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Chiao-lin Chen
*University of Texas M.D. Anderson Cancer Center*

Molly C. Schroeder
*Baylor College of Medicine*

Madhuri Kango-Singh
*University of Dayton, mkangosingh1@udayton.edu*

Chunyao Tao
*University of Texas M.D. Anderson Cancer Center*

Georg Halder
*University of Texas M.D. Anderson Cancer Center*

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Tumor suppression by cell competition through regulation of the Hippo pathway

Chiao-Lin Chen1,2, Molly C. Schroeder1,2, Madhuri Kango-Singh3, Chunyao Tao3, and Georg Halder1,b,c,e

*Department of Biochemistry and Molecular Biology and 1Program in Genes and Development, University of Texas MD Anderson Cancer Center, Houston, TX 77030; 2Program in Developmental Biology, Baylor College of Medicine, Houston, TX 77030; 3Department of Biology, Center for Tissue Regeneration and Engineering at Dayton, University of Dayton, Dayton, OH 45469; and 4Vlaams Instituut voor Biotechnologie (VIB) Center for the Biology of Disease, and Center for Human Genetics, Katholieke Universiteit Leuven, 3000 Leuven, Belgium

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Homeostatic mechanisms can eliminate abnormal cells to prevent diseases such as cancer. However, the underlying mechanisms of this surveillance are poorly understood. Here we investigated how clones of cells mutant for the neoplastic tumor suppressor gene scribble (scrib) are eliminated from Drosophila imaginal discs. When all cells in imaginal discs are mutant for scribb, they hyper-activate the Hippo pathway effector Yorkie (Yki), which drives growth of the discs into large neoplastic masses. Strikingly, when discs also contain normal cells, the scribb cells do not overproliferate and eventually undergo apoptosis through JNK-dependent mechanisms. However, induction of apoptosis does not explain how scribb cells are prevented from overproliferation. We report that cell competition between scribb and wild-type cells prevents hyperproliferation by suppressing Yki activity in scribb cells. Suppressing Yki activation is critical for scribb clone elimination by cell competition, and experimental elevation of Yki activity in scribb cells is sufficient to fuel their neoplastic growth. Thus, cell competition acts as a tumor-suppressing mechanism by regulating the Hippo pathway in scribb cells.

Animals have evolved homeostatic mechanisms to eliminate abnormal and cancerous cells, protecting the animal from harm (1). A prominent example of an organism removing abnormal cells that have the potential to form tumors is the elimination of scribble mutant (scrib) cells from Drosophila imaginal discs (2–8). scribb is a conserved tumor-suppressor gene that is essential for the establishment of apical–basal cell polarity (8–10). scribb is a scaffold protein that localizes to basolateral cell junctions and functions together with the Discs large (Dlg) and Lethal giant larvae (Lgl) adaptor proteins to govern apical–basal cell polarity in epithelial cells (8, 10). Imaginal discs from Drosophila larvae that are homozygous mutant for scribb, dlg, or lgl grow into large tumorous masses of neoplastic cells that display several hallmarks of carcinomas: They lose apical–basal cell polarity, hyperproliferate, and have defects in differentiation (10). Interestingly, the neoplastic phenotype of scribb cells depends on their cellular environment. When scribb cells are produced in patches (clones) of mutant cells that are surrounded by normal cells, they do not hyperproliferate, remain small, and eventually are eliminated (2–7, 11–13). Similar effects are observed for lgl and dlg clones, although they may not be eliminated very efficiently (11, 14, 15). Thus, the presence of wild-type cells prevents scribb, lgl, and dlg cells from manifesting their tumorigenic potential (2–7, 11–15). Several groups have shown that the JNK stress–response pathway is activated in scribb clones, leading to engulfment and death or extrusion of mutant cells from the epithelium (2–4, 6, 11, 16). Activation of JNK is required for the elimination of scribb cells because blocking JNK activity in scribb cells results in massive overgrowth of clones that is reminiscent of the tumors overgrowth of entirely mutant discs (2–4, 6, 12, 13). However, blocking apoptosis does not cause overproliferation of scribb clones (2, 3). Therefore, in addition to inducing apoptosis, JNK suppresses the potential of scribb cells to hyperproliferate (2, 3). However, how scribb cells are prevented from hyperproliferating is not known.

