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Tissue-Intrinsic Tumor Hotspots: Terroir for Tumorigenesis

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Abstract

Epithelial tissues are highly organized systems with a remarkable homeostatic ability to maintain morphology through regulation of cellular proliferation and tissue integrity. This robust self-organizing system is progressively disrupted during tumor development. Recent studies of conserved tumor-suppressor genes in *Drosophila* showed how pro-tumor cells deviate from the robustly organized tissue microenvironment to take the first steps into becoming aggressive tumors. Here we review the '*tumor hotspot*' hypothesis that explains how the tissue-intrinsic local microenvironment has a pivotal role in the initial stage of tumorigenesis in *Drosophila* epithelia and discuss comparable mechanisms in mammalian tissues.

The 'Seed and Soil' Theory in Tumorigenesis

Throughout lifespan, a vast number of cells continually experience various stressors and mutagens from exogenous and endogenous sources, many of which cause genetic mutations in the cell. If the mutation involves activation of an oncogene, or inactivation of a tumor-suppressor gene, the mutant cell could act as a cancer 'seed.' In fact, recent studies of healthy human tissues showed that somatic mutations including nucleotide substitutions and chromosomal anomalies increase with age, suggesting that cells carrying cancer-causing mutations accumulate over time in various types of tissue [1–4]. Considering the amount of cells in a human body, the number of transformed cells should accumulate at a significant pace every day. Nevertheless, many of these 'seeds' do not grow into tumors as would be expected from the mutational load [5]. In 1889, Stephen Paget postulated "*When a plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil.*"- This "Seed and Soil" hypothesis proposed the important role of the microenvironment in metastasis formation [6]. Paget suggested that metastasis do not occur by chance, but rather, that certain tumor cells with metastatic activity (the 'seed') have a special affinity for growth-enhancing milieu within specific organs (the 'soil') [7]. His

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theory is the basis for the metastatic niche model that explains organ-preference patterns and local microenvironment cues that support the survival and outgrowth of disseminated tumor cells [8]. Can the “Seed and Soil” hypothesis explain the beginning of a primary tumor? Recent studies of initial stages of tumor induction in *Drosophila* epithelial tissue revealed that the local ‘soil’ has a critical role in determining the life-or-death fate of the ‘seeds’ of primary tumors [9]. These studies analyzed conserved neoplastic tumor-suppressor genes (nTSGs) in the *Drosophila* wing imaginal disc epithelia and showed that tumor initiation depends on tissue-intrinsic local cytoarchitectures and endogenous local growth signaling activities, and that tumors consistently originate in a specific tissue region. Here we present a review of the recently proposed ‘*tumor hotspot*’ hypothesis that explains how ‘terroir,’ the environmental conditions including soil and climate in which a plant is grown, has a decisive role in the initial stages of tumorigenesis.

Elimination of Pro-tumor Cells by Cell Competition

Epithelial tissues are composed of epithelial cells communicating with their neighbors and environment in order to regulate cellular proliferation and tissue integrity. This highly organized system has a remarkable homeostatic ability to maintain tissue integrity and organ size through turnover of its units and repair of damaged parts after injury. During cancer development, however, this robust self-organizing system is progressively disrupted. At the beginning of carcinogenesis, individual mutant cells with activated oncogenes or inactivated tumor suppressors emerge within the epithelial layer [10]. When these transformed “pro-tumor cells” evade the organized environment, disrupt epithelial organization, and begin uncontrolled proliferation, tumorigenesis occurs. Recent studies, however, revealed that pro-tumor cells bearing mutant tumor-suppressor genes are frequently eliminated from epithelial tissues in which they are surrounded by normal epithelial cells [11].

Detection and removal of aberrant or relatively less fit cells by their neighbors involve cell competition, a remarkable homeostatic process at the cellular level [12–14]. It has been reported that various types of mutant cells that are defective in growth rate, anabolic activity, or cellular structure are out-competed by the surrounding normal cells and are eventually eliminated by cell competition [11]. Interestingly, cells with mutations in a group of tumor-suppressor genes in *Drosophila* - *lethal giant larvae* (*lgl*), *discs large* (*dlg*), and *scribble* (*scrib*) - are also frequently eliminated by cell competition [15–17]. These genes play key roles in the regulation of apical-basal cell polarity and proliferation in epithelial tissues [18]. When imaginal-disc epithelial cells in *Drosophila* larvae have a homozygous mutation for any of these three genes, the normally monolayered epithelia lose the organized structure, fail to differentiate, overproliferate, and thus become multilayered amorphous masses that fuse with adjacent tissues [18]. Loss or alteration in expression of the homologs of these genes in mammals was also shown to be associated with development of malignant tumors [19,20]. The neoplastic phenotypes exhibited by mutant tissues led to the classification of these three genes as conserved neoplastic tumor-suppressor genes (nTSGs) [18,19,21,22]. However, when sporadic nTSG mutant clones are generated in the developing imaginal epithelium using the FLP/FRT-mediated mitotic recombination technique, the mutant cells adjacent to wild-type cells are eliminated through JNK-dependent apoptosis and basal extrusion [15,16,23–25], or by engulfment and phagocytosis by neighbors [26] (Figure 1). It

was observed that some of those apoptotic nTSG-mutant cells stayed in the epithelial layer, suggesting that apoptosis was not caused by basal extrusion [16]. In the genetically mosaic epithelia, apoptosis was mostly detected in nTSG mutant cells that were at the clone boundary, suggesting that the presence of adjacent normal cells triggered competition-dependent apoptosis of nTSG mutant cells [15–17]. This cell-competition-dependent elimination of pro-tumor cells has also been confirmed in mammalian cells [27–29]. For example, in a monolayer of cultured Madin-Darby Canine Kidney (MDCK) epithelial cells, Scrib-knockdown cells undergo apoptosis when surrounded by normal MDCK cells. Contrary to this, apoptosis does not occur when Scrib-knockdown cells are cultured alone [29]. More recently, another study using the MDCK cell culture system showed that apoptosis of *Scrib*-knockdown cells on contact with wild-type cells is induced by compaction into higher cell densities [30]. These studies suggest that elimination of pro-tumor cells such as nTSG mutant cells from normal tissues is an evolutionarily conserved epithelial self-defense mechanism against cancer [31].

