Mechanism of tumor-suppressive cell competition in flies

Hiroshi Kanda | Tatsushi Igaki

Abstract
Oncogenic mutations often trigger antitumor cellular response such as induction of apoptosis or cellular senescence. Studies in the last decade have identified the presence of the third guardian against mutation-induced tumorigenesis, namely "cell competition." Cell competition is a context-dependent cell elimination whereby cells with higher fitness eliminate neighboring cells with lower fitness by inducing cell death. While oncogene-induced apoptosis or oncogene-induced senescence acts as a cell-autonomous tumor suppressor, cell competition protects the tissue from tumorigenesis via cell-cell communication. For instance, in Drosophila epithelium, oncogenic cells with cell polarity mutations overproliferate and develop into tumors on their own but are eliminated from the tissue when surrounded by wild-type cells. Genetic studies in flies have unraveled that such tumor-suppressive cell competition is regulated by at least three mechanisms: direct cell-cell interaction between polarity-deficient cells and wild-type cells, secreted factors from epithelial cells, and systemic factors from distant organs. Molecular manipulation of tumor-suppressive cell competition could provide a novel therapeutic strategy against human cancers.

KEYWORDS
cell competition, Drosophila, tumor suppression

1 | INTRODUCTION

Oncogenic mutations not only confer cells with proliferative advantage but also trigger antiproliferative effects that suppress tumorigenesis, a phenomenon called "intrinsic tumor suppression." One such mechanism is oncogene-induced apoptosis, which is triggered by upregulation of oncogenes such as Myc and E1A. Another important mechanism of intrinsic tumor suppression is oncogene-induced cellular senescence, an irreversible cell cycle arrest induced by the activation of oncogenes such as Ras, Braf, Akt, E2F1, mos, and Cdc6 or inactivation of tumor suppressor genes such as PTEN and NF1. These tumor-suppressive machineries eliminate or inactivate premalignant cells emerged in the tissue in a cell-autonomous manner. Apart from these classical tumor-suppressive mechanisms, studies in the last decade have identified a prominent role of surrounding wild-type cells to eliminate premalignant mutant cells: the third machinery of intrinsic tumor suppression via cell-cell interaction. This phenomenon is called cell competition, a context-dependent cell elimination whereby cells with higher fitness eliminate neighboring cells with lower fitness by inducing cell death. While oncogene-induced apoptosis or oncogene-induced senescence acts as a cell-autonomous tumor suppressor, cell competition protects the tissue from tumorigenesis via cell-cell communication. For instance, in Drosophila epithelium, oncogenic cells with cell polarity mutations overproliferate and develop into tumors on their own but are eliminated from the tissue when surrounded by wild-type cells. Genetic studies in flies have unraveled that such tumor-suppressive cell competition is regulated by at least three mechanisms: direct cell-cell interaction between polarity-deficient cells and wild-type cells, secreted factors from epithelial cells, and systemic factors from distant organs. Molecular manipulation of tumor-suppressive cell competition could provide a novel therapeutic strategy against human cancers.
Twenty years later, it was found in Drosophila epithelium that oncogenic polarity-deficient cells such as scribble (scrib) mutant cells overproliferate on their own but are eliminated by cell competition when surrounded by wild-type cells. This underscores the significance of cell competition in epithelial tumor suppression. Notably, similar elimination of oncogenic mutant cells via cell-cell interaction has been observed in mammalian systems, a phenomenon called epithelial defense against cancer (EDAC). In this review, we summarize recent studies on the mechanism of tumor-suppressive cell competition in flies, which is regulated by at least three factors including direct cell-cell interaction, secreted factors from epithelial cells, and systemic factors from distant organs (Figure 2). We also discuss how it is relevant to cancer regulation.

**Figure 1** Oncogenic mutations trigger intrinsic tumor-suppressive programs. A variety of oncogenic mutations not only promote tumorigenesis but simultaneously activate intrinsic tumor-suppressive mechanisms such as induction of apoptosis, cellular senescence, and cell competition.