The presence of normal cells is required for the elimination of tumorigenic scribb clones because genetically ablating the normal tissue surrounding scribb cells results in hyperproliferation of the scribb cells (2, 3). It has been suggested that cell competition, a process by which viable cells of lower fitness are removed from a tissue and replaced through extra proliferation of fitter neighbors (17), is responsible for the elimination of scribb and lgl cell clones (2, 14). However, the hypothesis that scribb and lgl clones are eliminated by cell competition is in conflict with other reports and thus is controversial.

It has been reported that cells with compromised Scrib or Lgl function exhibit elevated activity of Yorkie (Yki), a transcriptional coactivator and downstream effector of the Hippo growth-control pathway (13, 14, 18–20). The Hippo pathway is a conserved tumor-suppressor pathway that suppresses growth by antagonizing the activity of Yki (21). Thus, loss of Hippo pathway activity or elevated levels of Yki activity result in hyperproliferation of imaginal disc cells and resistance to apoptosis that normally would eliminate extra cells (21). Notably, an increase in Yki activity can rescue weak cells, such as cells heterozygous for Minute (M) mutations, from being eliminated by cell competition (22). M mutations occur in ribosomal protein-encoding genes and were the first class of genes identified as having cell-competition phenotypes (23). Homozygous M mutations are lethal, but heterozygous M animals are viable, although their cells have reduced growth rates (23). In genetic mosaics, however, interaction between wild-type and M M cells leads to the elimination of the M+/− cells and expansion of the wild-type population, a phenomenon termed “cell competition” (17). Thus, M+/− cells are less competitive than wild-type cells. Importantly, elevated levels of Yki can rescue M+/− cells from being eliminated by cell competition and also can transform normal cells into supercompetitors that induce apoptosis in their neighbors and proliferate at their neighbors’ expense (22, 24, 25). Yki may increase the competitiveness of cells by inducing the expression of Myc, a known regulator of cell competition (24–27). However, the reports that scribb cells have high levels of Yki activity and the hypothesis that cell competition present a paradox. If scribb cells indeed have elevated levels of Yki activity, why does that elevated Yki activity not protect scribb cells from cell competition?

Here we investigated this paradox further. We show that scribb cells are indeed eliminated by cell competition. We found that for this elimination to occur, scribb cells undergo a JNK-dependent suppression of Yki activity; this suppression of Yki activity


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1C.-L.C. and M.C.S. contributed equally to this work.

2To whom correspondence should be addressed. E-mail: ghalder@mdanderson.org.

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prevents scrib− cells from hyperproliferating and enables their removal. The modulation of Yki activity in scrib− cells thus is a critical effect of the JNK-dependent cell-competition process that removes such tumorigenic cells from imaginal discs. Finally, we show that the Myc and Ras oncoproteins, which can rescue scrib− clones from elimination (2, 4, 15), do so by conferring competitive fitness to scrib− cells and thereby prevent the down-regulation of Yki activity in scrib− cells. Our results thus further characterize the effects of cell-competition pathways in removing tumorigenic scrib− cells from imaginal discs.