Spindle Misorientation, Basal Delamination, and Apoptosis of Mutant Cells

The elimination of nTSG mutant cells in *Drosophila* imaginal discs might also be explained by a different mechanism. Epithelial tissues are composed of apico-basally polarized cells. To maintain the morphological architecture of the epithelial sheet wherein epithelial cells are arranged in a plane, the direction of cell division needs to be parallel to the plane of the epithelial sheet. Several genetic analysis and *in vivo* live-imaging studies of nTSGs in *Drosophila* showed that the protein products of *lgl*, *scrib* and *dlg* have a key role in determining the planar orientation of the mitotic spindle that interacts with mitotic apparatuses in proliferating epithelial cells [32–34]. This function of nTSG proteins coordinates the geometry of chromosome segregation with the architecture of polarized cell-cell junctions, thereby ensuring epithelial integrity [32]. In vertebrates, Dlg1 has been shown to be localized at the basolateral cell cortex during mitosis, and is necessary for planar spindle orientation in chick neuroepithelium and in human HeLa cells [35]. Conditional deletion of *Scrib* in mice mammary gland results in excess growth of atypical luminal cells, and development of ductal and alveolar hyperplasia associated with aberrant spindle orientation [36]. These findings indicate an evolutionarily conserved function for nTSG proteins in maintaining mitotic spindle orientation.

When nTSG genes are disrupted, *Drosophila* imaginal disc cells show fluctuation in the direction of mitotic spindles and abnormal planar orientation, which in turn cause misoriented cell division orthogonal to the plane of the epithelium [32]. Consequently, basally-located daughter cells delaminate from the epithelial layer and undergo apoptosis (Figure 2). This spindle misorientation-induced elimination of nTSG mutant cells is triggered by live-cell delamination, since apoptosis of nTSG mutant cells was observed in the basal side of epithelial layer after delamination. Although delamination-induced cell death differs from cell competition, which actively eliminates loser cells, most of pro-tumor cells are also eliminated from the tissue.

Local Cytoarchitecture and Direction of Delamination of Pro-tumor Cells

Both cell competition and spindle misorientation-induced delamination are involved in eliminating deleterious pro-tumor cells, and thus decrease the possibility of tumor formation in epithelial tissues. Interestingly, we have shown in *Drosophila* wing imaginal discs that loss-of-nTSG-induced tumor formation is dependent on the location of the pro-tumor cells. Tumors induced by nTSG knockdown were always found initially in the peripheral “hinge” region and never observed in the central “pouch” region of the disc epithelium [9] (Figure 3A and 3B). The tumorigenic potential of nTSG-knockdown cells therefore depends on their local environment in the epithelial tissue. The central pouch region acts like a “tumor coldspot” where pro-tumor cells do not show dysplastic overgrowth, while the peripheral hinge region behaves as a “tumor hotspot” [9].

A key difference between nTSG-knockdown cells located in coldspots and hotspots is the direction of delamination. In the hotspot hinge area, pro-tumor cells delaminate from the apical side and show dysplastic growth. In contrast, in coldspot areas, nTSG-knockdown cells delaminate from the basal side of epithelial layer and undergo apoptosis (Figure 3C). Cell competition-induced apoptosis, however, occurs in both the pouch coldspot and hinge hotspot regions. These findings suggesting that some nTSG-knockdown cells in the hinge area survive cell competition and delaminate from the apical side prompted a close examination of the tissue and cellular organization between these different regions of the wing disc. One key difference is the morphology of cells composing the pseudostratified monolayer, such that cells in the flat wing pouch coldspot are elongated along their apical-basal axis, whereas cells in the folded hinge hotspot regions are shorter [9]. A follow-up analysis using transmission electron micrographs revealed marked differences in the basal side of the epithelial layer: (1) in the valley-folded hotspot, cellular membranes display a complicated set of bends at the basal side, whereas in the coldspot they appear straight along the apical-basal axis; (2) hotspot cells show filopodia-like protrusions at the basal surface that elongate laterally and intertwined intricately with the protrusions from neighboring cells; and (3) the basement membrane composed of approximately ten thin laminae is organized loosely in the coldspot, but aligned tightly in the hotspot. These basal-specific structures of the hotspot are hypothesized to prevent delamination of pro-tumor cells from its basal surface [9] (Figure 4).

