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Mechanisms of cell competition emerging from *Drosophila* studies

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Abstract

Cell competition was described in *Drosophila* as the loss from mosaic tissues of otherwise viable cells heterozygous for Ribosomal protein mutations ('*Minutes*'). Cell competition has now been described to occur between multiple other genotypes, such as cells differing in *myc* expression levels, or mutated for neoplastic tumor suppressors. Recent studies implicate innate immunity components, and possibly mechanical stress, compression and cell intercalation as a consequence of differential growth rates in competitive cell death. Competition to eliminate pre-neoplastic tumors makes use of signals and receptors also used in patterning the nervous system including Slit/Robo2 and Sas/PTP10D to recognize and extrude clones of mutant cells, at least where local epithelial cyto-architecture is favorable. Cell competition facilitates expansion of *Drosophila* tumors through host tissue, and in normal development may promote developmental robustness and longevity by selecting for optimal progenitor cells.

INTRODUCTION

In 1975 Morata and Ripoll reported an interesting observation concerning a class of '*Minute*' mutations that they were studying in *Drosophila* [1]. '*Minute*' mutations dominantly slow cell growth and division rate. Almost all correspond to loss of function mutations of cytoplasmic ribosomal proteins [2,3]. Morata and Ripoll reported that although animals of these slow-growing genotypes were clearly viable, clones of cells of these genotypes generally could not survive in mosaics with wild type cells. They called this sensitivity of the Minute cells to a wild type cellular environment 'cell competition' [1] (Figure 1A).

It was soon suggested that mutations in other genes might also lead to cell competition [4]. Genotypes for which cell competition has now been specifically characterized are listed in Table 1. Some examples involve 'super-competitor' cells that out-compete normal, wild type cells. For example cells that express relatively more Myc are super-competitors [5,6](Figure 1B). In *Drosophila*, cell competition has only been observed in vivo in proliferating

epithelial tissues, such as the undifferentiated imaginal discs that comprise the precursor cells for adult structures, in the ovarian follicle, and in the adult gut [1,7,8*]. Several examples of cell competition have now also been described in mammalian systems [9].

Many interesting questions about cell competition remain incompletely resolved. How are cells targeted for elimination only in mosaic environments? What is the adaptive significance of cell competition? Is cell competition conserved in mammals, and if so does it contribute to health or disease? There is particular interest in possible cell competition in cancer, because tumors generally differ genetically from surrounding cells and because many cell competition genes have mammalian homologs that are proto-oncogenes or tumor suppressors (Table 1). Cell competition has been comprehensively reviewed previously [10–13,9,14] and the present review serves to summarize recent developments in *Drosophila*, where there has been progress in understanding both apoptotic cell competition mechanisms and in mechanisms that involve cell extrusion from epithelia.

Competitive apoptosis

The competition of *Minute* cells by wild type cells relies on the induction of apoptosis. *Minute* cells survive in mosaic tissues when apoptosis is blocked [15,16]. Competition between cells that differ in *myc* expression level is similar. *Myc* mutant cells die in mosaics with wild type cells, and wild type cells undergo apoptosis in the presence of cells with higher *myc* gene copy numbers, or mildly elevated *myc* expression level [5,6]. Competitive apoptosis is concentrated near the interfaces between the genotypes, supporting earlier conclusions that competition is a local phenomenon [17,5,18].

Is competitive apoptosis triggered by a molecular distinction that is recognized at the interface between the competing cells? Early reports focused on gene transcripts (or isoforms) that were up-regulated in cells under competition by *Myc*, which encoded Flower, Sparc and Azot proteins [19–21*]. If these expression changes reflect responses to a competitive situation, not apparent before cells with different *Myc* levels encounter one another, they may not represent the initial differences that first trigger the competition. More recently, it has been shown that components of innate immune pathways, including Toll-related receptors and NF-KB transcription factors, are required during both *Minute* and *Myc* competition and stimulate cell death genes [22]. Although this is suggestive of altered-self recognition at competitive interfaces, no directional receptor-ligand interaction has yet been found. Perhaps molecular recognition of the competed cells occurs, but this is not the only possibility. It should particularly be noted that cells expressing more *Myc* are out-competing wild type, which could not ordinarily express a ‘non-self’ marker.