Results

Normal Cells Inhibit the Proliferation of scrib− Clones. scrib− clones activate JNK signaling and induce JNK-dependent apoptosis (2–4, 6). However, the induction of apoptosis is not sufficient to explain how scrib− clones are eliminated, because blocking apoptosis by coexpression of the caspase inhibitor p35 does not rescue the small clone size of scrib− clones to the size observed when JNK activity is inhibited (2, 3). To confirm that apoptosis is not sufficient for the removal of scrib− clones, we generated large and consistent numbers of GFP-marked scrib− cell clones by combining an eye-specific source of Flippase (ey-Flp) using the mosaic analysis with a repressible cell marker (MARCM) system (28) and examined the contribution of these mutant cells to third-instar eye discs as a measure of their proliferation and survival. Corroborating previous observations, scrib− clones comprised only a small fraction of eye discs compared with wild-type control clones (Fig. 1A and B) (2–5, 7), as did scrib− clones that coexpressed p35 or the antiapoptotic gene Drosophila inhibitor of apoptosis 1 (Diap1) (Fig. 1C and Fig. S1 A–C) (3). Blocking apoptosis thus is not sufficient to rescue the growth defects of scrib− clones. In contrast, scrib− cells in which JNK signaling was blocked by coexpressing a dominant-negative form of the Drosophila JNK basket (BskDN), overexpression is indicated as +BskDN) or because they were generated in animals that were homozygous mutants for eiger (egr−/−), an extracellular ligand that activates JNK signaling (16), were no longer eliminated and grew into large clones (Fig. 1D and G and Fig. S1D) (2–4, 12). In addition to surviving, these clones hyperproliferated, as revealed by an excess of BrdU-incorporating cells in third-instar eye discs, in which all cells are proliferating, we analyzed the activity of pathways known to misregulate these signaling pathways (Fig. S3) (3). Therefore, in addition to triggering apoptosis, JNK signaling counteracts the potential of scrib− cells to hyperproliferate (2, 3). Notably, scrib− cells that cannot activate JNK still showed defects in photoreceptor differentiation, observed through ELAV expression, and in cell polarity, observed through anti-Patj staining, forming multilayered structures of tumorigenic cells (Fig. 1 H and I) (12). These data show that scrib− cells have the potential to hyperproliferate and in genetic mosaics this potential is counteracted by JNK activity (2, 3).

Cell Competition Regulates Hippo Pathway Activity in scrib− Cells. The observation that the proliferation of scrib− cells is restricted in the presence of wild-type neighbors raised questions about the role of neighboring cells in maintaining homeostasis and eliminating scrib− cells. Removal of scrib− clones may depend on cell competition (2), on the presence of neighboring cells with normal apical–basal polarity (3), or on circulating hemocytes that attach to scrib− cells and secrete Egr (6). To determine the contribution of cell competition to the elimination of scrib− cells, we decreased the fitness of the surrounding scrib− cells by making them heterozygous for M scrib− cells with M+ neighbors formed large clones of proliferating cells, revealed by high levels of BrdU incorporation, that often resulted in deformed and overgrown imaginal discs (Fig. 1J and Fig. S2 B and G). This result demonstrates that the suppression of the tumorigenic potential of scrib− cell clones depends on the fitness of their neighboring cells rather than on the mere presence of cells with normal polarity. Thus, cell competition between scrib− cells and neighboring wild-type cells is essential for the elimination of scrib− cells.

To gain insight into the effects of cell competition on scrib− cells and to explore how scrib− cells are prevented from hyperproliferating, we analyzed the activity of pathways known to regulate imaginal disc growth in scrib− cells that were protected from cell competition and then compared that activity with that of scrib− cells facing cell competition. Readouts for the Decapentaplegic (Dpp) and Hedgehog (Hh) pathways (29) were not affected significantly in scrib− clones in egr−/− discs, demonstrating that scrib− cells protected from cell competition do not misregulate these signaling pathways (Fig. S3). In contrast, expanded-lacZ (ex-lacZ), a reporter for the Hippo tumor-suppressor pathway and Yki activity (30), was dramatically up-regulated in scrib− clones in egr−/− discs as well as in scrib− +BskDN clones (Fig. 2A–C and E–H and Figs. S2H and S4 A and B) (13). In addition, Yki was more concentrated in the nuclei of scrib− clones showing the normal pattern of BrdU incorporation (grey in Fig. 1E) compared with wild-type clones (Fig. 2J). Genotypes are listed in SI Methods.