Delamination Direction: Apoptosis vs Survival

Why do apically delaminated pro-tumor cells survive and proliferate, but basally delaminated ones die? One plausible reason for the death of the basally delaminated cells is apoptosis induced by tumor necrosis factor (TNF). TNF, a ligand of the death receptors, functions as an extracellular signal to activate pro-apoptotic cell surface receptors [37]. In the *Drosophila* imaginal discs, apoptosis of nTSG mutant pro-tumor cells is induced by Eiger, the *Drosophila* homolog of tumor necrosis factor (TNF)-α, which is produced by circulating hemocytes recruited to tumor tissues [38]. Because hemocytes associate directly with cells at the basal side of the epithelial layer [39], basally delaminated pro-tumor cells can receive the death signal from hemocytes. Another possible reason that pro-tumor cells undergo apoptosis after basal delamination is anoikis, a specialized form of apoptosis

triggered by inappropriate cell-extracellular matrix interaction [40,41]. For example, MDCK cells or HaCat cells (spontaneously immortalized but nontransformed human keratinocytes) undergo apoptosis when their anchorage to extracellular matrix is lost [42]. The pro-apoptotic protein Bim is upregulated by cell detachment and knockdown of Bim expression inhibits anoikis, implying that Bim-induced apoptosis is a critical mechanism of anoikis in epithelial cells. Expression of Bim induced by detachment requires a lack of β1-integrin engagement, downregulation of EGF receptor (EGFR) expression, and inhibition of Erk signaling [43]. These systems could cooperatively induce apoptosis of basally delaminated nTSG-mutant pro-tumor cells. In contrast, apically delaminated pro-tumor cells in the *Drosophila* imaginal discs do not receive the death signal from hemocytes that associate directly with cells along the basal side of the epithelial layer. How do then pro-tumor cells become refractory to anoikis in hotspots?

Endogenous Oncogenic Signaling and Survival of Delaminated Cells

Cancer cells show significant resistance to apoptosis, and can survive and proliferate in the absence of appropriate adhesion to extracellular matrix [44]. This correlates closely with tumorigenicity and metastaticity, and reflects the tendency of tumor cells to survive and grow in inappropriate locations *in vivo* [45]. A recent study using MCF-10A cells (a non-tumorigenic human breast epithelial cell line) showed that sporadic ErbB2-overexpressing cells in organotypic mammary acini dissociate from the epithelial layer, translocate to the lumen and initiate luminal outgrowth [46]. Although suppression of local cell-matrix adhesion in acini is sufficient to induce cell translocation to the lumen, translocated MT1-MMP-overexpressing cells do not proliferate in the lumen. ErbB2-overexpressing cells that stayed in the epithelial layer as a result of MMP inhibition are also unable to proliferate. Luminal translocation thus allows oncogene-expressing cells to evade the suppressive epithelial environment and unleash their tumorigenicity, yet translocation alone is not sufficient to induce luminal outgrowth.

But why can apically delaminated nTSG-knockdown cells survive and show tumorigenic overgrowth in the luminal region in the absence of an oncogenic mutation? It turns out that the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway is endogenously active specifically in the tumor-hotspot region of developing wing imaginal discs [9]. Dysregulation of JAK/STAT signaling has been implicated in diverse types of human cancers [47,48], and in several tumor models in *Drosophila* [49]. In fact, STAT3 activity has been shown to be required for anchorage-independent growth in a ErbB2-overexpressing tumor-derived cell line [50]. Its secreted cytokine-like ligand Unpaired (Upd) [51], a *Drosophila* homolog of mammalian Interleukin-6 (IL-6), is endogenously expressed in the hinge area of wing imaginal discs [52]. The expression pattern of 10xSTAT92E-GFP, a JAK/STAT activity reporter, in wild-type tissues also confirms the endogenous activity of this pathway in these same tumor hotspots, particularly in the dorsal hinge region where its endogenous activity appears to be the highest. The dorsal hinge has three epithelial folds: proximal, medial, and distal (Figure 3C). Endogenous activity of the JAK/STAT pathway is high in the medial fold, weak in the proximal fold, and barely detectable in the distal fold. Indeed, dysplastic tumor growth induced by nTSG-knockdown was mostly observed in the medial fold (Figure 3C). Further analysis showed that depletion of STAT92E, the *Drosophila*

homolog of mammalian STAT3 and STAT5 [53,54], blocked the dysplastic tumor growth in nTSG-knockdown cells, indicating that STAT activation is necessary for nTSG-knockdown-induced tumorigenesis. Moreover, over-activation of STAT in nTSG-knockdown cells in tumor hotspots, including the distal fold of the dorsal hinge, dramatically enhanced tumor size when compared with nTSG-knockdown-induced tumors without STAT over-activation from the same time point post-clone induction. These results indicate that nTSG-knockdown cells exploit local endogenous activity of the JAK/STAT pathway to survive and proliferate [9] (Figure 4). Similar to reports in mammalian epithelial cells [55,56], *Drosophila* Upd is secreted from the apical surface of epithelial cells to transduce the signal to the neighboring cells, where it binds the receptor Domeless, which is also localized on the apical membrane [57]. Therefore, apical delamination in the valley-folded hotspot where the proinflammatory JAK/STAT ligand abundantly accumulates provides the pro-tumor cells with a crucial survival advantage.

Evolutionary conservancy of tumor hotspots

Pro-tumor cells are eliminated through basal extrusion and subsequent apoptosis in *Drosophila* imaginal disc epithelia except the tumor hotspot regions [9]. In vertebrate epithelia, instead of basal extrusion, oncogenic pro-tumor cells predominantly undergo apical extrusion into the lumen, which basically eliminates pro-tumor cells [27,58,59]. Defects in apical extrusion could enhance basal extrusion and survival of tumor cells, which enable their invasion beneath the epithelium [60,61]. These facts suggest an important aspect of apical extrusion especially in digestive tracts, because apical extrusion removes pro-tumor cells into the waste canal [59]. Similarly, *Drosophila* intestinal epithelia also eliminate cells apically in maintaining tissue homeostasis [62]. However, oncogenic cells in the human mammary acinar culture are apically extruded and undergo luminal outgrowth, resembling the tumor growth in hotspots in *Drosophila* imaginal discs [46]. Taken together, the direction of pro-tumor cell extrusion and its implication in tumor cell survival are highly context dependent. What matters is whether the pro-tumor cells will enter a suitable microenvironment for their survival and growth.

