Interaction between \textit{Ras}^{V12} and \textit{scribble} clones induces tumour growth and invasion

Ming Wu\textsuperscript{*}, José Carlos Pastor-Pareja\textsuperscript{*}, and Tian Xu
Howard Hughes Medical Institute, Department of Genetics, Yale University School of Medicine, 295 Congress Avenue, New Haven CT 06519, USA

Abstract

Human tumours exhibit a large degree of cellular and genetic heterogeneity. Complex cell interactions in the tumour and its microenvironment are thought to play a significant role in tumourigenesis and cancer progression. It is also known that cooperation between oncogenic genetic lesions is required for tumour development. However, it is not known how cell interactions contribute to oncogenic cooperation. The genetic techniques available in the fruit fly \textit{Drosophila melanogaster} allow analysis of the behavior of cells with distinct mutations, giving this model organism a privileged position to study cell interactions and oncogenic cooperation. In \textit{Drosophila} eye-antennal discs, cooperation between the oncogenic protein \textit{Ras}^{V12} and loss-of-function mutations in the conserved tumour suppressor \textit{scribble} \cite{6,7} gives rise to metastatic tumours that display many characteristics observed in human cancers \cite{8-11}. Here we show that clones of cells bearing different mutations can cooperate to promote tumour growth and invasion in \textit{Drosophila}. We found that the \textit{Ras}^{V12} and \textit{scrib}\textsuperscript{−} mutations can also cause tumours when they affect different adjacent epithelial cells. We show that this interaction between \textit{Ras}^{V12} and \textit{scrib}\textsuperscript{−} clones involves JNK signalling propagation and JNK-induced upregulation of JAK/STAT-activating cytokines, a compensatory growth mechanism for tissue homeostasis. The development of \textit{Ras}^{V12} tumours can also be triggered by tissue damage, a stress condition that activates JNK signalling. Given the conservation of the pathways examined here, similar cooperative mechanisms could play a role in the development of human cancers.

Keywords

Interclonal oncogenic cooperation; Ras; Cell Polarity Mutants; JNK-induced Cytokines; JAK/STAT

Authors may view, print, copy, download and text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use: http://www.nature.com/authors/editorial_policies/license.html#terms

Correspondence and requests for materials should be addressed to T.X. (tian.xu@yale.edu).

Equal contribution, in random order

Tian Xu Yale University School of Medicine Department of Genetics 295 Congress Ave New Haven, CT 06519 USA tian.xu@yale.edu Telephone: (203)737-2623 Fax: (203)737-1762

Author contributions M.W., J.C.P.-P. and T.X designed research, M.W. and J.C.P.-P. performed experiments and analyzed the data. M.W., J.C.P.-P. and T.X wrote the manuscript.

Competing interests statement The authors declare that they have no competing financial interest.
Clones of mutant cells marked with green fluorescent protein (GFP) can be generated in the eye-antenna imaginal discs of Drosophila larvae by mitotic recombination. Clones expressing the oncogenic protein Ras\textsuperscript{V12} moderately overgrow 12 (Fig. 1a, b). Clones mutant for scrib lose apico-basal polarity and die 6,13 (Fig. 1c). In contrast, scrib clones simultaneously expressing Ras\textsuperscript{V12} grow into large metastatic tumours (Fig. 1d) 8. To better understand cooperation between these two mutations, we produced animals in which cell division after a mitotic recombination event creates two daughter cells, one expressing Ras\textsuperscript{V12} and the other mutant for scrib. Discs containing adjacent Ras\textsuperscript{V12} (GFP-positive) and scrib\textsuperscript{−} clones developed into large tumours, capable of invading the ventral nerve cord (VNC) (Fig. 1e). This shows that Ras\textsuperscript{V12} and scrib cooperate for tumour induction also when they occur in different cells. We will refer to these tumours as Ras\textsuperscript{V12}/scrib\textsuperscript{−} tumours, to denote interclonal oncogenic cooperation and distinguish them from Ras\textsuperscript{V12}/scrib\textsuperscript{−} tumours, in which cooperation occurs in the same cells intraclonally.

