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Review Article

Non-autonomous overgrowth by oncogenic niche cells: Cellular cooperation and competition in tumorigenesis

Masato Enomoto,[1,3] John Vaughen[1,3] and Tatsushi Igaki[1,2]

[1]Laboratory of Genetics, Graduate School of Biostudies, Kyoto University, Kyoto; [2]PRESTO, Japan Science and Technology Agency, Saitama, Japan

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Correspondence
Tatsushi Igaki, Laboratory of Genetics, Graduate School of Biostudies, Kyoto University, Yoshida-Konoecho, Sakyo-ku, Kyoto 606-8501, Japan.
Tel.: +81-75-753-7684; Fax: +81-75-753-7686;
E-mail: igaki@lif.kyoto-u.ac.jp

[3]These authors contributed equally to this work.

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Tumor progression is classically viewed as the Darwinian evolution of subclones that sequentially acquire genetic mutations and autonomously overproliferate. However, growing evidence suggests that tumor microenvironment and subclone heterogeneity contribute to non-autonomous tumor progression. Recent *Drosophila* studies revealed a common mechanism by which clones of genetically altered cells trigger non-autonomous overgrowth. Such "oncogenic niche cells" (ONCs) do not overgrow but instead stimulate neighbor overgrowth and metastasis. Establishment of ONCs depends on competition and cooperation between heterogeneous cell populations. This review characterizes diverse ONCs identified in *Drosophila* and describes the genetic basis of non-autonomous tumor progression. Similar mechanisms may contribute to mammalian cancer progression and recurrence.

Cancers progress through clonal evolution, the sequential acquisition of oncogenic mutations through Darwinian selection of advantaged subclones. For example, in colon cancers, adenomatous polyposis coli (*APC*) mutant clones acquire malignancy after further mutation of *K-Ras*, *p53*, and other genes.[1] While genetic sequencing corroborated cancer's clonal evolution, it also revealed remarkable clonal heterogeneity.[2,3] A recent study found rampant intratumor genetic divergence, with ~60% of mutations not universally distributed among subclones.[3] Furthermore, in human glioblastoma, coexisting subclones amplified distinct oncogenic receptor tyrosine kinases (epidermal growth factor receptor [*EGFR*], *PDGFR*, and *c-Met*).[4] Clonal heterogeneity could therefore promote subclone cooperation alongside clonal competition. Indeed, interclonal cooperation between subclones potentiated tumorigenesis in mouse-modeled breast cancer.[5] Excitingly, clonal population dynamics of cancer-derived, heterogeneous subclones were reproducible across independent xenografts,[6] suggesting that tumor heterogeneity is a tractable problem. While interclonal communication is considered crucial to cancer's etiology, detailed *in vivo* mechanisms are lacking.

Drosophila genetics enables manipulation of oncogenic cell clones *in vivo*.[7] Remarkably, *Drosophila* tumorigenesis recapitulates aspects of human cancer, including polarity loss, basement-membrane degradation, and invasion.[8] Accordingly, genetic screens in *Drosophila* have identified evolutionarily conserved tumor-suppressor genes, including Hippo pathway components.[9–11] Genetic mosaic analysis also revealed an unusual tumor-promoting cell population that can be called "oncogenic niche cells" (ONCs). Oncogenic niche cells drive non-autonomous tumor progression through cellular competition and cooperation with surrounding cells (Fig. 1a). This review describes mechanisms by which ONCs regulate *Drosophila* tumorigenesis and discusses putative ONCs in mammalian cancers.

Non-autonomous tumor progression by ONCs

Epithelial cells harboring oncogenic mutations can promote their own growth through interactions with surrounding stroma.[12] However, oncogenic mutations can also promote non-autonomous proliferation as ONCs. ONCs can be induced

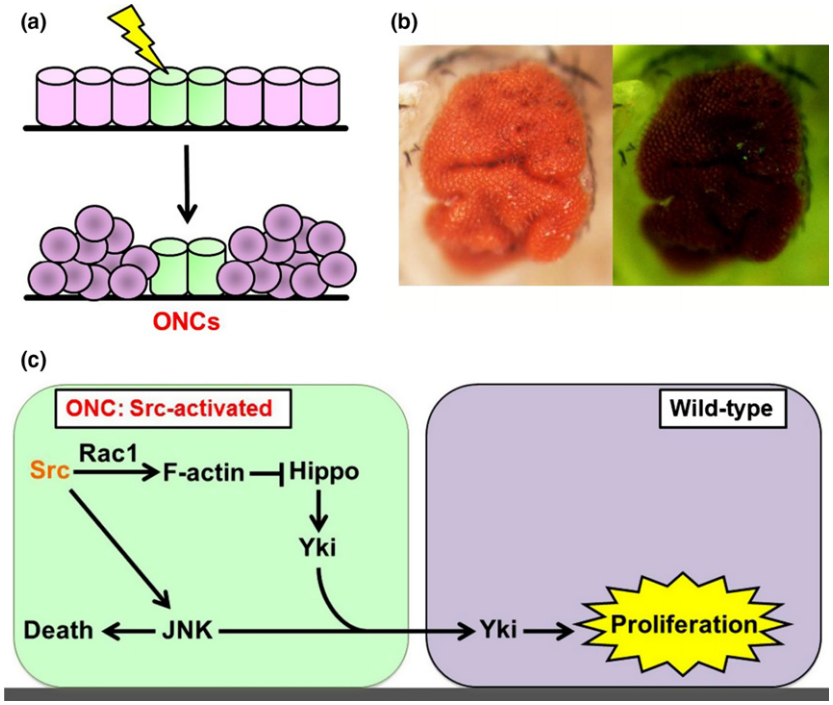

Fig. 1. Oncogenic niche cells (ONCs) activated by oncoprotein Src. (a) General ONC scheme showing genetically altered clones (green) become ONCs, stimulating surrounding cell overgrowth. (b) Src64B-overexpressing cells (GFP+) are scarce yet wild-type tissue overgrows, causing tissue folding. (c) Src64B-overexpressing cells inactivate Hippo signaling through F-actin accumulation. Src activation simultaneously triggers JNK, inducing intraclonal death but propagating Yorkie (Yki) to neighboring cells, causing overproliferation.

by cell competition, a process in which normally viable "loser" cells are eliminated by neighboring "winner" cells. Cell competition is triggered by lower translation rates, disrupted apicobasal polarity, or aberrant signal transduction, and thus functions as a tumor suppressor and developmental regulator.[13–16] Alongside cell competition, ONCs commonly feature cooperation between the JNK and Hippo pathways. Below, we describe five classes of ONCs characterized in *Drosophila* imaginal epithelia.