**Figure 2** Factors that regulate scrib cell elimination by cell competition. scrib mutant cell (red) is eliminated when surrounded by wild-type cells (blue) via at least three mechanisms including (1) direct cell-cell interaction with neighboring wild-type cells, (2) microenvironmental regulation by locally provided secreted factors such as Slit and Spz, and (3) systemic regulation by factors such as Drosophila insulinlike peptides (Dilps).
CyclinE levels and BrdU incorporation, they do not overgrow but are eliminated from the tissue by apoptosis.\textsuperscript{10} This suggests that elimination of \textit{scrib} clones is led by an active, regulated mechanism rather than passive consequence of impaired cell survival or cell growth. Genetic studies in \textit{Drosophila} have uncovered the molecular basis for how \textit{scrib} clones are eliminated from the tissue when surrounded by wild-type cells. It was first shown that \textit{scrib} clone elimination is mediated by c-Jun-N-terminal kinase (JNK) as blocking \textit{Drosophila} JNK Bsk abolished the elimination and led to \textit{scrib} cell overproliferation.\textsuperscript{10} This JNK-dependent \textit{scrib} elimination is triggered by Eiger,\textsuperscript{34} the sole tumor necrosis factor (TNF) in \textit{Drosophila}\textsuperscript{35,36} and its receptor Grindelwald.\textsuperscript{37} It was found that \textit{scrib} clones elevate endocytosis, which translocates Eiger from the plasma membrane to endosomes, thereby leading to activation of downstream JNK signaling (Figure 3).\textsuperscript{34} It has also been reported that Eiger expression in the hemocytes attached to the imaginal discs activate JNK signaling in polarity-deficient imaginal cells.\textsuperscript{28}

While the elimination of \textit{scrib} clones essentially depends on JNK signaling, JNK-induced cell death does not fully account for the cell elimination as blocking cell death does not cause as drastic tumorigenesis as blocking JNK.\textsuperscript{10,39} It was found through a genetic screen that JNK activation in \textit{scrib} clones upregulates the evolutionarily conserved repulsive axon guidance ligand, receptor, and downstream target, namely Slit, Roundabout2 (Robo2), and Enabled (Ena)/VASP, respectively. This causes downregulation of E-Cadherin and thus disruption of cell-cell adhesion, which promotes extrusion of \textit{scrib} cells from epithelium (Figure 3).\textsuperscript{39}

\section*{CELL ELIMINATION BY DIRECT CELL-CELL INTERACTION}

\textit{scrib} mutant cells overproliferate in the absence of wild-type neighbors, suggesting a non-cell-autonomous antitumor effect by juxtaposed wild-type cells. Intriguingly, when \textit{scrib} clones are induced in the eye imaginal discs, JNK activation is observed not only in \textit{scrib} cells but also in surrounding wild-type cells right next to the mutant cells.\textsuperscript{27,40} JNK activation in surrounding wild-type cells does not cause apoptosis, but instead, it upregulates the \textit{Drosophila} platelet-derived growth factor (PDGF)/vascular endothelial growth factor (VEGF) receptor (PVR), resulting in activation of ELMO (engulfment and cell motility, a Ced-12 homolog)/Mbc (myoblast city, a Ced-5/DOCK180 homolog)-mediated engulfment pathway. As a consequence, wild-type cells phagocytose nearby \textit{scrib} cells, thereby promoting \textit{scrib} cell elimination\textsuperscript{40} (Figure 3). This mechanism first provided the molecular basis for the significance of neighboring wild-type cells in the execution of tumor-suppressive cell competition. An ethyl methanesulfonate (EMS)-based genetic screen in \textit{Drosophila} identified cell-surface ligand-receptor proteins that regulate \textit{scrib} cell elimination via cell-cell interaction. The ligand Sas