Fig. 1. Activation of JNK restrains the proliferation of scrib− cells. Shown are confocal images of mosaic eye imaginal discs. Anterior is to the left in all panels. (A–D) Clones generated using the MARCM system to label mutant clones by GFP expression (green) and ey-Flp to induce recombination in eye discs. Nuclei are labeled with DAPI (blue). (A) Wild-type clones. (B) scrib− clones. (C) scrib− clones overexpressing p35 (+p35) are prevented from undergoing apoptosis. (D) scrib− +BskDN clones. scrib− clones lacking JNK activity overgrow. (E–J) Mosaic eye imaginal discs containing clones marked by the absence of GFP expression (green in E–J) and by the absence of egr expression (green in I) and egr expression (green in J). (E) Discs with wild-type clones showing the normal pattern of BrdU incorporation (grey in E). (F) scrib− clones do not show proliferation defects. (G) scrib− clones in homozygous egr−/− discs have an excess of BrdU-incorporating (grey in E) cells posterior to the second mitotic wave, indicating hyperproliferation. (H) scrib− clones, marked by lack of GFP, in an egr−/− animal stained for ELAV, a marker of differentiated neurons (grey in H). (I) Optical cross-section through a wing disc with a scrib− clone, marked by absence of GFP, in an egr−/− animal stained for Patj (red) and DAPI (blue). Patj (grey in I) is mislocalized, indicating cell polarity defects. (J) scrib− cells surrounded by M+ cells with BrdU staining (grey in J). Genotypes are listed in SI Methods.
competition, displayed high levels of the Yki activity reporters ex-lacZ and Diap1-GFP (Fig. 3 E and F and Fig. S4 C and D) (31). Thus, scrib cells not facing cell competition have abnormally high levels of Yki activity.

To test whether these elevated levels of Yki activity are required for the hyperproliferation phenotype of “noncompeted” scrib cells, we decreased Yki activity in scrib+ BskDN cells by coexpressing Warts (Wts), a Hippo pathway serine threonine kinase that phosphorylates Yki and inactivates it (Fig. 3). We found that such cells made only small contributions to third-instar eye discs, indicating that Yki is important for the proliferation of noncompeted scrib+ clones. Thus, scrib+ cells not facing cell competition have high levels of Yki activity, which is required for their hyperproliferation.

The finding that Yki activity is elevated in noncompeted scrib+ cells raised the question of what happens to Yki in scrib− cells that do face cell competition. Elevation of Yki levels is sufficient to protect M− cells from cell competition and can even transform normal cells into supercompetitors (22, 24, 25). Remarkably, ex-lacZ, which was up-regulated in noncompeted scrib+ clones, was not induced in scrib− clones surrounded by wild-type cells in most regions of eye and wing discs (Figs. 2C and 3G). Thus, ex-lacZ generally was not elevated in scrib− cells that faced cell competition, whereas scrib− clones rescued from cell competition (scrib− + BskDN clones) had elevated ex-lacZ levels in all regions of eye and wing discs (Fig. 2, quantified in Fig. S6). The failure of competed scrib+ clones to up-regulate Yki activity may be caused by the perdurance of Scrib, because competed scrib− clones generally were much smaller than rescued clones. However, ex-lacZ also was up-regulated in noncompeted scrib+ clones that were small (Figs. S7 and S8B). Small scrib+ clones also had the polarity and differentiation defects seen in big clones, indicating that it is not Scrib perdurance that prevents the up-regulation of Yki activity in scrib− clones subject to cell competition. Thus, these data show that cell competition prevents the up-regulation of Yki activity in scrib− cells.

We noted that a minority of scrib− clones in the hinge region of wing discs and in the posterior region of eye discs displayed some increase in ex-lacZ expression, which has been observed by other groups (13, 20). Thirty-one percent of clones in the hinge and 16% of clones in the posterior eye had at least one cell in which ex-lacZ was up-regulated (Fig. S6). Notably, the hinge region has been proposed to be a less competitive environment than the wing pouch, and the posterior region of eye discs may similarly be a less competitive environment, since cells in that region start to differentiate earlier than those located more anteriorly (15, 26, 27). Therefore, some scrib− clones may face less cell competition in these regions, allowing them to elevate ex-
lacZ levels. However, even in the wing hinge region and posterior eye disc region there was a significant difference in ex-lacZ expression profiles between scrib- clones that were subjected to or protected from cell competition (Fig. S6).