Oncoprotein Src. Elevation of oncoprotein Src often correlates with tumor malignancy, yet Src's role in tumorigenesis remains unclear.[17] Clones of cells overexpressing Src64B (Src; c-Src homolog) in the *Drosophila* imaginal disc are eliminated by JNK-dependent cell competition.[18,19] However, Src clones also function as ONCs to cause non-autonomous overgrowth of surrounding tissue (Fig. 1b).[19] Src-activated cells accumulate intracellular F-actin and activate the Hippo pathway effector Yorkie (Yki; YAP homolog). Simultaneously, JNK signaling induces cell death in a cell-autonomous manner but propagates Yki to neighboring cells, causing overgrowth of surrounding tissue (Fig. 1c). Blocking Yki inside Src-activated cells abolished neighboring Yki activation, implying propagation of Yki from ONCs. Thus, while JNK-mediated cell competition restrains Src-activated ONC autonomous growth, JNK–Yki cooperation contributes to non-autonomous tumorigenesis.

Endocytic dysregulation. Endocytic trafficking controls internalization and sorting of extracellular molecules and transmembrane proteins. Consequently, endocytic dysregulation disrupts signaling pathways and cell polarity, contributing to human cancers.[20–22] Multiple genetic screens in *Drosophila* identified endosomal sorting complex components *vps25* and *erupted* (*ept*; *tsg101* homolog) as causing non-autonomous overgrowth.[23–26] Endocytic ONCs accumulated endosomal Notch, inducing the cytokine Unpaired (Upd; interleukin [IL]-6 homolog) and triggering JAK– signal transducer and activator of transcription (STAT) signaling in surrounding cells (Fig. 2a). A similar but distinct endocytic ONC was formed by mutating *Rab5*, an early endosome component. Rab5-deficient ONCs accumulated EGFR and Eiger (tumor necrosis factor homolog), activating Ras and JNK pathways, respectively.[27] JNK and Ras signaling cooperatively activated Yki, inducing

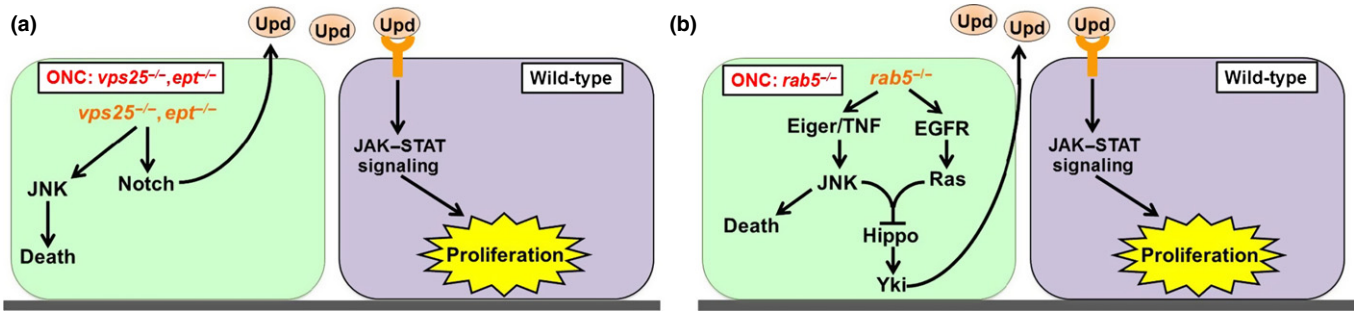

Fig. 2. Endocytic dysregulated oncogenic niche cells (ONCs). (a) *vps25* or *ept* mutant clones accumulate Notch, stimulating secretion of the cytokine Unpaired (Upd) and non-autonomous overgrowth. (b) *Rab5* mutant cells activate epidermal growth factor receptor (EGFR)–Ras and Eiger–JNK signaling, cooperatively activating Yorkie (Yki) and inducing Upd. STAT, signal transducer and activator of transcription; TNF, tumor necrosis factor.

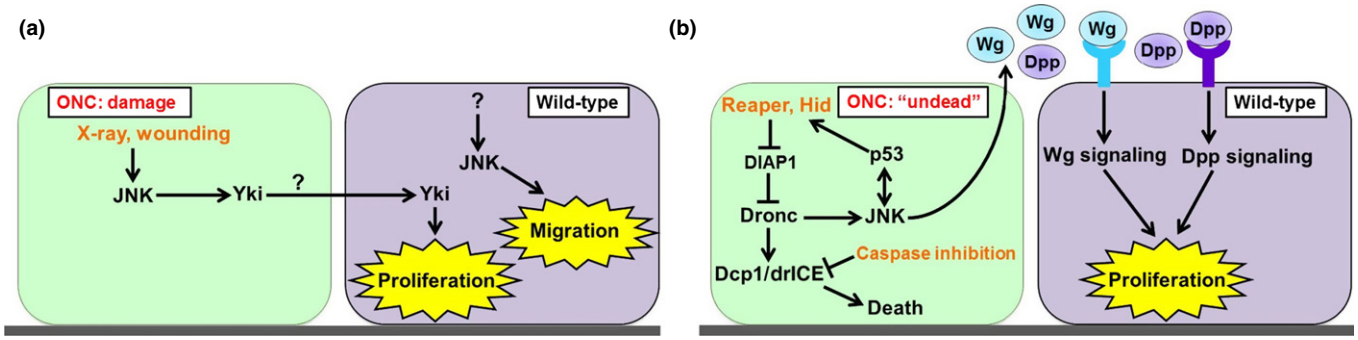

Fig. 3. Apoptotic oncogenic niche cells (ONCs). (a) Damage-induced JNK activates Yorkie (Yki) in wild-type cells, triggering compensatory proliferation. Wild-type cell JNK stimulates migration to the damaged area. (b) "Undead" ONCs formed by overexpression of pro-apoptotic genes (*Reaper*, *Hid*) and caspase inhibition. Resultant JNK activity stimulates surrounding cell proliferation through ONC Decapentaplegic (Dpp)/Wingless (Wg) secretion. Dcp1, death caspase 1; DIAP1, death-associated inhibitor of apoptosis 1; drICE, *Drosophila* ICE; Dronc, *Drosophila* NEDD2-like caspase.

Upd expression and subsequent non-autonomous overgrowth (Fig. 2b). Non-autonomous phenotypes are dependent on cell competition: *vps25* clones prevented from dying autonomously overgrow,[24,25] and growth of Rab5 dominant-negative ($Rab5^{DN}$)-expressing cells was constrained by JNK-mediated cell competition.[27,28] Interestingly, sufficiently large $Rab5^{DN}$-expressing cell clones autonomously overgrow,[28] suggesting that establishment of endocytic ONCs is contingent on cell competition and clone size.