In the late stages of the development of Ras\textsuperscript{V12}/scrib\textsuperscript{−} tumours, most cells in the tumour mass are Ras\textsuperscript{V12} cells (Fig. 1h, i). scrib\textsuperscript{−} cells, as well as residual wild-type cells, are almost completely absent from the tissue, similar to the absence of wild-type cells in late Ras\textsuperscript{V12}/scrib\textsuperscript{−} tumours (Fig. 1f, g). To test the possibility that Ras\textsuperscript{V12}/scrib\textsuperscript{−} tumours are caused by unopposed growth of Ras\textsuperscript{V12} cells, we examined eye-antennal discs where all cells expressed Ras\textsuperscript{V12}. Dramatic overgrowth or invasion did not occur (Supplementary Fig. 1), showing that interaction between Ras\textsuperscript{V12} and scrib\textsuperscript{−} cells is required for tumour development. Interclonal cooperation between Ras\textsuperscript{V12} and lethal giant larvae (lgl) also produced tumours (Supplementary Fig. 2), suggesting that other polarity mutations can cooperate interclonally with Ras\textsuperscript{V12}. Intrigued by these findings, we decided to investigate the mechanisms underlying non-autonomous oncogenic cooperation and sustained growth in Ras\textsuperscript{V12}/scrib\textsuperscript{−} tumours.

JAK/STAT signaling promotes cell proliferation in different contexts in mammals and flies 14, including the overgrowth caused by mutation of several tumour suppressors 15. In a cDNA microarray analysis of Ras\textsuperscript{V12}/scrib\textsuperscript{−} tumours, we discovered upregulation of the unpaired genes (upd, upd2 and upd3; data not shown), which encode JAK/STAT-activating cytokines related to Interleukin 6 16-18. We confirmed the upregulation of the unpaired genes in Ras\textsuperscript{V12}/scrib\textsuperscript{−} and Ras\textsuperscript{V12}/scrib\textsuperscript{−} tumours by real-time RT-PCR (Fig. 2a). Furthermore, we observed elevated expression of the JAK/STAT reporter STAT-GFP 19 in both Ras\textsuperscript{V12}/scrib\textsuperscript{−} and Ras\textsuperscript{V12}/scrib\textsuperscript{−} tumours (Fig. 2b-e), thus correlating high expression of Upd cytokines with increased JAK/STAT activity.

To test the involvement of JAK/STAT signaling in the growth of Ras\textsuperscript{V12}/scrib\textsuperscript{−} and Ras\textsuperscript{V12}/scrib\textsuperscript{−} tumours, we used a dominant negative form of the JAK/STAT receptor Domeless (Dome\textsuperscript{DN}) 20. Expression of Dome\textsuperscript{DN} achieved suppression of overgrowth and invasion of the VNC in Ras\textsuperscript{V12}/scrib\textsuperscript{−} tumours (Fig. 2f). Also Ras\textsuperscript{V12}/scrib\textsuperscript{−} tumours were suppressed by expression of Dome\textsuperscript{DN} in Ras\textsuperscript{V12} cells (Fig. 2g). A loss-of-function mutation in stat\textsuperscript{92E}, encoding the Drosophila STAT transcriptional activator, reduced growth and invasiveness of Ras\textsuperscript{V12}/scrib\textsuperscript{−} and Ras\textsuperscript{V12}/scrib\textsuperscript{−} tumours (Supplementary Fig. 3). From these experiments, we conclude that JAK/STAT signaling is required for the development of Ras\textsuperscript{V12}/scrib\textsuperscript{−} and Ras\textsuperscript{V12}/scrib\textsuperscript{−} tumours.
The suppression of RasV12scrib− and RasV12scrib− tumours by reducing JAK/STAT activity in RasV12 cells points to cooperation between Ras and JAK/STAT signaling as a cause of tumour growth. To confirm this, we generated clones of cells co-expressing Upd cytokines and RasV12. While Upd overexpression in wild-type cells (Fig. 2h), in scrib− cells or in wild-type cells adjacent to scrib− cells (Supplementary Fig. 4) did not cause tumours, co-expression of RasV12 and Upd produced large invasive tumours (Fig. 2i). Similar results were obtained co-expressing RasV12 and Upd2 (Fig. 2j), whereas co-expression of RasV12 and Upd3 caused smaller, non-invasive tumours (Fig. 2k). Finally, RasV12Upd Upd2 tumours were larger than RasV12Upd and RasV12Upd2 tumours (Fig. 2l), suggesting an additive effect of the expression of different Upd cytokines (see also Supplementary Fig. 5).