Consequences of differential growth rates

In many cases the out-competed cells are slower growing than their competitors. Also, cell competition occurs most efficiently in rapidly growing tissues where growth differences are accentuated. This raises the possibility that growth differences themselves are the direct cause of cell competition, not necessarily requiring any cell recognition event [17,23,24]. Hyperplastic clones result in local mechanical crowding, and increasing evidence points to

crowding as a cause of cell delamination and death in epithelia [23,25,26]. Clones of cells expressing activated Ras (Ras^{V12}) are hyperplastic, compress the neighboring epithelium and eliminate cells lacking Ras^{V12} expression up to several cell diameters away [27*]. Faster growth does not seem sufficient to define a super-competitor cell, however, because hyperplastic cells do not always outcompete normal cells (for example hyperplastic cells activating the Insulin/IGF1 pathway, or over-expressing of CycD and Cdk4, are not super-competitors) [5]. One possibility is that differences in the compression (or sensitivity to compression) among genotypes affect the outcome of differential growth.

The molecular pathway of cell death in response to crowding is not yet known in *Drosophila*. A form of mechanical competition that occurs between mammalian cells is mediated by p53. Certain out-competed mammalian genotypes, such as MDCK cells knocked-down for the apicobasal polarity gene *scrib*, elevate p53 expression, which renders them hyper-sensitive to their mechanical compaction in mixed epithelia [28**]. *Drosophila* p53 seems to lack this role, however, because Minute cells lacking *p53* can be competed and killed by faster-growing wild type cells [16], and otherwise wild type cells lacking *p53* can be killed by hyperplastic supercompetitor cells expressing more Myc [29]. In *Drosophila*, on the other hand, p53 is required for cells over-expressing Myc to shift their metabolism towards glycolysis and super-compete wild type cells, and apparently protects the Myc over-expressing cells from DNA damage and death in the presence of wild type cells [29].

In *Drosophila*, compression due to differential growth activates the Salvador-Warts-Hippo (SWH) pathway of tumor suppressors that restrain the growth-promoting transcription factor Yorkie (Yki). This appears to confer homeostatic feedback that is thought to promote uniform growth in normal development [30*]. Reduction of Yki activity occurs in the core of faster growing cell populations, such as wild type clones in Minute discs [30*], however, which does not correspond to the location of competitive cell death, close to the clone boundaries. How cell competition may be linked to compression is also questioned by evidence that soluble factors generated in mixed tissue cultures lacking epithelial organization and associated mechanics can mediate competition [31,22].

Intermingling of competing cells

Cell competition appears to influence epithelial cell mixing. Boundaries between both wild type and Minute cells, and between cells expressing different *myc* levels, become more irregular with time as heterotypic interfaces are favored during cell rearrangements [17,32,33*]. Mixing between the populations is hypothesized to occur as a result of differential growth rates and enhance cell competition by increasing the area of competitive interactions [33*]. A possible mechanism is that competing cells may have different levels of PI3K activity, since PI3K activity affects epithelial cell rearrangements [34].

Tumor-suppressive competition

A further class of cell competition affects neoplastic tumor suppressors. These include genes that help define the polarity of epithelial cells (*scrib*, *lgl*, *dlg*) as well as components of the endocytic system (*vps25*, *erupted*, *Rab5^{DN}*) (Table 1). Mutants for these genes fail to

recognize developmental growth arrest signals, leading to oversized imaginal discs that lose normal epithelial organization and grow as disorganized tumors [35]. By contrast to the whole animal mutants, clones of cells mutant for neoplastic tumor suppressors are eliminated from mosaics with wild type cells before they can become neoplastic. This ‘tumor-suppressive competition’ is partially independent of caspase-dependent apoptosis [36,37]. Although apoptosis does contribute, mutant cells are also removed by extrusion from mosaic epithelia, in both apical and basal directions [38**,39**].

Recent studies describe a web of cell interactions in tumor-suppressive competition (Figure 2). Extrusion of *scrib* mutant cells depends on Slit-Robo2 signaling through Enabled/Vasp (Ena), an actin nucleator that downregulates E-cadherin and also affects the actomyosin network [39**]. Slit-Robo2 signaling is not induced by wild type neighbor cells, but as a cell autonomous response to elevated Jnk signaling in all the *scrib* mutant cells [39**]. Jnk is activated by the TNF-alpha factor Eiger and its receptor Grindelwald, again cell autonomously, apparently as a consequence of altered endocytic activity in *scrib* cells [37,40](Figure 2).

Wild type cells are required for Slit-Robo2-Ena mediated extrusion indirectly [41**] (Figure 2). Wild type cells use Stranded at Second (Sas) to activate PTP10D, a receptor tyrosine phosphatase, in *scrib* cells at the clone borders. PTP10D signaling suppresses EGFR activity. Without PTP10D signaling, EGFR is also hyperactivated in *scrib* clones and synergizes with Jnk signaling to inhibit SWH signaling and promote proliferation, survival, and tumorigenesis; it is only Jnk signaling by itself that promotes extrusion [41**].