Apoptotic stimulus. Apoptosis is a hallmark of many cancers and often correlates with increased proliferation and worse prognosis.[29] In *Drosophila* wing discs, massive cell death triggers non-autonomous "compensatory proliferation", yielding normal adult wings.[30] Yki is activated in dying and neighboring cells and is essential for wing disc regeneration.[31,32] Notably, in this case, JNK activation is necessary and sufficient for Yki induction in wing discs,[31] and JNK activity non-autonomously propagates following local wounding.[33] JNK also stimulates cell migration to the wound site,[34] similar to JNK-driven developmental or tumorigenic invasion (Fig. 3a).[35,36]

In a similar phenomenon, when cell death is induced but not executed, typically through overexpression of caspase inhibitor p35, persistent "undead" cells become ONCs and trigger non-autonomous overgrowth through the growth factors Decapentaplegic (Dpp; bone morphogenetic protein/transforming growth factor-β homolog) and Wingless (Wg; Wnt homolog).[37–39] In wing discs, this is dependent on a p53-JNK positive feedback loop activated by the initiator caspase Dronc (Fig. 3b).[40] Undead cells generated through genomic instability also function as ONCs, secreting Wg and triggering JNK-dependent non-autonomous hyperplasia.[41] Here, JNK-activated MMP1 activity also induces basement membrane degradation and invasion. Intriguingly, undead cells can also cause non-autonomous apoptosis propagated by Eiger–JNK.[42] Dying ONCs may unleash an autocatalytic wave of JNK, death, growth factor secretion, and proliferation.

Polarity loss. Apico-basal polarity is essential for epithelial cell function and homeostasis. Polarity loss underlies many cancers and is often critical for cancer progression.[43] Imaginal epithelia entirely mutant for conserved apico-basal polarity genes scribble (*scrib*) or discs large (*dlg*) develop into tumors. However, clones of these mutant cells surrounded by wild-type cells are eliminated through Eiger–JNK signaling in "tumor-suppressive cell competition".[44] Competition-induced JNK also suppresses Yki activity in *scrib* clones.[45] Although JNK restrains overgrowth of *scrib* tissue, it is required for tumor progression and metastasis of *scrib*+Ras^{V12} clones.[35] Surprisingly, distinct *scrib* and Ras^{V12} mosaic clones induced in the same imaginal disc still trigger metastasis of Ras^{V12} cells,[33] suggesting that *scrib* cells function as ONCs (Fig. 4a). JNK activity propagates from *scrib* to Ras^{V12} cells, inducing Upd and JAK–STAT signaling that cooperates with Ras signaling to induce metastasis. Remarkably, JAK–STAT signaling is also required within *scrib*-neighboring cells to eliminate *scrib* clones.[33,46] As JAK–STAT and JNK both suppress and promote *scrib* tumor formation, competition-triggered interclonal cooperation likely underlies polarity-defective ONCs.

Ras, mitochondrial dysfunction, and senescence. Metastasis of Ras^{V12}-expressing cells can be triggered by different ONCs, such as *scrib* clones.[8,47] A genetic screen for Ras^{V12}-induced non-autonomous growth identified mutations in genes required for mitochondrial respiratory function, which are frequently downregulated in various cancers.[48] Clones harboring Ras^{V12} and mitochondrial dysfunction ($Ras^{V12}/mito^{-/-}$) produce reactive oxygen species (ROS), activating JNK. JNK and Ras cooperatively activate Yki, which upregulates Upd and Wg to induce surrounding tissue overgrowth.[49] Thus, $Ras^{V12}/mito^{-/-}$ cells act as ONCs. Interestingly, $Ras^{V12}/mito^{-/-}$ cells cause cell-cycle arrest through ROS production and undergo p53-dependent cellular senescence (Fig. 4a).[50] Overexpression of p53 inside Ras^{V12} clones is sufficient to induce ONCs and non-autonomous overgrowth. Therefore, senescent cells may function as ONCs through inflammatory cytokine release,[51] a conserved phenomenon called the senescence-associated secretory phenotype (SASP).[52] Indeed, paralleling *scrib* interclonal cooperation, $Ras^{V12}/mito^{-/-}$-produced Upd triggers adjacent Ras^{V12} metastasis (Fig. 4b).[49] Interestingly, activated Ras signaling stimulates Eiger exocytosis, causing JNK accumulation and JAK–STAT activation at clonal boundaries between Ras^{V12} and wild-type cells.[53] JNK transcytosis could underlie JNK propagation and cell competition's role in oncogenic cooperation.

Oncogenic niche cell themes: JNK-mediated cell competition and cooperation

There are several shared ONC themes (Table 1). Notably, Src-activated, endocytic, and polarity-defective ONCs undergo JNK-mediated cell competition. Thus, tumor-suppressive cell