Prevention of actual cell death in cells apoptotically stimulated has been shown to potently promote overgrowth 21. To assess a possible involvement of apoptosis prevention in the synergy between Ras and JAK/STAT signaling, we coexpressed the apoptosis inhibitor p35 with RasV12 or Upd. Neither conditions produced tumours (Supplementary Fig. 6), suggesting that cooperation between Ras and JAK/STAT involves a mechanism other than apoptosis prevention. RasV12Upd tumours were suppressed by expression of DomeDN (Fig. 2m), thus confirming that their development requires JAK/STAT activity. In all, both loss- and gain-of-function experiments lead us to conclude that Ras and JAK/STAT signaling exhibit a strong synergistic tumour-promoting interaction, responsible for the development of RasV12scrib− and RasV12scrib− tumours.

Having established the involvement of JAK/STAT signaling in the growth of RasV12scrib− and RasV12scrib− tumours, we decided to investigate how expression of the Upd cytokines is upregulated. We previously showed that expression of the unpaired genes is elevated in wounds in a JNK-dependent manner 22. It has been shown as well that JNK signaling can induce non-autonomous overgrowth 23,24, and that JNK signaling is upregulated in scrib− clones 13,25 and scrib− discs 22, which develop as tumours in scrib− larvae 6. To test the possibility that JNK activation causes ectopic JAK/STAT signaling in scrib− cells, we monitored STAT-GFP expression in discs double mutant for scrib and hep, coding for the Drosophila JNK-kinase Hemipterous. In these discs, STAT-GFP expression was reduced and overgrowth suppressed (Fig. 3a-c), showing that JAK/STAT elevation in scrib− cells depends on JNK activity.

The induction of Upd cytokines by JNK in scrib− cells can explain the growth of RasV12scrib− tumours, placing JAK-STAT signaling downstream of JNK. In support of this, a dominant negative form of the Jun-kinase Basket (BskDN) suppressed RasV12scrib− tumours 9 (Fig. 3d, e), but not RasV12Upd tumours (Fig. 3f, g). In the case of RasV12scrib− tumours, few scrib− cells remain in the tissue at late stages (Fig. 1i). Therefore, Upd induction in scrib− cells cannot fully account for tumour development. Indeed, expression of BskDN in RasV12 cells partially suppressed the growth of RasV12scrib− tumours (Fig. 3h, i) and expression of the unpaired genes (Fig. 3j). This shows that in RasV12scrib− tumours expression of Upd cytokines downstream of JNK signaling also occurs in RasV12 cells.

scrib− clones cause JNK activation both autonomously and non-autonomously 25. Furthermore, in wing discs, wounding induces JNK activation away from the site of

Nature. Author manuscript; available in PMC 2010 July 28.
wounding 22,26, suggesting that JNK activity can propagate. To investigate this, we wounded wing discs and examined the expression of puckered (puc), a JNK downstream gene encoding a JNK-phosphatase that negatively regulates the pathway 27. When wounds were induced in the anterior or posterior wing regions, JNK activation, revealed by puc-lacZ expression, was observed across the disc in the opposite compartment (Fig. 3k). In contrast, overexpression of Puc in a central stripe of cells prevented expansion of JNK to the opposite compartment (Fig. 3l, Supplemental Fig. 7). This indicates that JNK activity propagates through a feed-forward loop and, together with previous findings, suggests that in RasV12//scrib− tumours, scrib− cells trigger JNK activation and that this activation propagates to adjacent RasV12 cells. JNK-dependent upregulation of Upd cytokines in RasV12 cells, thus, can sustain tumour growth when the original source of JNK activity, the scrib− cells, is no longer present.