In pathological histology, it is known that tumors frequently arise in transition zones where two different types of epithelial tissue meet, resulting in the appearance of a distinct abrupt transition, which can be found in numerous locations within various tissues such as the junction of the cervix with the uterus and the junction of the esophagus and the stomach [63]. In addition, it is also known that epithelial carcinogenesis frequently arises from metaplasia, where one type of normal cell layer is displaced by cells of another type that are normally resident in a different organ [64]. In fact, metaplasia is most frequent in epithelial transition zones. Even though these metaplastic cells have quite a normal appearance, metaplasia is considered an early step in the development of carcinomas. For example, cervical cancers associated with human papillomavirus (HPV) infection are frequently observed at the transformation zone, a region where metaplastic squamous cells are detected in otherwise columnar epithelial-lined endocervical glands [65]. Another example is tumors in the anal canal that arise primarily in a transition zone between stratified squamous epithelium of anal skin and mucosal epithelium of the large intestine [66]. Intriguingly, even in wild-type mice, the epithelium of the transitional zone in the anus intrinsically shows

many features reminiscent of hyperproliferative epidermis including aberrant expression of differentiation markers, enhanced Ras-MAPK signaling, and locally increased inflammation [66,67]. Future studies will determine if the transition zones in mammalian tissues have similar functions to the tumor-hotspots found in *Drosophila* imaginal discs.

Concluding remarks

Although a number of causative genetic backgrounds of tumor development have been discovered, still little is known about how pro-tumor cells deviate from the robustly organized tissue environment to take the first steps in their evolution into aggressive tumors. The study of tumor initiation in *Drosophila* imaginal discs suggests that two different processes are closely involved in tumorigenesis by nTSG defects in tumor hotspots: delamination of surviving pro-tumor cells, and proliferation outside of the epithelial layer promoted by endogenous JAK/STAT activity [9]. Apical delamination of pro-tumor cells is caused by intrinsic local cytoarchitectures specifically observed in the basal side of hotspots. Tumor-promoting local JAK/STAT activity is also endogenously patterned in the tissue. These facts suggest that tissue-intrinsic factors are responsible for the fate of pro-tumor cells. Although it is still unclear how these two factors are related to each other, it is possible that coexistence of the two causal factors creates a local ‘terroir’ suitable for the survival and growth of pro-tumor seeds. It is also possible that pro-tumor cells may utilize additionally occurring mutations to create hotspot-like terroir by themselves during multi-step tumor progression. To determine the significance of local terroir for tumorigenesis in human, first we need to ask whether tumor hotspots exist in human epithelial tissues (see Outstanding Questions). The transition zones in mammalian tissues are reminiscent of tumor hotspots found in *Drosophila*. Furthermore, given the fact that tumor hotspots are formed in small local areas (smaller than 50 cells in diameter) in *Drosophila* imaginal disc epithelia, it is possible that a substantial number of local ‘terroir’ exist in human epithelial tissues. Because human tumors are comprised of a billion or more cells when clinically detected [68], the specific location where the tumor arose is difficult to determine. However, it might be possible to quantify the regional frequency of tumorous lesion in an organ. Examination of specific cytoarchitectures and endogenous activities of oncogenic or inflammatory signaling pathways in regions where primary tumors are frequently observed will help us identify tumor hotspots in human tissues, which may allow better prediction and earlier detection of malignancies, and ultimately lead to preventive therapies. Future studies to identify the causal factors that facilitate formation of tumor hotspots in various types of tissues or to clarify the behaviors of different types of pro-tumor cells in tumor hotspots will lead to a better understanding of tumor initiation.

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Glossary

Terroir

environmental conditions including soil and climate in which a plant is grown

Tumor hotspot
tissue-intrinsic microenvironment where tumorigenesis preferentially occur

Tumor coldspot
regions where pro-tumor cells do not undergo tumorigenesis

Imaginal disc
developing epithelial tissues in the larva of insects

Wing pouch
center region of wing imaginal discs which develop into the wing blade

Hinge region
peripheral region of wing imaginal discs which develop into the hinge of wing

Cytoarchitecture
structures and arrangements of cells in a tissue

Neoplastic tumor suppressor genes (nTSGs)
tumor suppressor genes, *lethal giant larvae* (*lgl*), *discs large* (*dlg*), and *scribble* (*scrib*), that control cell polarity and proliferation in epithelia and neuroblasts

Cell competition
fitter cells kill and eliminate less-fit neighbors in a tissue

Spindle misorientation
improper alignment of mitotic spindles which induce a misdirected cell division

References

1. Jacobs KB, et al. Detectable clonal mosaicism and its relationship to aging and cancer. Nat Genet. 2012; 44:651–658. [PubMed: 22561519]
2. Laurie CC, et al. Detectable clonal mosaicism from birth to old age and its relationship to cancer. Nat Genet. 2012; 44:642–650. [PubMed: 22561516]
3. Hsieh JCF, et al. Large chromosome deletions, duplications, and gene conversion events accumulate with age in normal human colon crypts. Aging Cell. 2013; 12:269–279. [PubMed: 23425690]
4. Martincorena I, et al. High burden and pervasive positive selection of somatic mutations in normal human skin. Science. 2015; 348:880–886. [PubMed: 25999502]
5. Bissell MJ, Hines WC. Why don’t we get more cancer? A proposed role of the microenvironment in restraining cancer progression. Nat Med. 2011; 17:320–329. [PubMed: 21383745]
6. Paget S. THE DISTRIBUTION OF SECONDARY GROWTHS IN CANCER OF THE BREAST. The Lancet. 1889; 133:571–573.
7. Ribatti D, et al. Stephen Paget and the “seed and soil” theory of metastatic dissemination. Clin Exper Med. 2006; 6:145–149. [PubMed: 17191105]
8. Psaila B, Lyden D. The metastatic niche: adapting the foreign soil. Nat Rev Cancer. 2009; 9:285–293. [PubMed: 19308068]
9. Tamori Y, et al. Epithelial Tumors Originate in Tumor Hotspots, a Tissue-Intrinsic Microenvironment. PLoS Biol. 2016; 14:e1002537–23. [PubMed: 27584724]
10. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100:57–70. [PubMed: 10647931]