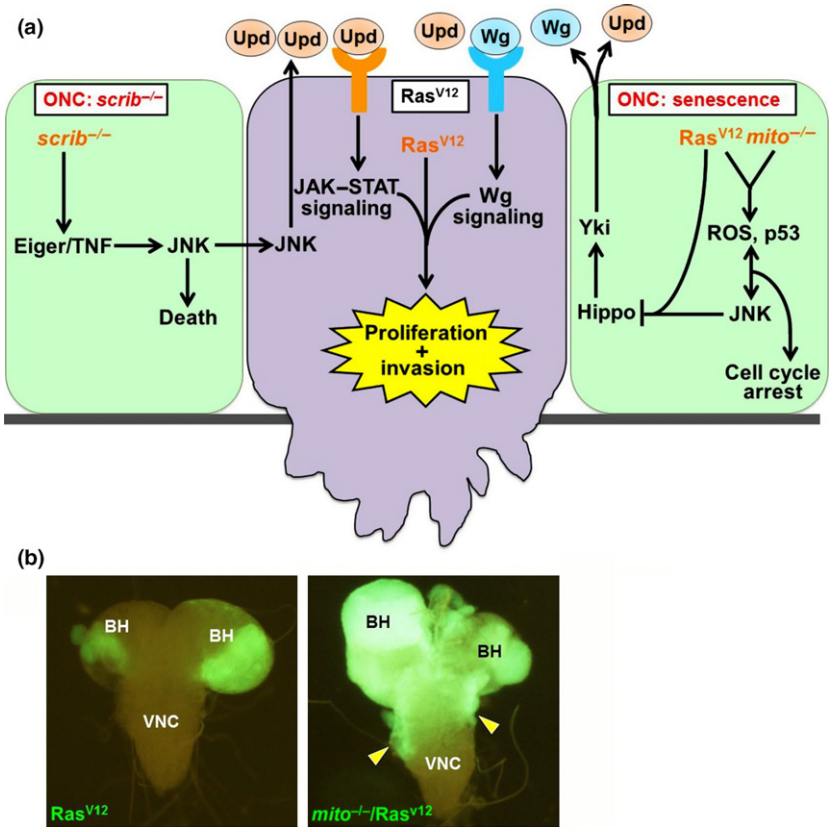

Fig. 4. Cooperation between Ras^{V12} and oncogenic niche cells (ONCs). (a) *scrib* cells activate Eiger/JNK signaling, causing neighboring Ras^{V12} cells (purple) to secrete Unpaired (Upd) and metastasize. Alternatively, $Ras^{V12}/mito^{-/-}$ cells (mitochondrial dysfunction, senescent ONC) generate reactive oxygen species (ROS) and activate p53, which cooperatively activate JNK. JNK and Ras^{V12} inactivate the Hippo pathway, activating Yorkie (Yki) and triggering secretion of Upd and Wingless (Wg). ONC-induced senescence-associated secretory phenotype stimulates neighboring Ras^{V12} cell invasion. (b) While Ras^{V12} clones (GFP+) fail to metastasize from the larval brain hemisphere (BH) to the ventral nerve cord (VNC), senescent ONCs ($Ras^{V12}/mito^{-/-}$) stimulate Ras^{V12} invasion (arrowheads). STAT, signal transducer and activator of transcription; TNF, tumor necrosis factor.

Table 1. Common oncogenic niche cell (ONC) themes

ONCs	Competition and cell death	Cooperation	Growth factors	Cellular senescence or p53 accumulation (p53+)
Src-activated	JNK→cell death	JNK→Yki	–	–
Endocytic dysregulation	JNK→cell death	Notch→JAK–STAT JNK/Ras→Yki	Upd Wg Dpp	p53+
Apoptotic stimulus	Cell death	JNK→Dpp, Wg JNK→Yki JNK↔p53 JNK→JNK/death	Wg Dpp	p53+
Polarity defect	JNK→cell death	JNK→JAK–STAT	Upd	–
$Ras^{V12}/mito^{-/-}$	–	JNK/Ras→Yki ROS/p53→JNK	Upd Wg	p53+, cellular senescence

Dpp, Decapentaplegic; ROS, reactive oxygen species; STAT, signal transducer and activator of transcription; Upd, UNPAIRED; Wg, Wingless; Yki, Yorkie.

competition is subverted to promote cancer. Cancers may hijack cell competition machinery to "sweep" through populations in field cancerization,[13,54] and cell competition also generates ONCs. Ironically, neighboring cells that activate tumor-suppressive machinery become the cancerous entity they sought to destroy (Fig. 5). Additionally, JNK is activated following apoptosis, causing non-autonomous death and proliferation. Thus, a feed-forward loop of JNK and death could propagate to potentiate tumor progression and metastasis.

How does JNK drive ONC-mediated overgrowth? Another ONC theme is JNK-dependent Yki activation (Table 1), which can induce growth factors and anti-apoptotic genes like *Diap1*. Notably, JNK directly impinges on Hippo through Ajuba-mediated Warts regulation, activating Yki and driving overgrowth.[55,56] Therefore, non-autonomous JNK propagation in certain ONCs could directly induce non-autonomous Yki. However, within Src-activated ONCs, non-autonomous JNK does not induce Yki. Instead, autonomous JNK cooperatively propagates Yki activity to neighboring cells (Fig. 1c).[19] Additionally, polarity loss can still induce non-autonomous Yki activation in tissues lacking the JNK ligand Eiger,[45] suggesting that JNK-independent pathways may activate non-autonomous Yki in polarity ONCs. Thus, specific mechanisms of non-autonomous Yki activation remain unclear. Surprisingly,