To test whether the suppression of Yki activity by cell competition is required for the elimination of scrib- clones, we experimentally increased Yki activity in scrib- cells by overexpression of Yki (+Yki) or loss of wts. Both these manipulations were sufficient to rescue scrib- clones from being outcompeted (Fig. 3 J and K and Fig. S5 B and C). Therefore, the prevention of Yki up-regulation is key to the elimination of scrib- clones. We conclude that cell competition acts as a tumor-suppression mechanism by preventing Yki activation in scrib- cells.

scrib- Cells Not Subjected to Cell Competition Have Enhanced Non-Cell-Autonomous Effects on the Hippo Pathway. scrib- clones can cause non-cell-autonomous up-regulation of ex-lacZ in neighboring wild-type cells (Fig. 3G) (20). This non-cell-autonomous effect on Hippo signaling also was observed around scrib- clones rescued from elimination: scrib- clones in M"- tissues showed non-cell-autonomous effects on ex-lacZ (Fig. 3 B-D). Such non-cell-autonomous induction of ex-lacZ was observed most dramatically around scrib- clones that coexpressed oncogenic RasV12, which can rescue scrib- cells from being outcompeted and acts synergistically with loss of scrib to form tumors (Figs. S7 A-D and S8A) (2, 4, 7). Clones of scrib- cells overexpressing RasV12 (scrib-+RasV12) expressed high levels of ex-lacZ and also showed strong non-cell-autonomous up-regulation of ex-lacZ expression (Figs. S7 A-D and S8A) (13, 14). Such rescued scrib- clones grew into multilayered masses that expanded beyond the epithelial monolayer. This effect, combined with extra growth caused by non-cell-autonomous Hippo pathway regulation, caused non-competed scrib- clones to distort the morphology of the discs (Fig. S7 A-D). Non-cell-autonomous regulation of Hippo signaling by abnormal or damaged cells has been observed previously and has been suggested as a mechanism for ensuring that compensatory growth restores the tissue (19, 20).

This regenerative signal has been proposed to depend upon JNK signaling (19, 20). In contrast to these reports, however, we observe non-cell-autonomous effects on ex-lacZ in scrib-+BskDN clones and in scrib- clones in egr-1 animals (Fig. 2 E-H and Fig. S4B). Therefore, scrib- cells that are not cleared efficiently from imaginal discs are competent to elevate Yki activity in their normal neighbors via a JNK-independent signal.

Increased Relative Myc Levels Protect scrib- Cells from Cell Competition. To test further the importance of cell competition in the elimination of scrib- cells, we increased their fitness by overexpressing Myc (+Myc), which turns cells into supercompetitors (26, 27). We found that overexpression of Myc in scrib- cells rescued their poor growth and resulted in strong up-regulation of ex-lacZ expression (Fig. 4 A and C and Fig. S8B). This result is striking because overexpression of Myc in wild-type cells did not cause up-regulation of ex-lacZ expression; rather, it slightly suppressed ex-lacZ expression levels (Fig. 4 B and D) (26). This indicates that the increase of Yki activity in scrib-+Myc clones is an indirect consequence of these cells being able to evade cell competition due to the increased fitness conferred by Myc overexpression, rather than Myc directly inducing Yki activity. Thus, Myc has different effects on Hippo signaling in scrib- and wild-type cells and the oncogenic potential of Myc is more dramatically realized in scrib- cells than in wild-type cells. This suggests that elevated Myc may most potently increase the proliferation of tumorigenic cells by counteracting the growth suppressing effects of cell competition that they may face.

This result could be explained by two different kinds of effects. One possibility is that the absolute level of Myc in scrib- cells determines whether scrib- cells can survive in the presence of normal neighbors. Alternatively, it could be that high levels of Myc in scrib- cells transform them into supercompetitors. In the latter case, the relative levels of Myc between scrib- cells and their neighbors would determine whether the scrib- cells will be eliminated. To distinguish between these two possibilities, we overexpressed Myc throughout the posterior wing compartment and produced scrib- clones in this uniformly high-Myc environment. If Myc contributes to the absolute growth ability of scrib- clones rather than to relative growth ability, we would expect that scrib- clones would not be eliminated and would be able to grow when Myc is overexpressed in the entire tissue. We observed that scrib- clones generated in compartments in which Myc is overexpressed are not rescued from elimination (Fig. 4E). This result indicates that high levels of Myc are insufficient to rescue scrib- clones from being eliminated by cell competition if surrounding normal cells also have high levels of Myc. Thus, the effects of Myc on the survival of scrib- clones are not a simple result of a cell-autonomous increase in proliferation rate. Rather, the relative level of Myc in scrib- cells compared with their normal neighbors is important. When scrib- cells have more Myc...
than their neighbors, they are protected from elimination; when both populations have high Myc levels, the scrib" cells are eliminated. This result confirms that scrib" cells are eliminated by cell competition. In contrast to these results with Myc overexpression, scrib" clones were rescued from elimination when BskDN was overexpressed in entire posterior compartments, showing that the overexpression in this system is early enough to rescue scrib" clones (Fig. 4F). Altogether, we conclude that Myc acts as an oncogene in scrib" cells by increasing their relative fitness.