The previous experiments reveal a central role for JNK in the cooperation of RasV12 and scrib−. Since both wounds and scrib− induce JNK activation, we tested the possibility that tissue damage could cooperate with RasV12 to promote tumour overgrowth. We wounded larval right wing discs and examined them 48 hours later. In wild-type discs, compared to the unwounded left disc, woundinduced in size reduction (Fig 4a, b; Supplementary Fig. 8; wounded/unwounded size ratio ±5SD=0.70±0.18). In contrast, wounding of RasV12-expressing discs caused a marked increase in RasV12-induced overgrowth (Fig. 4c, d; Supplementary Fig. 8; 1.46±0.31). No metastasis was detected in this experiment (not shown). Finally, the wounded/unwounded ratio in p35-expressing discs (1.09±0.14, Supplementary Fig. 8) shows that apoptosis prevention by RasV12 cannot completely account for its cooperation with mechanically-induced damage.

The fact that both scrib− clones and tissue damage induce overgrowth of RasV12 tissue suggests that compensatory proliferation in response to scrib− cells could underlie cooperation in RasV12//scrib− tumours. To test this, we studied the effect of confronting scrib− cells with cells mutant for stat92E. When scrib− clones are generated in eye-antennal discs, scrib− cells in the adult eye are mostly absent 13 and the eye appears normal in size (Fig. 4e, f). When stat92E− cells confront scrib− cells, in contrast, the eye is greatly reduced (Fig. 4g, h; Supplemental Fig. 9), showing that stat92E− cells cannot compensate for the loss of scrib− cells. These data indicate a role for JAK/STAT signaling in tissue homeostasis through compensatory proliferation (see also Supplemental Figs. 9 and 10). Therefore, a mechanism to ensure recovery after damage explains the development of RasV12 scrib− and RasV12//scrib− tumours and can mediate interclonal oncogenic cooperation (Fig. 4i).

We have used Drosophila to investigate how oncogenic cooperation between different cells can promote tumour growth and invasion. Our experiments, addressed to understanding interclonal cooperation in RasV12//scrib− tumours, uncovered a two-tier mechanism by which scrib− cells promote neoplastic development of RasV12 cells: (1) propagation of stress-induced JNK activity from scrib− cells to RasV12 cells and (2) expression of the JAK/STAT-activating Unpaired cytokines downstream of JNK. Our findings, therefore, highlight the importance of cell interactions in oncogenic cooperation and tumour development. We also show that stress-induced JNK signaling and epigenetic factors such as tissue damage can contribute to tumour development in flies. Interestingly, tissue damage caused by
conditions such as chronic inflammation has been linked to tumourigenesis in humans 28,29. Furthermore, expression of the Unpaired cytokines promotes tumour growth (this study) as well as an antitumoural immune response 22, which parallels the situation in mice and humans 30. Future research into phenomena such as compensatory growth and interclonal cooperation in Drosophila will provide valuable insights into the biology of cancer.

METHODS SUMMARY

Clones of mutant cells in the eye-antennal discs were generated as previously described 8. Detailed genotypes of the experimental individuals are described in Supplemental Information. The following antibodies and dyes were used: mouse monoclonal anti-βgal (1:500, Sigma), goat Alexa-488-conjugated anti-mouse IgG (1:200, Molecular Probes), phalloidin Texas Red (Molecular Probes). Wounds were performed with forceps in mid third-instar larvae as described previously 22.