11. Tamori Y, Deng WM. Cell competition and its implications for development and cancer. Journal of Genetics and Genomics. 2011; 38:483–495. [PubMed: 22035869]
12. Baker NE. Cell competition. Curr Biol. 2011; 21:R11–5. [PubMed: 21215926]
13. Johnston L. Competitive interactions between cells: Death, growth, and geography. Science. 2009; 324:1679. [PubMed: 19556501]
14. Clavería C, Torres M. Cell Competition: Mechanisms and Physiological Roles. Annu Rev Cell Dev Biol. 2016; 32:411–439. [PubMed: 27501445]
15. Igaki T, et al. Intrinsic tumor suppression and epithelial maintenance by endocytic activation of Eiger/TNF signaling in Drosophila. Dev Cell. 2009; 16:458–465. [PubMed: 19289090]
16. Tamori Y, et al. Involvement of Lgl and Mahjong/VprBP in cell competition. PLoS Biol. 2010; 8:e1000422. [PubMed: 20644714]
17. Menéndez J, et al. A tumor-suppressing mechanism in Drosophila involving cell competition and the Hippo pathway. Proc Natl Acad Sci USA. 2010; 107:14651–14656. [PubMed: 20679206]
18. Bilder D. Epithelial polarity and proliferation control: links from the Drosophila neoplastic tumor suppressors. Genes Dev. 2004; 18:1909–1925. [PubMed: 15314019]
19. Humbert PO, et al. Control of tumourigenesis by the Scribble/Dlg/Lgl polarity module. Oncogene. 2008; 27:6888–6907. [PubMed: 19029932]
20. Muthuswamy SK, Xue B. Cell polarity as a regulator of cancer cell behavior plasticity. Annu Rev Cell Dev Biol. 2012; 28:599–625. [PubMed: 22881459]
21. Navarro C, et al. Junctional recruitment of mammalian Scribble relies on E-cadherin engagement. Oncogene. 2005; 24:4330–4339. [PubMed: 15806148]
22. Zhan L, et al. Deregulation of scribble promotes mammary tumorigenesis and reveals a role for cell polarity in carcinoma. Cell. 2008; 135:865–878. [PubMed: 19041750]
23. Brumby AM, Richardson HE. scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. EMBO J. 2003; 22:5769–5779. [PubMed: 14592975]
24. Yamamoto M, et al. The ligand Sas and its receptor PTP10D drive tumour-suppressive cell competition. Nature. 2017; doi: 10.1038/nature21033
25. Vaughen J, Igaki T. Slit-Robo Repulsive Signaling Extrudes Tumorigenic Cells from Epithelia. Dev Cell. 2016; 39:683–695. [PubMed: 27997825]
26. Ohsawa S, et al. Elimination of oncogenic neighbors by JNK-mediated engulfment in Drosophila. Dev Cell. 2011; 20:315–328. [PubMed: 21397843]
27. Hogan C, et al. Characterization of the interface between normal and transformed epithelial cells. Nat Cell Biol. 2009; 11:460–467. [PubMed: 19287376]
28. Kajita M, et al. Interaction with surrounding normal epithelial cells influences signalling pathways and behaviour of Src-transformed cells. J Cell Sci. 2010; 123:171–180. [PubMed: 20026643]
29. Norman M, et al. Loss of Scribble causes cell competition in mammalian cells. J Cell Sci. 2012; 125:59–66. [PubMed: 22250205]
30. Wagstaff L, et al. Mechanical cell competition kills cells via induction of lethal p53 levels. Nat Commun. 2016; 7:1–14.
31. Kajita M, Fujita Y. EDAC: Epithelial defence against cancer-cell competition between normal and transformed epithelial cells in mammals. J Biochem. 2015; 158:15–23. [PubMed: 25991731]
32. Nakajima YI, et al. Epithelial junctions maintain tissue architecture by directing planar spindle orientation. Nature. 2013; 500:359–362. [PubMed: 23873041]
33. Bell GP, et al. Aurora kinases phosphorylate Lgl to induce mitotic spindle orientation in Drosophila epithelia. Curr Biol. 2015; 25:61–68. [PubMed: 25484300]
34. Carvalho CA, et al. Aurora A triggers Lgl cortical release during symmetric division to control planar spindle orientation. Curr Biol. 2015; 25:53–60. [PubMed: 25484294]
35. Saadaoui M, et al. Dlg1 controls planar spindle orientation in the neuroepithelium through direct interaction with LGN. J Cell Biol. 2014; 206:707–717. [PubMed: 25202028]
36. Godde NJ, et al. Scribble modulates the MAPK/Fra1 pathway to disrupt luminal and ductal integrity and suppress tumour formation in the mammary gland. PLoS Genet. 2014; 10:e1004323. [PubMed: 24852022]