Both PTP10D and Sas are apical transmembrane proteins that normally do not interact. Wild type cells abutting *scrib* clones relocate Sas to basolateral membranes, and *scrib* cells abutting wild type cells relocate PTP10D, so that trans signaling occurs only at the boundaries of *scrib* clones [41**](Figure 2). Defects in *scrib* cell polarity and endocytosis might account for basolateral PTP10D relocation but how Sas is relocated in wild type cells is uncertain. Interestingly, Egfr is also concentrated basolaterally at the boundaries of *scrib* clones [41**], suggesting a ligand is being presented basolaterally [42].

Another feature of the *scrib* clone boundary is that Eiger also activates JNK in the wild type cells adjacent to the clones [43]. This JNK activity promotes Pvr expression and activates Rac through ELMO to promote apoptotic engulfment of *scrib* mutant cells. This contributes to eliminating *scrib* mutant cells, although to a lesser extent than extrusion [43](Figure 2). How *scrib* cells activate TNF-alpha signaling to JNK in nearby wild type cells is unclear.

Although basal extrusion and apoptosis are tumor-suppressive, they are disfavored in certain tissue ‘hotspots’ defined by more robust basal matrix and microtubule networks. Here cells delaminate apically and can grow as neoplastic tumors by exploiting apical Jak-Stat signaling [38**].

These studies provide an outline for how wild type cells interact with *scrib* mutant clones (and *dlg*, *vps25*, *eru* and *Rab5^{DN}* clones) using Sas and PTP10D but many mechanistic questions are unanswered (Figure 2). Perhaps TNF signaling could be coupled to the apical determinant Crumbs, whose levels can also affect competition [44,40]. Although apoptosis

contributes to tumor-suppressive competition [36,37,43], apoptotic cells are also sources of proliferative signals, which can be tumor sustaining under favorable conditions [45]. It has not been reported whether *Drosophila* cells lacking *scrib* activate p53, as in mammals [28**], or whether divergent mechanisms apply in *Drosophila* and mammals.

Cell competition and cancer

Many cell competition genes have mammalian homologs that are proto-oncogenes or tumor suppressors (Table 1). It is envisaged that cell competition could be exploited by tumors to overgrow wild type tissues (like super-competitors do: Figure 1B), in addition to tumor-suppressive competition (Figure 1A). Recent studies of *Drosophila* tumors have provided examples of super-competitor tumors.

As in mammals, clones of *apc* mutant cells in the *Drosophila* intestine grow as hyperplastic adenomas [46**]. The adenomas induce apoptosis of other nearby intestinal cells. Wild type clones near to adenomas show growth reduced by 75% and are often completely out-competed. Blocking apoptosis restores normal growth to cells near the tumors and restricts adenoma expansion. In fact, it seems that hyperplasia of *apc* mutant clones is entirely dependent on killing neighboring cells to permit clonal expansion, so that the reaction of the surrounding normal tissue is determinative of tumor growth. Surrounding wild type cells were extremely sensitive to the SWH pathway, and even slight changes in SWH signaling that have no effect on normal intestines were sufficient to bolster wild type cells against competition and thereby prevent adenoma expansion [46**].

Another invasive tumor model results from overexpression of EGFR along with miR8. EGFR promotes hyperplasia while miR8 targets septin-7 to induce cytokinesis defects. The overgrowing EGFR+miR8 clones induce cell death in nearby wild type cells. However, EGFR+miR8 clones cannot expand through cells that have higher Myc levels, showing that these tumors are susceptible to suppression by competition, and might use competition themselves to expand through wild type cells [47].

What is the function of cell competition?

Why has cell competition evolved? Although the advantage of tumor-suppressive competition seems plain, Sas and PTP10D are not required for competition of Minute cells or by Myc supercompetitors, which may have evolved separately [41**]. Cell competition is able to buffer organ size in response to hyperplasia induced by elevated Myc levels by reducing the contribution of unaffected cells [5]. Some competition phenomena may occur as consequences of homeostatic responses to mechanical forces in epithelia; these would be important to maintain homogenous organ growth and to dissipate stress within epithelia [30*]. Mutations in *azot*, a putative calcium binding protein gene induced by Myc cell competition, are reported to shorten lifespan and accumulate developmental defects, illustrating the possibility that cell competition optimizes progenitor cell pools during development and aging by eliminating defective cells [21*]. Cell competition phenomena in mammals seem to fit this paradigm [9]. It is suggested in the DNA damage field that ribosomal protein genes, being predominantly single copy and distributed throughout the

genome in *Drosophila* as in mammals, might be ‘counted’ as sentinels of aneuploidy and other major genome alterations, and that Minute cells are eliminated due to their resemblance to aneuploid cells [48,49]. Such a surveillance function for cell competition is one possible explanation for cancer predisposition in some ribosomopathies, although it would not explain why some human ribosomal protein genes are recurrently mutated in tumors themselves [50–52].