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\caption{Mechanisms that eliminate \textit{scrib} cells by cell competition. Sas-PTP10D signaling activated by direct cell-cell interaction with neighboring wild-type cells inhibits EGFR signaling, thereby suppressing oncogenic cooperation between EGFR-Ras and Eiger/TNF-JNK signaling that activates the Hippo effector Yki.\textsuperscript{51} Slit-Robo2-Ena/Vasp signaling activated by Eiger/TNF-JNK signaling promotes extrusion of \textit{scrib} cells by downregulating E-Cadherin.\textsuperscript{39} Eiger/TNF-JNK signaling activated in wild-type cells elevates Pvr-Elmo-Mbc signaling and thereby promotes engulfment of neighboring \textit{scrib} cells.\textsuperscript{40} Epithelial cells secrete a serine protease inhibitor Serpin S, which inhibits Toll signaling and subsequently Yki-mediated cell survival signaling in \textit{scrib} cells.\textsuperscript{42} Circulating blood insulin (Dilp) suppresses \textit{scrib} cell elimination by insulin receptor (InR)-mediated elevation of protein synthesis.\textsuperscript{45}}
\end{figure}
and its receptor PTP10D (a receptor tyrosine phosphatase) normally localize to the apical surface of epithelial cells, but at the interface between *scrib* and wild-type cells they relocalize to the lateral membrane, where the ligand and receptor meet with each other in trans. This leads to activation of PTP10D signaling in *scrib* cells, resulting in suppression of epidermal growth factor receptor (EGFR) signaling and subsequent elimination of *scrib* cells41 (Figure 3). In the absence of Sas-PTP10D signaling, *scrib* clones elevate both EGFR and JNK signaling, which cooperate to activate the Hippo pathway effector Yorkie (Yki) and thus cause overgrowth.41 It is likely that the lateral relocalization of apical proteins in oncogenic polarity-deficient cells, which would cause direct cell-cell interaction with nearby cells.

5 | MICROENVIRONMENTAL REGULATION OF CELL ELIMINATION

While genetic studies in flies have clearly shown the critical role of cell-cell interaction in driving tumor-suppressive cell competition, it had been unclear whether cell competition is solely regulated by direct cell-cell interaction. A genetic screen in *Drosophila* identified *serpin 5* (*spn5*), which encodes a secreted serine protease inhibitor as a suppressor of tumor-suppressive cell competition when mutated in surrounding wild-type cells.42 *spn5* is one of the most abundantly expressed *serpins* in the imaginal discs,42 whose protein product negatively regulates the Toll ligand, Spätzle (Spz). Therefore, downregulation of Spn5 in surrounding wild-type cells leads to activation of Toll signaling in *scrib* cells. It has previously been shown that activation of Spz/Toll signaling causes elimination of loser cells in *Minute* or Myc-induced cell competition.43,44 Intriguingly, however, Toll activation in *scrib* cells does not promote their elimination but rather causes JNK activation and F-actin accumulation, leading to activation of Yki and thus *scrib* overgrowth45 (Figure 3). This suggests that restricting the basal level of Toll signaling in the epithelium is crucial for the induction of tumor-suppressive cell competition and that Toll activation by infection may trigger tumorigenesis by abrogating cell competition. In this sense, Serpins act as microenvironmental "surveillance factors" that facilitate tumor-suppressive cell competition.

6 | SYSTEMIC REGULATION OF CELL ELIMINATION

Cancer development is comprehensively regulated by a variety of systemic factors within the human body. Significantly, a recent genetic study revealed that a systemic factor also critically regulates tumor-suppressive cell competition in flies. A dominant modifier screen identified *chico*, which encodes an evolutionarily conserved insulin receptor substrate as a suppressor of tumor-suppressive cell competition when deleted heterozygously in the animal.45 Unexpectedly, Chico was not required in competing cells in the imaginal discs but was essential in insulin-producing cells (IPCs) of the brain to execute cell competition remotely. Mechanistically, *chico* downregulation in IPCs causes hyperinsulinemia by upregulating a *Drosophila* insulin Dilp2, which activates insulin/target of rapamycin (TOR) signaling in *scrib* cells. Notably, *scrib* cells normally show decreased protein synthesis activity compared to wild-type neighbors, but hyperinsulinemia-induced insulin/TOR activation in *scrib* cells boosts protein synthesis and causes *scrib* overgrowth45 (Figure 3). These observations provide an in vivo mechanistic explanation for why metabolic diseases such as type 2 diabetes are associated with increased cancer incidence in humans. This study also highlights an unexpected mechanistic link between tumor-suppressive cell competition and classical *Minute* cell competition, both of which represent elimination of loser cells that have lower protein synthesis compared with neighboring winner cells.