37. Sedger LM, McDermott MF. TNF and TNF-receptors: From mediators of cell death and inflammation to therapeutic giants – past, present and future. Cytokine and Growth Factor Reviews. 2014; 25:453–472. [PubMed: 25169849]
38. Cordero JB, et al. Oncogenic Ras diverts a host TNF tumor suppressor activity into tumor promoter. Dev Cell. 2010; 18:999–1011. [PubMed: 20627081]
39. Pastor-Pareja JC, et al. An innate immune response of blood cells to tumors and tissue damage in Drosophila. Disease Models and Mechanisms. 2008; 1:144–54. discussion 153. [PubMed: 19048077]
40. Gilmore AP. Anoikis. Cell Death Differ. 2005; 12:1473–1477. [PubMed: 16247493]
41. Chiarugi P, Giannoni E. Anoikis: a necessary death program for anchorage-dependent cells. Biochem Pharmacol. 2008; 76:1352–1364. [PubMed: 18708031]
42. Frisch SM, Francis H. Disruption of epithelial cell-matrix interactions induces apoptosis. J Cell Biol. 1994; 124:619–626. [PubMed: 8106557]
43. Reginato MJ, et al. Integrins and EGFR coordinately regulate the pro-apoptotic protein Bim to prevent anoikis. Nat Cell Biol. 2003; 5:733–740. [PubMed: 12844146]
44. Buchheit CL, et al. Cancer cell survival during detachment from the ECM: multiple barriers to tumour progression. Nat Rev Cancer. 2014; 14:632–641. [PubMed: 25098270]
45. Schwartz MA. Integrins, oncogenes, and anchorage independence. J Cell Biol. 1997; 139:575–578. [PubMed: 9348275]
46. Leung CT, Brugge JS. Outgrowth of single oncogene-expressing cells from suppressive epithelial environments. Nature. 2012; 482:410–413. [PubMed: 22318515]
47. Thomas SJ, et al. The role of JAK/STAT signalling in the pathogenesis, prognosis and treatment of solid tumours. Br J Cancer. 2015; 113:365–371. [PubMed: 26151455]
48. Schafer ZT, Brugge JS. IL-6 involvement in epithelial cancers. J Clin Invest. 2007; 117:3660–3663. [PubMed: 18060028]
49. Amoyel M, et al. JAK/STAT pathway dysregulation in tumors: a Drosophila perspective. Semin Cell Dev Biol. 2014; 28:96–103. [PubMed: 24685611]
50. Barbieri I, et al. Stat3 is required for anchorage-independent growth and metastasis but not for mammary tumor development downstream of the ErbB-2 oncogene. Mol Carcinog. 2010; 49:114–120. [PubMed: 20027636]
51. Harrison DA, et al. Drosophila unpaired encodes a secreted protein that activates the JAK signaling pathway. Genes Dev. 1998; 12:3252–3263. [PubMed: 9784499]
52. Bach EA, et al. GFP reporters detect the activation of the Drosophila JAK/STAT pathway in vivo. Gene Expr Patterns. 2007; 7:323–331. [PubMed: 17008134]
53. Hou XS, et al. Marelle acts downstream of the Drosophila HOP/JAK kinase and encodes a protein similar to the mammalian STATs. Cell. 1996; 84:411–419. [PubMed: 8608595]
54. Yan R, et al. Identification of a Stat gene that functions in Drosophila development. Cell. 1996; 84:421–430. [PubMed: 8608596]
55. Rossi O, et al. Vectorial secretion of interleukin-8 mediates autocrine signalling in intestinal epithelial cells via apically located CXCR1. BMC Res Notes. 2013; 6:431. [PubMed: 24164922]
56. Healy LL, et al. Polarized Epithelial Cells Secrete Interleukin 6 Apically in the Bovine Endometrium. Biol Reprod. 2015; 92:151–151. [PubMed: 25740541]
57. Hombría JCG, Sotillos S. Disclosing JAK/STAT links to cell adhesion and cell polarity. Semin Cell Dev Biol. 2008; 19:370–378. [PubMed: 18590829]
58. Slattum G, et al. P115 RhoGEF and microtubules decide the direction apoptotic cells extrude from an epithelium. J Cell Biol. 2009; 186:693–702. [PubMed: 19720875]
59. Slattum GM, Rosenblatt J. nrc3767. Nat Rev Cancer. 2014; 14:495–501. [PubMed: 24943812]
60. Gu Y, et al. Defective apical extrusion signaling contributes to aggressive tumor hallmarks. Elife. 2014; 4:491.
61. Gudipaty SA, Rosenblatt J. Epithelial Cell Extrusion: pathways and pathologies. Semin Cell Dev Biol. 2016; doi: 10.1016/j.semcdb.2016.05.010
62. Patel PH, et al. Niche appropriation by Drosophila intestinal stem cell tumours. Nat Cell Biol. 2015; 17:1182–1192. [PubMed: 26237646]

63. Mcnairn AJ, Guasch G. Epithelial transition zones: merging microenvironments, niches, and cellular transformation. Eur J Dermatol. 2011; 21:21–28. [PubMed: 21628126]

64. Delvenne P, et al. Epithelial metaplasia: an inadequate environment for antitumour immunity? Trends in Immunology. 2004; 25:169–173. [PubMed: 15039042]

65. Elson DA, et al. Sensitivity of the cervical transformation zone to estrogen-induced squamous carcinogenesis. Cancer Res. 2000; 60:1267–1275. [PubMed: 10728686]

66. Guasch G, et al. Loss of TGFbeta signaling destabilizes homeostasis and promotes squamous cell carcinomas in stratified epithelia. Cancer Cell. 2007; 12:313–327. [PubMed: 17936557]

67. Wakefield LM, Stuelten C. Keeping order in the neighborhood: new roles for TGFbeta in maintaining epithelial homeostasis. Cancer Cell. 2007; 12:293–295. [PubMed: 17936552]

68. Moreno E. Is cell competition relevant to cancer? Nat Rev Cancer. 2008; 8:141–147. [PubMed: 18185517]

Trends Box

- Tumor initiation depends on tissue-intrinsic local cytoarchitectures and endogenous local growth signaling activities in *Drosophila* imaginal disc epithelia.
- Polarity-deficient pro-tumor cells bearing mutant neoplastic tumor-suppressor genes are frequently eliminated from epithelial tissues through cell competition-dependent apoptosis or spindle misorientation-induced delamination.
- Local cytoarchitecture of epithelial tissues is involved in determining delamination direction of polarity-deficient pro-tumor cells.
- Direction of delamination from epithelial layer has a decisive role for the life-or-death fate of pro-tumor cells.

