Tumor-promoting function of apoptotic caspases by an amplification loop involving ROS, macrophages and JNK in Drosophila

Ernesto Perez
University of Massachusetts Medical School

Let us know how access to this document benefits you.
Follow this and additional works at: https://escholarship.umassmed.edu/oapubs

Part of the Cancer Biology Commons, and the Neoplasms Commons

Repository Citation
Perez E, Lindblad JL, Bergmann A. (2017). Tumor-promoting function of apoptotic caspases by an amplification loop involving ROS, macrophages and JNK in Drosophila. Open Access Publications by UMass Chan Authors. https://doi.org/10.7554/eLife.26747. Retrieved from https://escholarship.umassmed.edu/oapubs/3226

Creative Commons License
This work is licensed under a Creative Commons Attribution 4.0 License.
This material is brought to you by eScholarship@UMassChan. It has been accepted for inclusion in Open Access Publications by UMass Chan Authors by an authorized administrator of eScholarship@UMassChan. For more information, please contact Lisa.Palmer@umassmed.edu.
Tumor-promoting function of apoptotic caspases by an amplification loop involving ROS, macrophages and JNK in Drosophila

Ernesto Pérez †‡, Jillian L Lindblad †, Andreas Bergmann *

Department of Molecular, Cell and Cancer Biology, University of Massachusetts Medical School, Worcester, United States

Abstract Apoptosis and its molecular mediators, the caspases, have long been regarded as tumor suppressors and one hallmark of cancer is ‘Evading Apoptosis’. However, recent work has suggested that apoptotic caspases can also promote proliferation and tumor growth under certain conditions. How caspases promote proliferation and how cells are protected from the potentially harmful action of apoptotic caspases is largely unknown. Here, we show that although caspases are activated in a well-studied neoplastic tumor model in Drosophila, oncogenic mutations of the proto-oncogene Ras (RasV12) maintain tumorous cells in an ‘undead’-like condition and transform caspases from tumor suppressors into tumor promotors. Instead of killing cells, caspases now promote the generation of intra- and extracellular reactive oxygen species (ROS). One function of the ROS is the recruitment and activation of macrophage-like immune cells which in turn signal back to tumorous epithelial cells to activate oncogenic JNK signaling. JNK further promotes and amplifies caspase activity, thereby constituting a feedback amplification loop. Interfering with the amplification loop strongly reduces the neoplastic behavior of these cells and significantly improves organismal survival. In conclusion, RasV12-modified caspases initiate a feedback amplification loop involving tumorous epithelial cells and macrophage-like immune cells that is necessary for uncontrolled tumor growth and invasive behavior.

Introduction Larval imaginal discs in Drosophila are single-cell layered sacs of epithelial cells that develop into the adult appendages such as eyes and wings, and are frequently used as genetic models for growth control and tumor development. Maintenance of apical-basal polarity of epithelial cells is critical for suppression of neoplastic tumor development (Elsum et al., 2012; Bergstrahl and St Johnston, 2012; Martin-Belmonte and Perez-Moreno, 2011). Mutations in genes encoding components of the Scribble complex including scribble (scrib), lethal giant larvae (lgl) and discs large (dlg) disrupt apical-basal polarity in epithelial cells which can trigger malignant neoplastic tumor growth (Bergstrahl and St Johnston, 2012; Elsum et al., 2012; Bilder et al., 2000; Gateff, 1978; Bilder and Perrimon, 2000). Drosophila larvae entirely mutant for scrib fail to respond to stop signals of growth, fail to pupariate and continue to grow as larvae (Gateff, 1994; Wodarz, 2000). They die as giant larvae with severely overgrown imaginal discs.

However, scrib mutant cells (clones) in otherwise wild-type imaginal discs are eliminated by cell competition mediated by neighboring wild-type cells (Brumby and Richardson, 2003; Menéndez et al., 2010; Igaki et al., 2009; Uhlirova et al., 2005; Ohsawa et al., 2011; Leong et al., 2009; Chen et al., 2012; Vaughan and Igaki, 2016). Mechanistically, in response to...
cell competition, Eiger, the Tumor Necrosis Factor alpha (TNFα)-like ligand in Drosophila, triggers Jun N-terminal kinase (JNK) activation and apoptosis in scrib mutant cells (Igaki et al., 2009; Brumby and Richardson, 2003; Uhlirova et al., 2005; Cordero et al., 2010; Ohsawa et al., 2011; Leong et al., 2009; Igaki et al., 2006; Chen et al., 2012). This tumor-suppressing function is dependent on Eiger and JNK through induction of apoptosis. Inhibition of Eiger or JNK restores the growth potential of scrib mutant cells which can then form large tumor masses in imaginal discs (Brumby and Richardson, 2003; Igaki et al., 2009; Uhlirova et al., 2005; Chen et al., 2012).