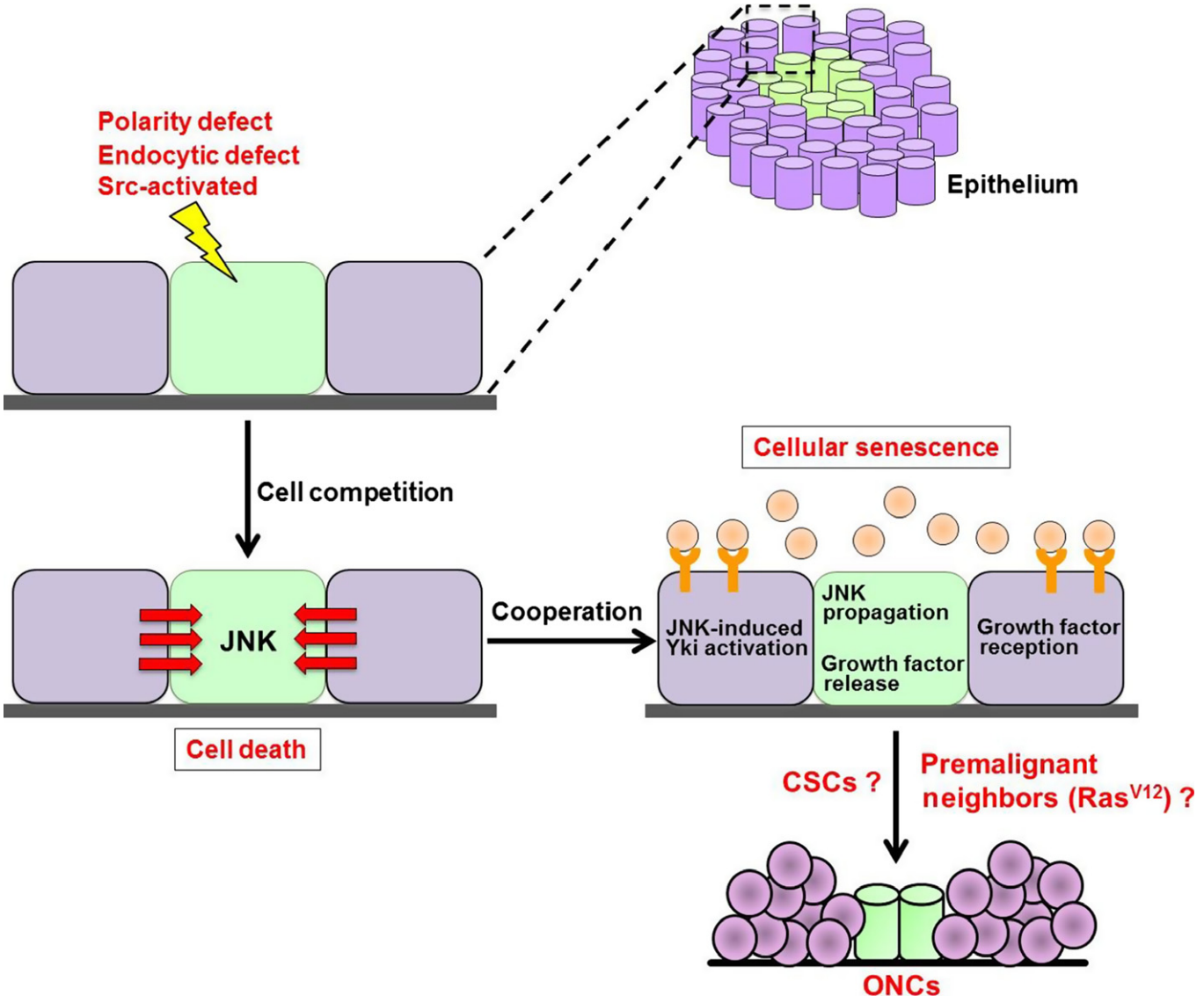

Fig. 5. Oncogenic niche cell (ONC) timeline. Potentially tumorigenic cells (green) are attacked by neighbors through JNK-based cell competition, causing autonomous cell death. However, dying cells stimulate cooperation between JNK and other pathways, triggering growth factor release and/or non-autonomous Yorkie (Yki) activation. Senescence also induces ONCs, causing growth factor secretion and potentiating metastasis of adjacent premalignant tissue (i.e. Ras^{V12}). Additionally, ONCs may stimulate neighboring cancer stem cell (CSC) proliferation or even induce CSCs, driving non-autonomous tumor progression. Chemotherapy-induced death or senescence may contribute to cancer recurrence through formation of ONCs.

core Hippo pathway components are not commonly disrupted in human cancer.[57] Instead, Hippo dysregulation may potentiate tumorigenesis through interclonal cooperation. Indeed, Yki/YAP is nuclear in ~60% of hepatocellular carcinomas and 65% of non-small-cell lung cancers.[57] Oncogenic niche cell-driven, non-autonomous Yki activation could contribute to mammalian cancer progression.

JNK may additionally act through Polycomb group proteins (PcG), which enforce epigenetic repression. During wound-healing, JNK inactivates PcG to allow fate reprogramming.[58] Therefore, JNK activity could alleviate PcG repression of growth factors like Wg or Upd. Notably, loss of PcG protein Polyhomeotic causes non-autonomous, Upd-mediated overgrowth.[59,60] Because mammalian JNK can act as a pro-apoptotic gene linked to tumor regulation,[61] JNK may function in both mammalian and *Drosophila* ONCs.

Oncogenic niche cell hypothesis: Crumbs in ONC establishment

A common ONC theme is cooperative Yki activation (Table 1), suggesting that Hippo pathway activity may influence ONCs. Indeed, differential Hippo pathway activity triggers cell competition: Yki-activated cells are super-competitors while *yki* mutants are eliminated.[13,14,16,62] Alongside JNK-based competition, could Hippo-mediated competition establish ONCs? The apical transmembrane protein Crumbs (Crb) is a good candidate for ONC establishment. Crb recruits Expanded (Ex),[63–66] activating Hippo and inhibiting Yki. Intriguingly, Crb accumulates in polarity and endocytic ONCs.[26,67] Furthermore, Crb-overexpressing clones are eliminated while *crb* mutants induce neighbor-cell death in a competitive phenomenon dependent on Crb–Crb extracellular interactions.[68] Therefore, in certain

ONCs, high Crb may cause JNK-dependent repression of Yki and "loser" status. Conversely, neighboring cells with lower Crb would overgrow through JNK-activated Yki. CRB3 is downregulated in human tumor-derived cell lines,[43] consistent with lower Crb permitting overgrowth.

However, while Crb-overexpressing cells are eliminated, overexpression of only Crb's intracellular domain (Crb-intra) causes autonomous overgrowth.[68] Intriguingly, Crb-intra overgrowth is rescued by depletion of Eiger's receptor *Grindelwald*, which colocalizes with Crb at apical membranes.[69] This supports the idea that Crb–JNK might contribute to ONCs, but highlights the complexity of Crb overexpression phenotypes. Indeed, although Crb recruits Ex and activates Hippo, Crb can also promote Ex degradation through ubiquitylation.[66] Moreover, Hippo pathway mutants accumulate Crb yet become super-competitors,[62,70,71] perhaps through elevation of anti-apoptotic DIAP1 or mechanisms paralleling Crb-intra induced overgrowth. While Crb's contribution to ONCs is highly complex, it is likely that Crb and JNK can cooperatively dictate Yki activity and competitive outcomes in ONCs.

Oncogenic niche cell themes: a role for cellular senescence?

Though cell death is common to ONCs, it is not induced in $Ras^{V12}/mito^{-/-}$ cells, which instead activate p53 and undergo cellular senescence.[50] JNK-activated Yki stimulates Upd/Wg secretion in a SASP,[50,52] reminiscent of growth factor secretion by "undead" ONCs. Intriguingly, p53 has been linked to cell competition. In murine hematopoietic stem cells (HSCs), irradiated $p53^{-/-}$ HSCs outcompete $p53^{+/+}$, but not $p53^{+/-}$ HSCs.[72] Separately, p53 knockdown in differentiated murine tissue allowed disproportionate expansion of $p53^{-/-}$ cells. Surprisingly, p53 knockdown in undifferentiated embryonic stem cells lead to $p53^{-/-}$ cell elimination, suggesting that p53 knockdown triggers context-dependent cell competition.[73] Supporting this, apoptosis of Myc-overexpressing, $p53^{-/-}$ *Drosophila* cells was dependent on surrounding neighbors.[74] Furthermore, p53 is intimately linked to JNK in *Drosophila*: p53 and JNK form a feed-forward loop in undead ONCs,[40] are sufficient to induce each other's expression,[40] and p53 directly binds JNK.[75] Indeed, blocking p53 in $Ras^{V12}/mito^{-/-}$ abrogated JNK activity and ONC formation.[50]

Therefore, p53 or cellular senescence may trigger cell competition and establish ONCs. p53 is elevated in *tsg101* null mice, likely from impaired ubiquitin-mediated degradation.[76] Ubiquitinated proteins accumulate in *vps25* and *ept* mutant ONCs,[23,24,26] and mutations in an E1 ubiquitin-activating enzyme cause non-autonomous overgrowth.[77] Moreover, p53 is required for undead ONCs' non-autonomous proliferation.[78] While these phenotypes can partly be explained by p53's pro-apoptotic role, p53 may fuel non-autonomous tumor progression through cell competition and SASP. As p53 is frequently mutated in human cancers,[79] dysregulated cell competition or SASP may contribute to mammalian cancer progression (see below).

Oncogenic niche cells in mammalian cancer

Do ONCs contribute to mammalian cancer progression? Although cellular senescence can suppress tumor formation, it can fuel non-autonomous overgrowth through cytokine secretion in a SASP.[52] Mammalian studies also suggest that SASP can drive non-autonomous tumor progression.[52] Senescence-associated secretory phenotype of murine hepatic stellate cells potentiated hepatocellular carcinoma formation.[80] Moreover, SASP in epithelial cells triggered malignancy through paracrine secretion of IL-6 and IL-8,[81] paralleling senescent ONCs' induction of neighboring Ras^{V12} metastasis (Fig. 4b). Thus, non-autonomous tumor progression through cellular senescence and SASP seems conserved in mammalian cancer progression. Like cell competition, cellular senescence can suppress or promote tumorigenesis. As chemotherapy often potentiates cellular senescence,[52,82] senescent ONCs could contribute to cancer recurrence (Fig. 5).