Outstanding Questions

- How are the two causal factors (local cytoarchitecture and endogenous JAK/STAT activity) involved in formation of tissue-intrinsic tumor-hotspots in *Drosophila* imaginal discs epithelia related to each other?
- Does a tissue-intrinsic microenvironment similar to the tumor hotspot found in *Drosophila* imaginal-disc epithelia also exist in human tissues?
- Is the regional frequency of tumorous lesions varied in a human organ?

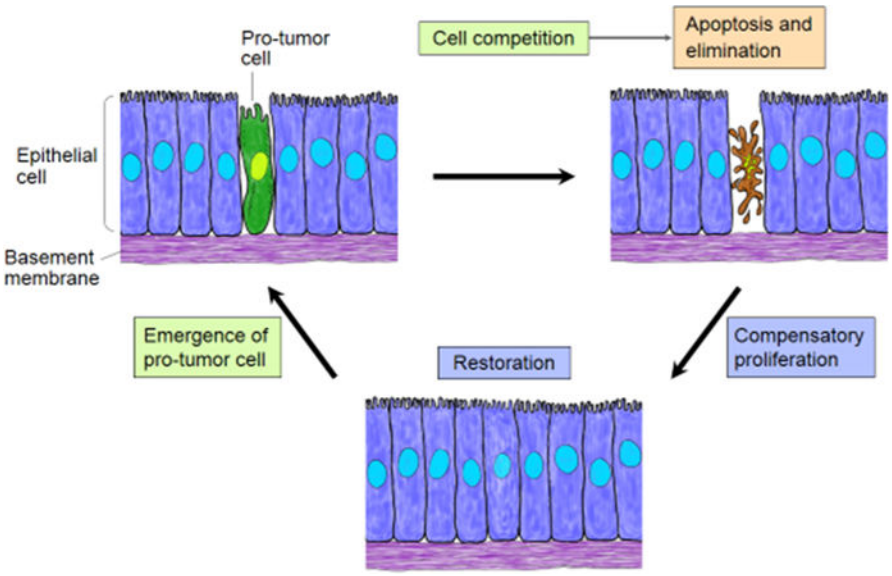

Figure 1. Cell competition eliminates pro-tumor nTSG mutant cells from epithelial tissues

When a transformed cell mutant for nTSGs (green) appears in the normal epithelial tissue, cell competition occurs. The mutant cell surrounded by normal wild-type cells (blue) undergoes apoptosis and is basally extruded. In some cases, engulfment and phagocytosis of nTSG mutant cells by adjacent normal cells has been reported. The lost mutant loser cells are normally replaced by neighboring normal cells through compensatory proliferation.

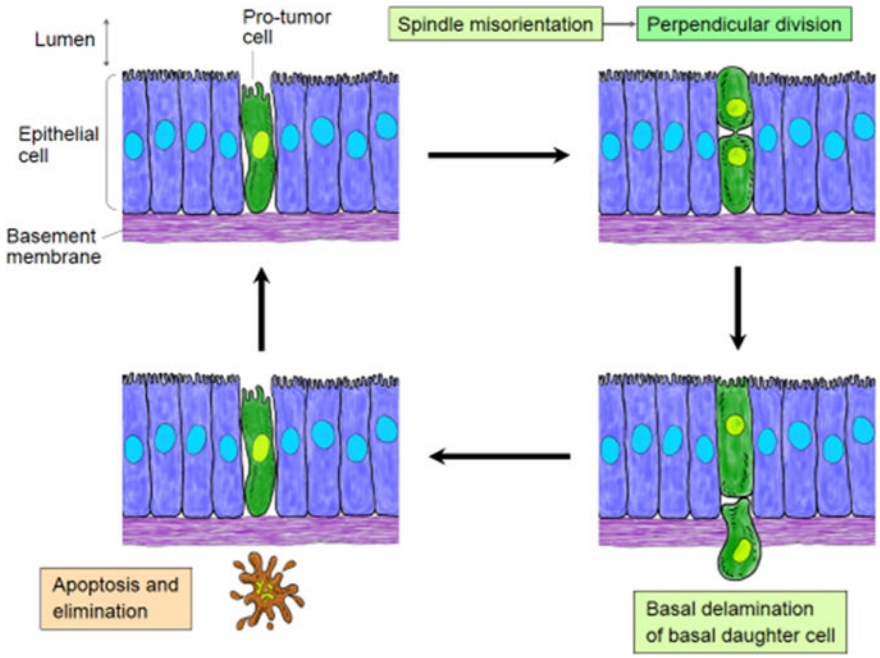

Figure 2. Spindle mis-orientation results in basal delamination and apoptosis of nTSG mutant cells

nTSG mutant pro-tumor cells (green) frequently show spindle mis-orientation during mitosis, which results in mis-oriented perpendicular cell division. In this case, one of the daughter cells located basally delaminates from the epithelial layer and undergoes apoptosis at the basal side.