Discussion
In this study we show that tumorigenic scrib" cells are removed from Drosophila imaginal discs by a cell–cell signaling event that suppresses elevated Yki activity in scrib" cells. Previous reports implicated JNK as a mediator of cell competition of scrib" clones, where it induces apoptosis and suppresses proliferation (2–5, 7). However, it was not known how JNK prevents scrib" clones from hyperproliferating. We now provide evidence that JNK prevents scrib" clones from hyperproliferating by regulating the activity of the Hippo pathway effector Yki. First, scrib" clones that do not face cell competition up-regulate Yki activity, which drives their hyperproliferation. Second, when scrib" clones do face cell competition, then JNK signaling prevents the up-regulation of Yki activity. Third, experimental up-regulation of Yki activity is sufficient to rescue scrib" clones from being eliminated by cell competition. Fourth, experimental suppression of Yki activity in scrib" clones not subjected to cell competition is sufficient to suppress their hyperproliferation. Therefore, cell competition suppresses up-regulation of Yki activity in scrib" cells, and this suppression is important for the elimination of scrib" clones by cell competition. Previous reports showed that Hippo pathway reporters can be up-regulated in scrib" and lgl" mutant discs and clones (13, 14, 18, 20) and that Yki is required for the overgrowth of scrib"+BskDN cells not subjected to cell competition (13). However, these studies did not analyze the effects of cell competition on Yki activity in scrib" cells. Our analysis now shows that scrib" cells facing cell competition do not up-regulate Yki activity and thereby identifies a mechanism that is critical for the elimination of scrib" cells.

Although it was reported that scrib" and lgl" cells can up-regulate ex-lacZ expression and Yki activity (13, 14, 18, 20). However, upon quantification we found that the majority of scrib" clones have normal or reduced levels of ex-lacZ expression, and only a small percentage of scrib" clones have elevated levels of ex-lacZ expression. Clones with elevated ex-lacZ expression were observed mainly in the hinge region of wing discs, which may provide an environment of reduced cell competition (15, 26, 27). Thus, outcompeted scrib" clones do not have elevated levels of Yki activity. In contrast, when scrib" clones are rescued from cell competition, they show highly elevated levels of ex-lacZ expression (this study and refs. 13 and 14). Similarly, discs that are entirely mutant for scrib, thereby creating an environment that does not have competing normal cells, show hyperactivation of Yki (this study and ref. 13). Cell competition thus prevents the hyperactivation of Yki in scrib" clones and turns a potential high-Yki “supercompetitor” scrib" cell into a cell of lower fitness and less resistance to apoptosis. Importantly, scrib" wts" and scrib"+Yki clones show greatly increased growth and survival compared with scrib" clones. These results show that elevated levels of Yki are sufficient to protect scrib" cells from being outcompeted. Thus, if Yki activity already was high in scrib" cells facing cell competition, those cells would not be outcompeted, and overexpression of Yki or loss of wts would not cause such dramatic effects on the survival and growth of scrib" clones. Apparently, Yki levels in scrib" cells facing cell competition are not high enough for these cells to evade cell competition. Thus, the amount of Yki activity in scrib" cells is a critical determinant of whether scrib" clones are eliminated or form tumorous tissue, and the suppression of Yki activity in scrib" clones is important for the elimination of scrib" clones by cell competition.