Alongside cellular senescence, cell death is commonly observed in human cancers and often correlates with increased tissue proliferation.[29] In a mouse model of hepatocellular carcinogenesis, dying hepatocytes activate JNK and ROS, inducing proliferation of surrounding cells through cytokine release.[83,84] Importantly, therapy-induced death can also trigger ONC-mediated proliferation. Following radiotherapy, dying cancer cells activate effector caspases 3/7, triggering prostaglandin E_2 (PGE_2) secretion. Subsequently, PGE_2 stimulates neighbor-cell proliferation.[85] Notably, tumor recurrence positively correlated with high activation of caspases 3/7 in human patients.[85] In human prostate cancer, chemotherapy-induced PGE_2 can trigger non-autonomous cell proliferation and tumor repopulation through cancer stem cells (see below).[86] Thus, cell death and cellular senescence in ONCs can initiate tumors and hinder treatment (Fig. 5).

Oncogenic niche cells may promote tumorigenesis through non-autonomous effects on cancer stem cells (CSCs). The CSC hypothesis proposes that specialized stem cells contribute disproportionately to cancerous populations.[87] Consequently, cancer heterogeneity can be partially attributed to differentiation hierarchies stemming from distinct CSC subpopulations.[2] Experimental evidence suggests that CSC progeny are surprisingly plastic in their fate and can dedifferentiate to CSCs.[87] Intriguingly, a pulse of Src activity was sufficient to convert a non-malignant breast cell line into a self-renewing, CSC-containing cancer through an IL-6/nuclear factor-κB positive feedback loop.[88] Furthermore, IL-6 addition to regular cancer cells induced dedifferentiation to CSCs.[89] As ONCs frequently induce Upd/IL-6 (Table 1) through JNK–Yki cooperation in *Drosophila*, ONC-secreted factors could promote non-autonomous CSC induction. Concomitantly, ONCs could promote CSC proliferation, such as in therapy-induced CSC proliferation and repopulation.[87] Notably, in human hepatocellular carcinoma, JNK and IL-6 markers are commonly associated with CSCs,[61] although no evidence directly links CSCs to ONCs. Additionally, as CSCs are often resistant to apoptosis,[87] it is possible that CSCs themselves could function as "undead" ONCs when challenged with apoptotic stimuli, such as chemotherapy.

Conclusions

Drosophila studies have brought insight to tumor progression through genetic dissection of ONCs. As senescent and dying ONCs are likely conserved in mammals, further ONC analysis will inform our understanding of mammalian cancer etiology. Despite diverse genetic triggers, ONCs share many characteristics, including cell death, competition, and cooperation. The role of JNK in cell competition and its cooperation with Yki merits further study in both *Drosophila* and mammalian cancer models. The relationships between ONCs, Crb, p53, cellular senescence, and cell competition are exciting areas for future

investigation. Notably, ONCs themselves do not directly contribute to cancer's effective population and hence do not undergo a Darwinian clonal selection. Instead, ONCs are created by the protective programs endowed with maintaining epithelial homeostasis: cell competition, cellular senescence, and apoptosis. We envision that ONCs contribute to non-autonomous tumor initiation and progression through cooperation with adjacent premalignant tissue or through CSC induction or stimulation (Fig. 5). The basic genetic mechanisms uncovered in *Drosophila* ONCs likely underlie interclonal cooperation in heterogeneous mammalian cancers. Moreover, mammalian evidence suggests that ONCs fuel tumor recurrence following chemotherapy-induced death or senescence. Therefore, cancer topography, the potential for ONC induction or subclone cooperation, must be carefully considered before individualized therapies become a reality.

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Disclosure Statement

The authors have no conflict of interest.