CONCLUSIONS

Recent progress begins to unravel the first steps in cell competition. It is notable that some pathways implicated are important in sculpting the nervous system (Slit/Robo2 and Sas/PTP10D), and in immunity, two processes that also involve recognition at the cellular level [53–55]. Changes in protein localization and endosomal processing that accompany disruption of epithelial polarity appear to initiate tumor-suppressive competition. In future, a mechanism of molecular recognition related to innate immunity may be found to recognize competed genotypes such as Minutes and cells with less Myc. However, it will be important to assess the contribution of mechanical stresses due to differential growth, once the stresses can be mapped in vivo and the genes that respond to them identified and manipulated. At present distinct molecular pathways are being implicated in cell competition phenomena in *Drosophila*, although it may be too early to rule out connections between the mechanisms, especially as crosstalk can occur between tumor suppressors, cell polarity pathways and innate immunity [56–58].

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FIGURE 1. The phenomenon of cell competition

(A) In the classic example of cell competition shown by cells heterozygous for mutations of dose-sensitive Ribosomal protein genes ('Minute' cells), these slow-growing cells are actively eliminated from mosaic tissues containing wild type cells [1]. Clones of the 'Minute' cells (white) attempt to grow in the presence of normal cells (black), but apoptosis (small cells) progressively removes them [15,16]. Other examples of out-competed cells, including the neoplastic tumor mutants like *scrib*, are extruded from the epithelium as well as subject to cell death (see Figure 2) (B) In the converse situation, a clone of more competitive cells, for example cells expressing elevated Myc levels (grey), expand within the growing imaginal disc at the expense of surrounding normal cells (black), which are themselves killed (small cells) [5,22]. Not shown in panels (A) and (B) are the mechanical consequences of differential growth in epithelia. Typically, differential growth in mosaics

leads to cell crowding and compaction in the vicinity of the faster-growing genotype [23,30*].

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FIGURE 2. Tumor-suppressive competition

Clones of cells mutant for neoplastic tumor mutations, exemplified by *scrib*, are eliminated from mosaic imaginal discs by apoptosis and extrusion, depending on JNK signaling in the mutant cells. JNK is activated by the *Drosophila* TNF- α factor Eiger and its receptor Grindelwald as an autocrine consequence of disrupted polarity or endosomal function [37,40]. Within *scrib* mutant cells, JNK promotes autocrine activation of Slit/Robo2 signaling [39**]. Slit/Robo2 signaling through Ena downregulates E-cadherin and also affects the cytoskeleton to promote extrusion from the epithelium. This only occurs in mosaic tissues also containing wild type cells, because interaction between Sas in neighboring wild type cells and PTP10D in *scrib* mutant cells holds EGFR signaling to Ras in check. Without this suppression of EGFR signaling, combined Ras and JNK activity promote tumor growth and survival, not extrusion [41**]. Simultaneously, JNK activity in surrounding wild type cells promotes expression of the PDGF-receptor Pvr, which signals through ELMO and Rac to promote apoptosis and engulfment of the *scrib* mutant cells [43].

TABLE 1Genotypes revealing cell competition in *Drosophila* imaginal discs

genotype	Outcompeted by	reference	Human homolog
Minute/+ (ie most Rp/+ genotypes)	Wild type	[1]	Rp
scribble	Wild type	[36]	scribble
Lethal giant larvae	Wild type	[59,60]	LLGL1,2
Discs large	Wild type	[37]	DLG1–4
Vps25	Wild type	[61]	VPS25
Ept/TSG101	Wild type	[62]	TSG101
Mahjong/VprBP/DCAF1	Wild type	[60]	VprBP/DCAF1
Rab5 ^{DN}	Wild type	[45]	RAB5A,B,C
Frizzled frizzled2	Wild type	[63]	FZD1–10
Squamous cell carcinoma-related oncogene ortholog (Scro)	Wild type	[64]	DCUN1D1,2
Troponin I (wupA)	Wild type	[65]	TNNI1,2,3
Wild type	Elevated myc	[5,6]	MYC
Wild type	Ex,sav,wts, hpo, or mats	[66]	FRMD1?/SAV1/LATS1,2/STK3,4/MOB1A,B
Wild type	Elevated Yki	[67]	YAP1
Wild type	Elevated Stat	[68]	STAT1–6
Wild type	Apc or axin	[63]	APC/APC2 or AXIN1,2
Wild type	<i>Crumbs</i> mutant	[44]	CRB1,2