METHODS

Strains and culture

Cultures were maintained at 25°C on standard medium. Whenever staging of larvae was required, parental flies were placed in a fresh culture vial and left there to lay eggs for 1 day; we considered the time of removing the flies from the vial 12h (±12) AEL (after egg laying). The following strains were used in this study:

- y w; FRT82B
- y w; FRT82B, scrib\(^1\)/TM6B
- w; UAS-Ras\(^{V12}\) (II)
- w; UAS-Ras\(^{V12}\) (III)
- y w, ey-FLP1; act>y>Gal4, UAS-GFP.S65T; FRT82B, tub-Gal80
- w; FRT82B, tub-Gal80, scrib\(^1\)/TM6B
- y w, ey-FLP1; act>y>Gal4, UAS-myrRFP; FRT82B, tub-Gal80
- tub-Gal80, FRT19A; eyFLP5, act>y>Gal4, UAS-GFP
- y w, ey-FLP1; tub-Gal80, FRT40A; act>y>Gal4, UAS-GFP.S65T
- y w, ey-FLP1; FRT82B, ubi-GFP
- w; 10XSTAT-GFP.1 (II)
- w; ey-FLP6 (III)
- w; UAS-upd (II)
- w; UAS-upd2 (III)
- w; UAS-upd3 (II)
- w; UAS-upd-IR(R-1) (III)
w; UAS-domeΔcyt1.1
w; UAS-domeΔcyt2.1 (II)
y w upd2Δ3-62
w; UAS-bskDN
w; UAS-bskDN (III)
y w hep75/FM7i,act-GFP
w; ptc-GAL
w; UAS-myRFP (II)
pucE69-lacZ,ry/TM3,Sb
w; UAS-puc (III)
w; nub-GAL4.K
w; UAS-p35 (II)
w; UAS-p35 (III)
w; FRT82B,stat92E06346/TM6B
w; FRT82B,stat92E397/TM3,Sb
w; FRT82B,stat92E85C9/TM3,Sb
w; FRT82B,abi-GFP,RpS3Plac92/TM6C, Sb
y w; ey-GAL4,UAS-Flp; FRT82B,GMR-hid,CL3R/TM6B.

Real-time RT-PCR
Total RNA from wild-type and tumour discs was isolated using Trizol (Invitrogen). cDNA was synthesized from 2 μg of RNA with the SuperScriptIII First-Strand Synthesis System (Invitrogen). Resulting DNA was subjected to real-time PCR with SYBR green fast kit (Applied Biosystems) according to manufacturers instructions. Relative gene expression was compared to rp49 as an internal control. Three experiments for each condition were averaged. The following primers were used: udp: 5′ TCCACACGCAACACTACAAGTTCTC 3′ and 5′ CCAGCGCTTTAGGGCAATC 3′; upd2: 5′
AGTGCGGTGAAGCTAAAGACTTG 3′ and 5′ GCCCGTCCCAGATATGAGAA 3′; upd3: 5′ TGCCCCGTCCTGAATCTCACT 3′ and 5′ GTGAAGCGCCCCACGTAA 3′; rp49: 5′ GGCCCAAGATCGTGAAGAAG 3′ and 5′ ATTTGTGCGACAGCTTAGATATC 3′.

Stainings and imaging
Images documenting tumour size and VNC invasion were taken in a Leica MZ FLIII fluorescence stereomicroscope with an Optronics Magnafire camera. Antibody staining was performed according to standard procedures for imaginal discs. The following antibodies and dyes were used: mouse monoclonal anti-βgal (1:500, Sigma), goat Alexa-488-conjugated anti-mouse IgG (1:200, Molecular Probes), phalloidin Texas Red (Molecular
Probes). Samples imaged through confocal microscopy were mounted in DAPI-Vectashield (Vector Labs). Confocal images were taken in a Zeiss LSM510 Meta confocal microscope. Adult eyes were imaged with a Leica DFC300FX camera in a Leica MZ FLIII stereomicroscope. Measurements of wing blade size were performed from confocal pictures using NIH Image-J software. Adult eye size measurements were performed for each genotype from pictures of at least ten female flies collected 1-3 days after hatching using NIH Image-J software.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgements**