References

1 Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759–67.
2 Marusyk A, Almendro V, Polyak K. Intra-tumour heterogeneity: a looking glass for cancer? *Nat Rev Cancer* 2012; **12**: 323–34.
3 Gerlinger M, Rowan AJ, Horswell S *et al.* Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med* 2012; **366**: 883–92.
4 Snuderl M, Fazlollahi L, Le LP *et al.* Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma. *Cancer Cell* 2011; **20**: 810–7.
5 Cleary AS, Leonard TL, Gestl SA, Gunther EJ. Tumour cell heterogeneity maintained by cooperating subclones in Wnt-driven mammary cancers. *Nature* 2014; **508**: 113–7.
6 Eirew P, Steif A, Khattra J *et al.* Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. *Nature* 2015; **518**: 422–6.
7 Xu T, Rubin GM. Analysis of genetic mosaics in developing and adult Drosophila tissues. *Development* 1993; **117**: 1223–37.
8 Pagliarini RA, Xu T. A genetic screen in Drosophila for metastatic behavior. *Science* 2003; **302**: 1227–31.
9 Enomoto M, Igaki T. Deciphering tumor-suppressor signaling in flies: genetic link between Scribble/Dlg/Lgl and the Hippo pathways. *J Genet Genomics* 2011; **38**: 461–70.
10 Pan D. The hippo signaling pathway in development and cancer. *Dev Cell* 2010; **19**: 491–505.
11 Halder G, Johnson RL. Hippo signaling: growth control and beyond. *Development* 2011; **138**: 9–22.
12 Junttila MR, de Sauvage FJ. Influence of tumour micro-environment heterogeneity on therapeutic response. *Nature* 2013; **501**: 346–54.
13 Amoyel M, Bach EA. Cell competition: how to eliminate your neighbours. *Development* 2014; **141**: 988–1000.
14 Levayer R, Moreno E. Mechanisms of cell competition: themes and variations. *J Cell Biol* 2013; **200**: 689–98.
15 de Beco S, Ziosi M, Johnston LA. New frontiers in cell competition†. *Dev Dyn* 2012; **241**: 831–41.
16 Baker NE. Cell competition. *Curr Biol* 2011; **21**: R11–5.
17 Yeatman TJ. A renaissance for SRC. *Nat Rev Cancer* 2004; **4**: 470–80.
18 Vidal M, Larson DE, Cagan RL. Csk-deficient boundary cells are eliminated from normal Drosophila epithelia by exclusion, migration, and apoptosis. *Dev Cell* 2006; **10**: 33–44.
19 Enomoto M, Igaki T. Src controls tumorigenesis via JNK-dependent regulation of the Hippo pathway in Drosophila. *EMBO Rep* 2013; **14**: 65–72.
20 Mosesson Y, Mills GB, Yarden Y. Derailed endocytosis: an emerging feature of cancer. *Nat Rev Cancer* 2008; **8**: 835–50.
21 Floyd S, De Camilli P. Endocytosis proteins and cancer: a potential link? *Trends Cell Biol* 1998; **8**: 299–301.
22 Lanzetti L, Di Fiore PP. Endocytosis and cancer: an 'Insider' network with dangerous liaisons. *Traffic* 2008; **9**: 2011–21.
23 Herz H-M, Chen Z, Scherr H, Lackey M, Bolduc C, Bergmann A. vps25 mosaics display non-autonomous cell survival and overgrowth, and autonomous apoptosis. *Development* 2006; **133**: 1871–80.
24 Thompson BJ, Mathieu J, Sung H-H, Loeser E, Rørth P, Cohen SM. Tumor suppressor properties of the ESCRT-II complex component vps25 in Drosophila. *Dev Cell* 2005; **9**: 711–20.
25 Vaccari T, Bilder D. The Drosophila tumor suppressor vps25 prevents nonautonomous overproliferation by regulating Notch trafficking. *Dev Cell* 2005; **9**: 687–98.
26 Moberg KH, Schelble S, Burdick SK, Hariharan IK. Mutations in erupted, the Drosophila ortholog of mammalian tumor susceptibility gene 101, elicit non-cell-autonomous overgrowth. *Dev Cell* 2005; **9**: 699–710.
27 Takino K, Ohsawa S, Igaki T. Loss of Rab5 drives non-autonomous cell proliferation through TNF and Ras signaling in Drosophila. *Dev Biol* 2014; **395**: 19–28.
28 Ballesteros-Arias L, Saavedra V, Morata G. Cell competition may function either as tumour-suppressing or as tumour-stimulating factor in Drosophila. *Oncogene* 2014; **33**: 4377–84.
29 Soini Y, Pääkkö P, Lehto VP. Histopathological evaluation of apoptosis in cancer. *Am J Pathol* 1998; **153**: 1041–53.
30 Haynie J, Bryant P. The effects of X-rays on the proliferation dynamics of cells in the imaginal wing disc of *Drosophila melanogaster*. *Rouxs Arch Dev Biol* 1977; **183**: 85–100.
31 Sun G, Irvine KD. Regulation of Hippo signaling by Jun kinase signaling during compensatory cell proliferation and regeneration, and in neoplastic tumors. *Dev Biol* 2011; **350**: 139–51.
32 Grusche FA, Degoutin JL, Richardson HE, Harvey KF. The Salvador/Warts/Hippo pathway controls regenerative tissue growth in *Drosophila melanogaster*. *Dev Biol* 2011; **350**: 255–66.
33 Wu M, Pastor-Pareja JC, Xu T. Interaction between RasV12 and scribbled clones induces tumour growth and invasion. *Nature* 2010; **463**: 545–8.
34 Herrera SC, Martín R, Morata G. Tissue homeostasis in the Wing Disc of *Drosophila melanogaster*: immediate response to massive damage during development. *PLoS Genet* 2013; **9**: e1003446.
35 Igaki T, Pagliarini RA, Xu T. Loss of cell polarity drives tumor growth and invasion through JNK activation in Drosophila. *Curr Biol* 2006; **16**: 1139–46.
36 Srivastava A, Pastor-Pareja JC, Igaki T, Pagliarini R, Xu T. Basement membrane remodeling is essential for Drosophila disc eversion and tumor invasion. *Proc Natl Acad Sci USA* 2007; **104**: 2721–6.
37 Huh JR, Guo M, Hay BA. Compensatory proliferation induced by cell death in the Drosophila Wing Disc requires activity of the apical cell death Caspase Dronc in a nonapoptotic role. *Curr Biol* 2004; **14**: 1262–6.
38 Pérez-Garijo A, Martín FA, Morata G. Caspase inhibition during apoptosis causes abnormal signalling and developmental aberrations in Drosophila. *Development* 2004; **131**: 5591–8.
39 Ryoo HD, Gorenc T, Steller H. Apoptotic cells can induce compensatory cell proliferation through the JNK and the Wingless signaling pathways. *Dev Cell* 2004; **7**: 491–501.
40 Shlevkov E, Morata G. A dp53/JNK-dependant feedback amplification loop is essential for the apoptotic response to stress in Drosophila. *Cell Death Differ* 2012; **19**: 451–60.
41 Dekanty A, Barrio L, Muzzopappa M, Auer H, Milán M. Aneuploidy-induced delaminating cells drive tumorigenesis in Drosophila epithelia. *Proc Natl Acad Sci USA* 2012; **109**: 20549–54.
42 Pérez-Garijo A, Fuchs Y, Steller H. Apoptotic cells can induce non-autonomous apoptosis through the TNF pathway. *eLife* 2013; **2**: e01004.
43 Martin-Belmonte F, Perez-Moreno M. Epithelial cell polarity, stem cells and cancer. *Nat Rev Cancer* 2012; **12**: 23–38.
44 Igaki T, Pastor-Pareja JC, Aonuma H, Miura M, Xu T. Intrinsic tumor suppression and epithelial maintenance by endocytic activation of Eiger/TNF signaling in Drosophila. *Dev Cell* 2009; **16**: 458–65.