Our studies show that JNK activity is required in scrib" cells for the suppression of Yki activity by cell competition. In contrast, JNK signaling can induce Yki activity during regeneration and compensatory proliferation in imaginal discs (19, 20). Therefore, the effects of JNK signaling on Yki activity in scrib" cells are different from those in normal cells: JNK signaling activates Yki in normal cells promoted to regenerate but suppresses Yki in scrib" cells induced to be eliminated. Interestingly, both these effects are observed in discs with scrib" clones. In scrib" cells, JNK activity suppresses the hyperactivation of Yki, but in neighboring cells that are stimulated to proliferate and compensate for the loss of scrib" cells, the activities of both JNK and Yki are elevated (11, 19, 20). However, we still observed non–cell-autonomous effects on Yki reporters in overexpression animals and in discs that ubiquitously inhibited JNK signaling by BskDN. Therefore, JNK-independent signals contribute to the non–cell-autonomous induction of Yki activity around scrib" clones. The regulation of Yki by JNK signaling thus is complex and context dependent and may involve several mechanisms.

The observation that wts" scrib" clones overgrow indicates that JNK and Wts function in parallel to regulate Yki or that JNK regulates the Hippo pathway upstream of Wts. JNK can phosphorylate and activate Yap1 to regulate apoptosis in mammalian cells (32, 33). Notably, the JNK phosphorylation sites of Yap1 are different from the Lats phosphorylation sites (21), supporting
a model in which JNK functions in parallel with Wts to regulate Yki activity. However, it is not known whether the same sites also act to suppress the activity of Yki in other contexts.

Although several models have been proposed to explain how cell–cell interactions between scribble and normal cells lead to the elimination of scribble clones from epithelia, it was not clear what properties normal cells must possess to perform this tumor-suppressive role (16, 17). Our data demonstrate that for scribble cells to be eliminated they must be juxtaposed with cells that have higher levels of competitive fitness, not just proper cellular architecture. Overexpression of the Myc or Ras oncogenes in scribble clones increases their fitness. As a result, in scribble clones cell competition does not suppress Yki activity, which protects these clones from being eliminated. Interestingly, Myc expression also synergizes with loss of scribble to form tumors in mammals (9), and our data offer a model to explain this phenomenon.

In addition to the cell-autonomous hyperproliferation, scribble cells that are not removed from imaginal discs have profound non–cell-autonomous effects on the Hippo pathway. This non–cell-autonomous Hippo pathway-regulating signal may serve normally as a regenerative growth signal that facilitates the replacement of eliminated or dying cells, such as outcompeted scribble cells (19, 20). If scribble clones are not eliminated efficiently, however, this signal may persist longer than required to restore the tissue, thereby causing overgrowth and deformation of neighboring tissue. Thus, continued residence of tumorigenic cells can stimulate growth beyond that needed for compensation, essentially hijacking the proliferation and regeneration programs of their normal neighbors. Therefore, the non–cell-autonomous activation of Yki by scribble cells may have important implications for tumor-stromal interactions in human cancers.

In summary, we conclude that cell competition is crucial in suppressing the tumorigenic capacity of scribble cells and does so by regulating their Yki activity (Fig. 5 A and B). Loss of this regulation results in overproliferation of both tumorigenic cells and neighboring wild-type cells (Fig. 5C). Efficient elimination of scribble clones by cell competition prevents Yki-fueled overgrowth of mutant cells and prevents them from disrupting proliferation control of their normal neighbors. Thus, we identified a tumor-suppression mechanism that depends on signaling between normal and tumorigenic cells. These data identify evasion of cell competition as a critical step toward malignancy and illustrate a role for wild-type tissue in preventing the formation of cancers.

Methods

Drosophila Stocks and Culture.

All crosses were maintained at 25 °C. Mutant clones were induced by mitotic recombination using the Flipase/Flipase recognition target (Flip/FRT) system. Flip recombination was expressed in a tissue-specific manner using ey-flp and ubx-flp or was induced conditionally using hs-flp. The Upstream Activation System (UAS)-Gal4 system was used to overexpress genes of interest. The scribble-null allele was flipped against corresponding ubi-GFP–marked FRT chromosomes to generate scribble clones. To express GFP and other genes of interest in mutant clones, the MARCM system was used (28). Heat shocks were performed at 37 °C for 30 min during the first or second larval stage. Information regarding immunostaining procedures and Drosophila strains used is given in SI Methods.

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