Bielmeier *et al.*, 2016). Thus, the elimination of aberrant cells can, in different cases, involve one or more processes that include apoptosis of mutant cells, their extrusion from the epithelium, and the engulfment of mutant cells by their wild-type neighbors (Figure 1, D–F).

EPITHELIAL HOMEOSTASIS IN MAMMALS

As in *Drosophila*, mammalian tissues also seem to have homeostatic mechanisms that can eliminate unfit cells. This has been primarily studied in the early epiblast. Similar to earlier experiments in imaginal discs, researchers have shown that cells heterozygous for mutations in genes encoding ribosomal proteins, or cells with lower Myc levels than their neighbors, are eliminated from the early epiblast (Oliver, 2004; Clavería *et al.*, 2013; Sancho *et al.*, 2013). Cell competition can also be induced in fetal and adult tissues by mosaic overexpression of Myc (Villa del Campo *et al.*, 2014) or loss of Notch (Alcolea *et al.*, 2014).

Similar to imaginal discs of *Drosophila*, it seems that mammalian epithelia have an innate ability to self-surveil and eliminate less fit cells. The elimination of aberrant clones from mosaic contexts has been seen in Madin-Darby canine kidney (MDCK) epithelial cells grown in culture (Hogan *et al.*, 2009) and in diverse mouse epithelia including the hair follicle, pancreas, gut, and lung (Brown *et al.*, 2017; Sasaki *et al.*, 2018). Mammalian epithelia have been shown to clear cells bearing some of the same abnormalities that have been investigated in *Drosophila*. For instance, clones of MDCK cells with decreased expression of Mahjong or Scribble undergo apoptosis only when surrounded by wild-type MDCK cells (Tamori *et al.*, 2010; Norman *et al.*, 2012).

Additionally, experiments in mammalian tissues suggest an ability to clear aberrantly overgrowing, preneoplastic clones. This clearance occurs by some of the same mechanisms that have been identified in *Drosophila* epithelial homeostasis, including induction of apoptosis of aberrant cells, or extrusion of these cells from the epithelial layer. When β-catenin–overexpressing, overgrowing clones are generated in the mouse hair follicle epithelium, these clones are surrounded by wild-type cells before undergoing apoptosis. These surrounding wild-type cells play an active role in inducing apoptosis; if wild-type cells are prevented from maintaining contact with the overgrowing clone, the clone can continue to grow in an uncontrolled manner (Brown *et al.*, 2017).

In other cases, single cells or small clones of cells are extruded from the epithelium, leaving behind a wild-type epithelium. Src and RasV12 mutant clones extrude from in vitro MDCK epithelia (Hogan *et al.*, 2009; Kajita *et al.*, 2010) as do clones expressing constitutively active YAP (Chiba *et al.*, 2016). Similar extrusion of RasV12 mutant clones has been seen in vivo in the *Drosophila* wing-imaginal disk epithelium (Prober and Edgar, 2002; Bielmeier *et al.*, 2016) and in diverse mouse tissues (Sasaki *et al.*, 2018). The Fujita group, which has studied this phenomenon intensely, has named this process Epithelial Defense Against Cancer, or EDAC. This may explain an earlier finding: when oncogenic Ras was expressed in random pancreatic precursor cells, the formation of invasive tumors was exceedingly rare (Hingorani *et al.*, 2003). The vast majority of preneoplastic clones were perhaps shed from the epithelium. Epithelial integrity is key to these processes, as loss of E-cadherin in wild-type MDCK cells drastically decreases the rate of RasV12 clone extrusion (Hogan *et al.*, 2009).

Two additional mechanisms of homeostatic tumor prevention have been suggested in mammals. In some cases, epithelia can simply prevent the overgrowth of a clone, without necessarily eliminating it. In three-dimensional cultures of human mammary epithelia, when a single cell was given a growth-promoting mutation, such as overexpression of Myc, or constitutively active AKT or CyclinD1, these cells simply remained quiescent, contained by their wild-type neighbors. Once again, when epithelial organization was perturbed, these cells regained their overproliferative behaviors (Leung and Brugge, 2012). Additionally, wild-type tissues may have some ability to induce the differentiation of cancerous cells. In rare cases, blastocysts that contain transplanted teratoma cells can grow into normal mosaic mice, with tissue originating from both the host blastocyst and the transplanted cells, suggesting that the teratoma cells were directed to normal developmental fates (Brinster, 1974; Mintz and Illmensee, 1975; Illmensee and Mintz, 1976).

Tantalizing evidence suggests that similar processes occur in human tissues, depressing the rate of tumor formation. Mutations that have been defined as "cancer-causing" are much more common in human skin cells than are skin cancers (Martincorena *et al.*, 2015). Additionally, autopsy studies of adults who died in their 40s of causes other than cancer show surprisingly high rates of histologically frank tumors (Nielsen *et al.*, 1987; Sakr *et al.*, 1993). Statistics on lifetime occurrence of tumors indicate that only a small portion of these tumors would have been likely to become invasive (Noone *et al.*, 2018). While the immune system likely has a role in cancer prevention, these observations suggest that nascent tumors might also be eliminated or contained by epithelial homeostatic mechanisms.

FUTURE DIRECTIONS

We suggest that more research is needed to unravel the molecular details of these epithelial homeostatic mechanisms and that genetic screens in model organisms could lead the way. For example, *Drosophila* is particularly suited to clonal screens where genetic manipulations in tumor-adjacent cells are possible either using chemical mutagenesis (Yamamoto *et al.*, 2017) or using RNA interference via methods such as CoinFLP (Bosch *et al.*, 2015). Similar systems could be engineered to enable screens involving mammalian cells. These could be conducted in epithelial monolayers in culture, in organoids, or even in intact organisms. A screen for small molecules that affect the extrusion of RasV12 clones in mammalian cell culture has already been described (Yamauchi *et al.*, 2015).

If we can find ways to enhance epithelial homeostatic mechanisms and thus empower normal epithelial cells to eliminate nascent cancers in their midst, it might be possible to devise preventative treatments whose goal is to cause the periodic shedding of preneoplastic lesions, long before they become life-threatening cancers. Such an approach could potentially obviate many of the problems associated with treating advanced, genetically heterogeneous cancers.

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