45 Chen CL, Schroeder MC, Kango-Singh M, Tao C, Halder G. Tumor suppression by cell competition through regulation of the Hippo pathway. *Proc Natl Acad Sci USA* 2012; **109**: 484–9.
46 Schroeder MC, Chen CL, Gajewski K, Halder G. A non-cell-autonomous tumor suppressor role for Stat in eliminating oncogenic scribble cells. *Oncogene* 2013; **32**: 4471–9.
47 Brumby AM, Richardson HE. scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. *EMBO J* 2003; **22**: 5769–79.
48 Brandon M, Baldi P, Wallace DC. Mitochondrial mutations in cancer. *Oncogene* 2006; **25**: 4647–62.
49 Ohsawa S, Sato Y, Enomoto M, Nakamura M, Betsumiya A, Igaki T. Mitochondrial defect drives non-autonomous tumour progression through Hippo signalling in Drosophila. *Nature* 2012; **490**: 547–51.
50 Nakamura M, Ohsawa S, Igaki T. Mitochondrial defects trigger proliferation of neighbouring cells via a senescence-associated secretory phenotype in Drosophila. *Nat Commun* 2014; **5**: 5264.
51 Ohsawa S, Takemoto D, Igaki T. Dissecting tumour heterogeneity in flies: genetic basis of interclonal oncogenic cooperation. *J Biochem* 2014; **156**: 129–36.
52 Coppé J-P, Desprez P-Y, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol* 2010; **5**: 99–118.
53 Chabu C, Xu T. Oncogenic Ras stimulates Eiger/TNF exocytosis to promote growth. *Development* 2014; **141**: 4729–39.
54 Moreno E. Is cell competition relevant to cancer? *Nat Rev Cancer* 2008; **8**: 141–7.
55 Sun G, Irvine KD. Ajuba family proteins link JNK to Hippo signaling. *Sci Signal* 2013; **6**: ra81.
56 Enomoto M, Kizawa D, Ohsawa S, Igaki T. JNK signaling is converted from anti- to pro-tumor pathway by Ras-mediated switch of Warts activity. *Dev Biol* 2015; **403**: 162–71.
57 Harvey KF, Zhang X, Thomas DM. The Hippo pathway and human cancer. *Nat Rev Cancer* 2013; **13**: 246–57.
58 Lee N, Maurange C, Ringrose L, Paro R. Suppression of Polycomb group proteins by JNK signalling induces transdetermination in Drosophila imaginal discs. *Nature* 2005; **438**: 234–7.
59 Classen A-K, Bunker BD, Harvey KF, Vaccari T, Bilder D. A tumor suppressor activity of Drosophila Polycomb genes mediated by JAK-STAT signaling. *Nat Genet* 2009; **41**: 1150–5.
60 Feng S, Huang J, Wang J. Loss of the Polycomb group gene polyhomeotic induces non-autonomous cell overproliferation. *EMBO Rep* 2010; **12**: 157–63.
61 Chen F. JNK-induced apoptosis, compensatory growth, and cancer stem cells. *Cancer Res* 2012; **72**: 379–86.
62 Tyler DM, Li W, Zhuo N, Pellock B, Baker NE. Genes affecting cell competition in Drosophila. *Genetics* 2007; **175**: 643–57.
63 Chen C-L, Gajewski KM, Hamaratoglu F *et al.* The apical-basal cell polarity determinant Crumbs regulates Hippo signaling in Drosophila. *Proc Natl Acad Sci USA* 2010; **107**: 15810–5.
64 Ling C, Zheng Y, Yin F *et al.* The apical transmembrane protein Crumbs functions as a tumor suppressor that regulates Hippo signaling by binding to Expanded. *Proc Natl Acad Sci USA* 2010; **107**: 10532–7.
65 Robinson BS, Huang J, Hong Y, Moberg KH. Crumbs regulates Salvador/Warts/Hippo signaling in Drosophila via the FERM-domain protein expanded. *Curr Biol* 2010; **20**: 582–90.
66 Ribeiro P, Holder M, Frith D, Snijders AP, Tapon N. Crumbs promotes expanded recognition and degradation by the SCFSlimb/β-TrCP ubiquitin ligase. *Proc Natl Acad Sci USA* 2014; **111**: E1980–9.
67 Bilder D, Perrimon N. Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. *Nature* 2000; **403**: 676–80.
68 Hafezi Y, Bosch JA, Hariharan IK. Differences in levels of the transmembrane protein Crumbs can influence cell survival at clonal boundaries. *Dev Biol* 2012; **368**: 358–69.
69 Andersen DS, Colombani J, Palmerini V *et al.* The Drosophila TNF receptor Grindelwald couples loss of cell polarity and neoplastic growth. *Nature* 2015; **522**: 482–6.
70 Genevet A, Polesello C, Blight K *et al.* The Hippo pathway regulates apical-domain size independently of its growth-control function. *J Cell Sci* 2009; **122**: 2360–70.
71 Hamaratoglu F, Gajewski K, Sansores-Garcia L, Morrison C, Tao C, Halder G. The Hippo tumor-suppressor pathway regulates apical-domain size in parallel to tissue growth. *J Cell Sci* 2009; **122**: 2351–9.
72 Bondar T, Medzhitov R. p53-mediated hematopoietic stem and progenitor cell competition. *Cell Stem Cell* 2010; **6**: 309–22.
73 Dejosez M, Ura H, Brandt VL, Zwaka TP. Safeguards for cell cooperation in mouse embryogenesis shown by genome-wide cheater screen. *Science* 2013; **341**: 1511–4.
74 de la Cova C, Senoo-Matsuda N, Ziosi M *et al.* Supercompetitor status of Drosophila Myc cells requires p53 as a fitness sensor to reprogram metabolism and promote viability. *Cell Metab* 2014; **19**: 470–83.
75 Gowda PS, Zhou F, Chadwell LV, McEwen DG. p53 binding prevents phosphatase-mediated inactivation of Diphosphorylated c-Jun N-terminal kinase. *J Biol Chem* 2012; **287**: 17554–67.
76 Ruland J, Sirard C, Elia A *et al.* p53 Accumulation, defective cell proliferation, and early embryonic lethality in mice lacking tsg101. *Proc Natl Acad Sci USA* 2001; **98**: 1859–64.
77 Lee TV, Ding T, Chen Z *et al.* The E1 ubiquitin-activating enzyme Uba1 in Drosophila controls apoptosis autonomously and tissue growth non-autonomously. *Development* 2008; **135**: 43–52.
78 Wells BS, Yoshida E, Johnston LA. Compensatory proliferation in Drosophila imaginal discs requires Dronc-dependent p53 activity. *Curr Biol* 2006; **16**: 1606–15.
79 Freed-Pastor WA, Prives C. Mutant p53: one name, many proteins. *Genes Dev* 2012; **26**: 1268–86.
80 Yoshimoto S, Loo TM, Atarashi K *et al.* Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 2013; **499**: 97–101.
81 Coppé J-P, Patil CK, Rodier F *et al.* Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol* 2008; **6**: e301.
82 Sun Y, Campisi J, Higano C *et al.* Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through WNT16B. *Nat Med* 2012; **18**: 1359–68.
83 Maeda S, Kamata H, Luo J-L, Leffert H, Karin M. IKKβ couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. *Cell* 2005; **121**: 977–90.
84 Sakurai T, He G, Matsuzawa A *et al.* Hepatocyte necrosis induced by oxidative stress and IL-1α release mediate carcinogen-induced compensatory proliferation and liver tumorigenesis. *Cancer Cell* 2008; **14**: 156–65.
85 Huang Q, Li F, Liu X *et al.* Caspase 3-mediated stimulation of tumor cell repopulation during cancer radiotherapy. *Nat Med* 2011; **17**: 860–6.
86 Kurtova AV, Xiao J, Mo Q *et al.* Blocking PGE2-induced tumour repopulation abrogates bladder cancer chemoresistance. *Nature* 2015; **517**: 209–13.
87 Clevers H. The cancer stem cell: premises, promises and challenges. *Nat Med* 2011; **17**: 313–9.
88 Iliopoulos D, Hirsch HA, Struhl K. An epigenetic switch involving NF-κB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell* 2009; **139**: 693–706.
89 Iliopoulos D, Hirsch HA, Wang G, Struhl K. Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion. *Proc Natl Acad Sci USA* 2011; **108**: 1397–402.