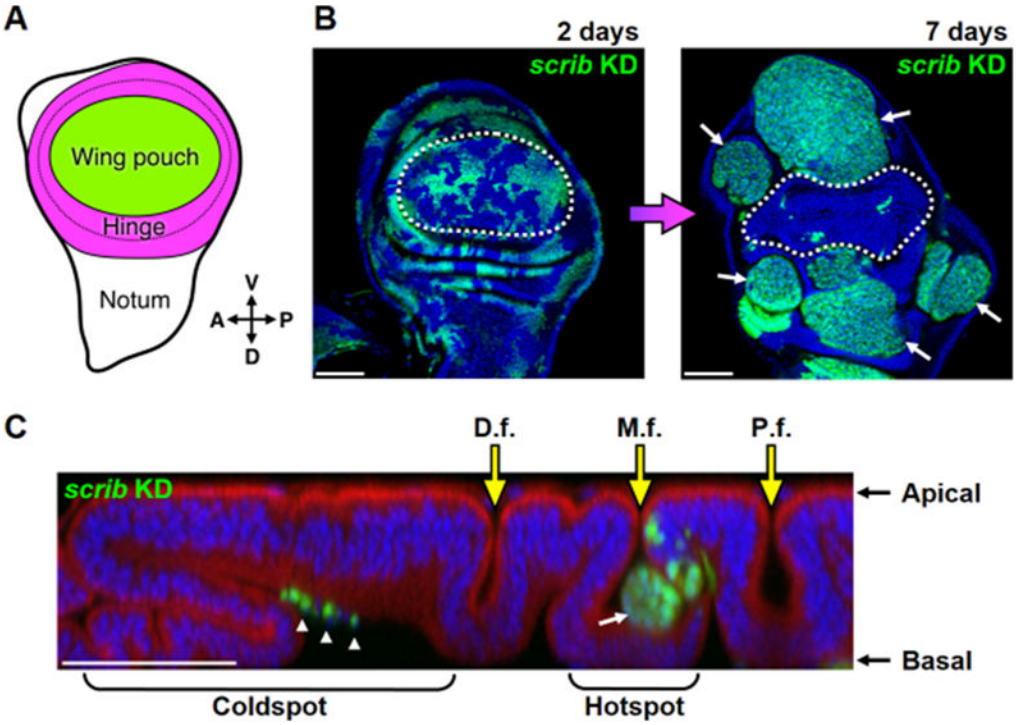

Figure 3. Site-specific tumorigenesis in *Drosophila* wing imaginal discs

(A) Schematic representation of *Drosophila* wing imaginal discs showing the wing pouch (green) and hinge (magenta) regions. (B) Genetically mosaic wing discs with cells expressing *scrib-RNAi* (marked with GFP expression, green) at the indicated time point after RNAi induction. White dotted lines mark the boundaries between wing pouch and hinge regions. (C) Vertical section of a mosaic wing disc with clones expressing *scrib-RNAi* (marked with GFP expression, green) along its anterior-posterior boundary 5 days after clone induction, stained for aPKC (red). Nuclei were labeled with DAPI (blue). White arrowheads indicate apoptotic clones. White arrows indicate dysplastic tumor growths in (B–C). Scale bars represent 50 μm in (B–C).

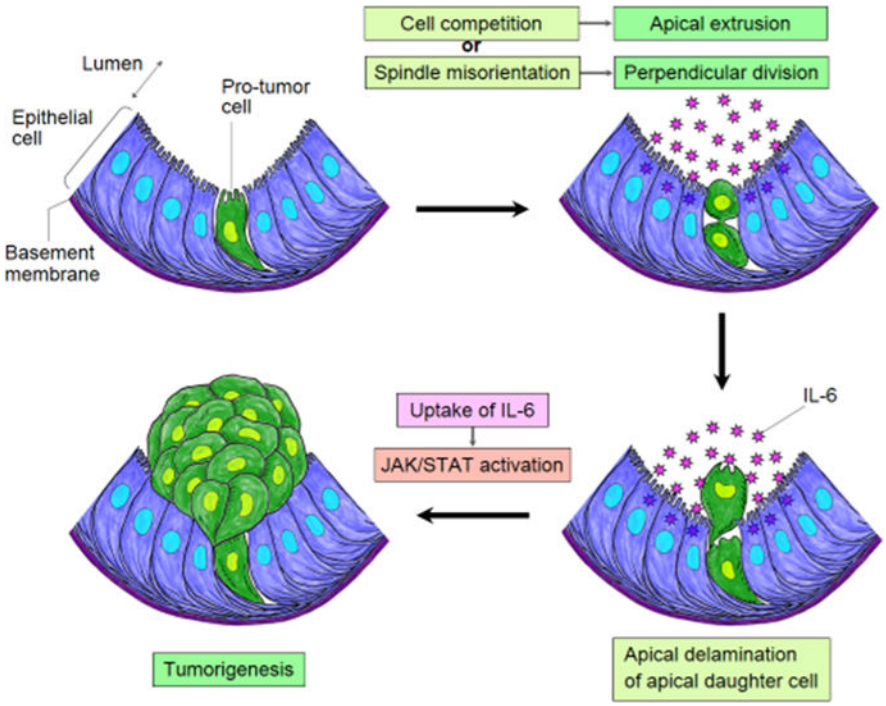

Figure 4. nTSG mutant cells delaminate apically and undergo tumorigenesis in hotspots

When an nTSG mutant pro-tumor cell (green) appears in tumor hotspots, mis-oriented perpendicular cell division results in apical delamination of one of the daughter cells because of the hotspot-specific robust basal structures. The hotspot-specific basal structures include a web of intertwining filopodia-like protrusions and tightly laminated basement membrane. An apically delaminated nTSG mutant cell survives and undergoes tumorigenic overgrowth by exploiting endogenous IL-6 (Upd in *Drosophila*) secreted in the lumen of tumor hotspots.