7 | ADDITIONAL ONCOGENIC ALTERATION REVERSES TUMOR-SUPPRESSIVE CELL COMPETITION

Accumulation of oncogenic alterations is a hallmark of malignant progression of tumors. This suggests that additional oncogenic alterations induced in *scrib* cells could reverse cell competition and cause *scrib* tumorigenesis. A prominent example of such phenomena is the activation of Ras or Notch signaling. While the activation of Ras or Notch in the eye imaginal discs only causes moderate overgrowth of benign tumors, its activation in *scrib* clones results in drastic, neoplastic overgrowth of malignant tumors that aggressively invade adjacent organs in the ventral nerve cord46 (Figure 4). The tumor malignancy of Ras-activated *scrib* (RasV12/*scrib*) cells is
caused by JNK activation, E-cadherin downregulation, and Yki activation. In addition, RasV12/scr ib cells undergo endoreplication and thus become polyplloid giant cells, which is essential for their malignant overgrowth. It has also been reported that RasV12/scr ib cells in the eye discs induce nonautonomous autophagy (NAA) in their surrounding wild-type cells and in distant tissues, which are essential to support aggressive growth and metastatic potential of RasV12/scr ib tumors, likely through nutrient supply. A similar NAA is observed in losers of Minute cell competition; in this case, NAA causes cell death, which may also promote the growth of neighboring winner cells like RasV12/scr ib-induced NAA. Interestingly, while RasV12/scr ib cells aggressively overproliferate, they also undergo apoptosis at the boundary between RasV12/scr ib and neighboring wild-type clones like losers of cell competition.

The elimination of scr ib clone is also reversed by overexpression of Myc, which is consistent with the fact that increased level of Myc turns cells into supercompetitors. In addition, it has been shown that clones defective in scr ib or lgl are eliminated when located in the wing pouch (therefore this region is referred to as "tumor cold spots"), while they evade cell competition and overgrow when located at the hinge region, where endogenous JAK/STAT activity is elevated (therefore referred to as "tumor hot spots"). This suggests that tissue-intrinsic local signaling activity or cytoarchitecture could regulate tumor-suppressive cell competition.

8 | CELL COMPETITION ELIMINATES SCRIB CELLS IN MAMMALS

The evolutionary conservation of the scr ib gene raises the question of whether elimination of scr ib mutant cells by cell competition is conserved in mammals. This was studied in mammalian epithelial cell line Madin-Darby canine kidney (MDCK) cells. While scr ib knockout MDCK cells are viable on their own, they undergo apoptosis and are extruded apically when cocultured with normal MDCK cells. Apoptosis of scr ib knockout MDCK cells when surrounded by normal cells depends on cell-autonomous activation of p38. A subsequent study showed that scr ib knockout MDCK cells surrounded by normal cells are hypersensitive to mechanical compaction due to elevated p53 activity. Compaction further upregulates p53 levels through Rho-associated kinase (ROCK) and p38 in scr ib knockout cells, thereby inducing cell death. This suggests that tumor-suppressive cell competition is also regulated by mechanical insults. Thus, while the underlying mechanisms are different, tumor-suppressive cell competition triggered by loss of scr ib seems to be conserved in mammalian systems.

9 | CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Genetic studies in Drosophila have established a concept that cell competition acts as an intrinsic tumor suppressor in the epithelium. Although the basal expansion of the apical domain is currently the sole hallmark of losers of tumor-suppressive cell competition, a recent study suggested that a reduction in protein synthesis may also contribute to establish the loser status. The fact that the elimination of scr ib cells is mediated not only by direct cell-cell interaction but by microenvironmental and systemic factors underscores that tumor-suppressive cell competition is regulated by comprehensive mechanisms and can therefore be affected by a variety of cellular or environmental changes within the animal, just like human cancers.

An important outstanding question is what the initial trigger is for tumor-suppressive cell competition. It would be important to clarify whether cellular changes other than loss of cell polarity can also trigger tumor-suppressive cell competition. It would also be interesting to investigate whether the machinery of tumor-suppressive cell competition is involved in physiological processes other than tumor suppressing. For instance, during normal development, there are a variety of cell-cell interactions that couple with cell elimination and extrusion, which may be regulated by the common machinery of tumor-suppressive cell competition.

Although there is experimental evidence that mammalian epithelial cells can eliminate scr ib cells via cell-cell interaction, it would need further investigations to clarify whether similar machinery also exists in mammalian epithelial tissues. Further studies in mammalian systems could lead to the development of a novel anticancer strategy that potentiates tumor-suppressive cell completion.

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ORCID

Hiroshi Kanda  https:/ /orcid.org/0000-0002-7922-7309
Tatsushi Igaki  https:/ /orcid.org/0000-0001-5839-9526

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