We thank E. Bach, D. Harrison, J. Castelli-Gair Hombria, M. Zeidler, T. Adachi-Yamada, M. Mlodzik, E. Matunis, D. Montell, H. Agaisse, the Bloomington Stock Center and the National Institute of Genetics (Kyoto) for fly strains, and T. Ni, S. Landrette and M. Rojas for comments. R. Pagliarini and S. Landrette helped with microarray analysis and RT-PCR. We thank T. Igaki for discussing the manuscript and providing FRT82B, tub-Gal80,scrib1/TM6B flies. We apologize for not being able to cite all the relevant references. M.W. is a Yale predoctoral fellow. J.C.P.-P. was funded by a Spanish Ministry of Education postdoctoral fellowship. This work was supported by a grant from NIH/NCI to T.X. T.X. is a Howard Hughes Medical Institute Investigator.

**REFERENCES**

1. Heppner GH. Tumour heterogeneity. Cancer Res. 1984; 44:2259–2265. [PubMed: 6372991]
2. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100:57–70. [PubMed: 10647931]
3. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell. 1996; 87:159–170. [PubMed: 8618899]
4. Xu T, Rubin GM. Analysis of genetic mosaics in developing and adult Drosophila tissues. Development. 1993; 117:1223–1237. [PubMed: 8404527]
5. Barbasid M. ras genes. Annual review of biochemistry. 1987; 56:779–827.
6. Bilder D, Perrimon N. Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. Nature. 2000; 403:676–680. [PubMed: 10688207]
7. Zhan L, et al. Deregulation of scribble promotes mammary tumourigenesis and reveals a role for cell polarity in carcinoma. Cell. 2008; 135:865–878. [PubMed: 19041750]
8. Pagliarini RA, Xu T. A genetic screen in Drosophila for metastatic behavior. Science. 2003; 302:1227–1231. [PubMed: 14551319]
9. Igaki T, Pagliarini RA, Xu T. Loss of cell polarity drives tumour growth and invasion through JNK activation in Drosophila. Curr Biol. 2006; 16:1139–1146. [PubMed: 16753569]
10. Uhlira M, Bohmann D. JNK- and Fos-regulated Mmp1 expression cooperates with Ras to induce invasive tumours in Drosophila. Embo J. 2006; 25:5294–5304. [PubMed: 17082773]
11. Srivastava A, Pastor-Pareja JC, Igaki T, Pagliarini R, Xu T. Basement membrane remodeling is essential for Drosophila disc eversion and tumour invasion. Proc Natl Acad Sci U S A. 2007; 104:2721–2726. [PubMed: 17301221]
12. Karim FD, Rubin GM. Ectopic expression of activated Ras induces hyperplastic growth and increased cell death in Drosophila imaginal tissues. Development. 1998; 125:1–9. [PubMed: 9389658]
13. Brumby AM, Richardson HE. scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. Embo J. 2003; 22:5769–5779. [PubMed: 14592975]
14. Zeidler MP, Bach EA, Perrimon N. The roles of the Drosophila JAK/STAT pathway. Oncogene. 2000; 19:2598–2606. [PubMed: 10851058]
15. Hariharan IK, Bilder D. Regulation of imaginal disc growth by tumour-suppressor genes in Drosophila. Annual review of genetics. 2006; 40:335–361.

*Nature. Author manuscript; available in PMC 2010 July 28.*
16. Harrison DA, McCoon PE, Binari R, Gilman M, Perrimon N. Drosophila unpaired encodes a secreted protein that activates the JAK signaling pathway. Genes Dev. 1998; 12:3252–3263. [PubMed: 9784499]

17. Agaisse H, Petersen UM, Boutros M, Mathey-Prevot B, Perrimon N. Signaling role of hemocytes in Drosophila JAK/STAT-dependent response to septic injury. Dev Cell. 2003; 5:441–450. [PubMed: 12967563]

18. Hombria JC, Brown S, Hader S, Zeidler MP. Characterisation of Upd2, a Drosophila JAK/STAT pathway ligand. Dev Biol. 2005; 288:420–433. [PubMed: 16277982]

19. Bach EA, et al. GFP reporters detect the activation of the Drosophila JAK/STAT pathway in vivo. Gene Expr Patterns. 2007; 7:323–331. [PubMed: 17008134]

20. Brown S, Hu N, Hombria JC. Identification of the first invertebrate interleukin JAK/STAT receptor, the Drosophila gene domeless. Curr Biol. 2001; 11:1700–1705. [PubMed: 11696329]

21. Martin FA, Perez-Garijo A, Morata G. Apoptosis in Drosophila: compensatory proliferation and undead cells. Int J Dev Biol. 2009; 53:1341–1347. [PubMed: 19247932]

22. Pastor-Pareja JC, Wu M, Xu T. An innate immune response of blood cells to tumours and tissue damage in Drosophila. Disease models & mechanisms. 2008; 1:144–154. [PubMed: 19048077]

23. 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. [PubMed: 15469838]

24. Uhlirova M, Jasper H, Bohmann D. Non-cell-autonomous induction of tissue overgrowth by JNK/Ras cooperation in a Drosophila tumour model. Proc Natl Acad Sci U S A. 2005; 102:13123–13128. [PubMed: 16150723]

25. Igaki T, Pastor-Pareja JC, Aonuma H, Miura M, Xu T. Intrinsic tumour suppression and epithelial maintenance by endocytic activation of Eiger/TNF signaling in Drosophila. Dev Cell. 2009; 16:458–465. [PubMed: 19289090]

26. Bosch M, Serras F, Martin-Blanco E, Baguna J. JNK signaling pathway required for wound healing in regenerating Drosophila wing imaginal discs. Dev Biol. 2005; 280:73–86. [PubMed: 15766749]

27. Martin-Blanco E, et al. puckered encodes a phosphatase that mediates a feedback loop regulating JNK activity during dorsal closure in Drosophila. Genes Dev. 1998; 12:557–570. [PubMed: 9472024]

28. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. The New England Journal of Medicine. 1986; 315:1650–1659. [PubMed: 3537791]

29. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001; 357:539–545. [PubMed: 11229684]

30. de Visser KE, Echten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer. 2006; 6:24–37. [PubMed: 16397525]
Figure 1. Interclonal cooperation between Ras\textsuperscript{V12} and scrib\textsuperscript{−} causes tumours

a-e. Clones of cells marked with GFP in the eye-antennal discs of third-instar larvae. Upper subpanels show the cephalic complex (CC), consisting of eye-antennal discs (EA), brain (B) and ventral nerve cord (VNC). Lower subpanels show the dissected VNC. Compared to wild-type clones (a), Ras\textsuperscript{V12} clones overgrow moderately (b). scrib\textsuperscript{−} clones are eliminated from the tissue (c). Double mutant Ras\textsuperscript{V12},scrib\textsuperscript{−} clones (d, intracloval cooperation), as well as Ras\textsuperscript{V12} clones confronted with scrib\textsuperscript{−} clones (e, interclonal cooperation), cause tumours that overgrow and invade the VNC. f-i, Confocal sections of the inner tumour mass in Ras\textsuperscript{V12},scrib\textsuperscript{−} (f, g) and Ras\textsuperscript{V12}//scrib\textsuperscript{−} tumours (h, i) at day 6 and 14 after egg laying (AEL). Non-Ras\textsuperscript{V12} cells (absence of GFP) are progressively eliminated from the tissue. Cell nuclei labeled with DAPI. Yellow arrowheads point to scattered remaining GFP-negative cells (insets in g and i).
Figure 2. Synergy between Ras and JAK/STAT signaling promotes growth and invasion in RasV12scrib− and RasV12//scrib− tumours

a, Quantification by real-time RT-PCR of expression of the upd genes, encoding the JAK/STAT-activating cytokines Upd, Upd2 and Upd3, in eye-antennal discs containing wild-type clones, RasV12-expressing clones, scrib− clones (day 6 AEL), RasV12scrib− tumours and RasV12//scrib− tumours (day 10 AEL). Expression is normalized to the housekeeping gene rp49. Error bars depict 95% confidence intervals (1.96 × s.e.m., n=3). b-e, Expression of the JAK/STAT reporter STAT-GFP (green) in day 6 AEL eye-antennal discs containing wild-type clones (b, red), RasV12 clones (c), RasV12scrib− (d) and RasV12//scrib− tumours (e). f, g, Suppression of RasV12scrib− (f) and RasV12//scrib− (g) tumours by expression of a dominant negative form of the JAK/STAT receptor Domeless (DomeDN). h, Clones overexpressing Upd. i-l, Tumours caused by RasV12 clones co-overexpressing Upd (i), Upd2 (j), Upd3 (k) and both Upd and Upd2 (l). m, RasV12Upd tumours are suppressed by DomeDN.
Figure 3. JNK signaling drives oncogenic cooperation upstream of JAK/STAT

a-c. STAT-GFP expression in wing discs of wild-type larvae (a) and scrib- larvae heterozygous (b) or hemizygous (male, c) for the JNK-kinase loss-of-function mutation hep^75. Overgrowth and STAT-GFP upregulation are suppressed by hep^75. Arrowheads point to normal STAT-GFP expression in the wing hinge. Discs stained with phalloidin (red).

d-g. Expression of a dominant negative form of the Jun-kinase Basket (Bsk^{DN}) suppresses Ras^{V12}_{scrib-} tumours (d, e), but not Ras^{V12}_{Upd} tumours (f, g). h, i. Expression of Bsk^{DN} in Ras^{V12} cells partially suppresses Ras^{V12}_{scrib-} tumours. j. Quantification by real-time RT-PCR of expression of the upd genes in Ras^{V12}_{scrib-} and Ras^{V12}_{Bsk^{DN}/scrib-} tumours (day 6 AEL). Error bars represent 95% confidence intervals (n=3).

k, l. Propagation of JNK activity (puc-lacZ reporter, green) into the posterior (P) compartment (arrowhead) in wing discs wounded in the anterior (A) compartment (asterisk) 24 hours after wounding. I, puc-lacZ expression in discs expressing the JNK-phosphatase Puc under control of ptc-GAL4 (red cells, expressing RFP), wounded as in k. Puc is both a downstream target and a negative regulator of JNK. Propagation of puc-lacZ expression into the P compartment is not observed (hollow arrowhead).
Figure 4. Tissue damage, compensatory growth and a model for interclonal oncogenic cooperation

a-d. Cooperation between Ras\textsuperscript{V12} and tissue damage. Right (wounded) and left (unwounded) wing discs of a wild-type larva (a, b) and a larva expressing Ras\textsuperscript{V12} under control of nub-GAL4 (c, d). Discs were wounded by repeated pinching and dissected 48h later. Expression of RFP driven by nub-GAL4 marks the wing blade region (red). Cell nuclei stained with DAPI (blue).

e-h. Requirement of JAK/STAT signaling in compensatory proliferation. Wild-type (e) and stat92E\textsuperscript{−} (g) clones in adult eyes, marked by absence of red pigment. In eyes containing scrib\textsuperscript{−} clones confronted with wild-type cells (f), scrib\textsuperscript{−} cells (red) are mostly absent and size of the eye is largely normal. In eyes containing scrib\textsuperscript{−} clones (red) confronted with stat92E\textsuperscript{−} cells (h), the size of the eye is reduced.

i. Model for the involvement of JNK and JAK/STAT signaling in intraclonal and interclonal cooperation between Ras\textsuperscript{V12} and scrib\textsuperscript{−}. See text for details.