However, if additional oncogenic mutations such as RasV12 are introduced into scrib mutant cells (referred to a scrib−/−RasV12), they can unleash their full malignant potential (Brumby and Richardson, 2003; Pagliarini and Xu, 2003). scrib−/− RasV12 mosaic eye/antennal imaginal discs display all neoplastic features observed in human tumors including unrestricted growth, failure to differentiate, tissue invasion and organisal lethality (Pagliarini and Xu, 2003; Brumby and Richardson, 2003). scrib−/− RasV12 clones occupy a large portion of the mosaic disc and trigger multi-layered overgrowth of the entire disc compared to wild-type controls (Figure 1H,I). scrib−/− RasV12 mutant cells also invade other tissues, most notably the ventral nerve cord (VNC) in the brain (Figure 1H,I) (Pagliarini and Xu, 2003). The scrib−/− RasV12 condition in ey-FLP-induced eye imaginal disc mosaics is 100% lethal. 95% of ey-FLP-induced scrib−/− RasV12 mosaic animals die as larvae; the remaining animals die during pupal stages.

Interestingly, RasV12 inhibits the apoptotic activity of JNK and converts the tumor-suppressor function of Eiger and JNK in scrib−/− cells into a tumor-promoting one in scrib−/− RasV12 cells (Enomoto et al., 2015; Cordero et al., 2010; Uhlirova et al., 2005; Igaki et al., 2006; Uhlirova and Bohmann, 2006). Therefore, the aggressive tumor growth of scrib−/− RasV12 mutant clones becomes dependent on Eiger and JNK (Igaki et al., 2006; Uhlirova and Bohmann, 2006; Brumby et al., 2011). Mechanistically, it is not understood how RasV12 promotes this oncogenic switch of Eiger and JNK.

Caspases are Cys-proteases that mediate the mechanistic events of apoptotic cell death (Shalini et al., 2015; Xu et al., 2009; Fuchs and Steller, 2011; Salvesen et al., 2016). They are synthesized aszymogens and depending on the length of their prodomains can be classified into initiator and effector caspases. Initiator caspases such as mammalian Caspase-9 or its Drosophila ortholog Dronc are controlled by upstream signaling events and when activated initiate apoptosis
Figure 1. Both intra- and extracellular ROS contribute to the strong neoplastic phenotype of scrib\textsuperscript{V12} mutant clones. Enlarged scrib\textsuperscript{V12} mutant clones significantly improves the pupariation rates of animals bearing scrib\textsuperscript{V12} mosaic eye/antennal imaginal discs. Expression of UAS-lacZ in scrib\textsuperscript{V12} clones as control has no effect on the pupariation rate. Pupariation rates were determined as the ratio of late stage mutant pupae vs total pupae.

DOI: https://doi.org/10.7554/eLife.26747.003

Figure 1 continued on next page
by activating effector caspases such as mammalian Caspase-3 or its Drosophila ortholog DrICE (Fuchs and Steller, 2011; Shalini et al., 2015; Salvesen et al., 2016). Caspases induce apoptosis of many cells to maintain homeostatic conditions, and are also thought to be critical for tumor suppression by eliminating malignant cells.

However, caspases can also have tumor-promoting roles, for example through apoptosis-induced proliferation (AiP), a caspase-driven process by which apoptotic cells produce mitogenic signals for proliferation of neighboring surviving cells (Mollereau et al., 2013) (reviewed in [Fogarty and Bergmann, 2017; Ryoo and Bergmann, 2012]). There are two types of AiP. During ‘genuine’ AiP, apoptotic cells release mitogenic factors before completing the apoptotic program. This type of AiP has been described for regeneration and wound healing both in vertebrates and invertebrates (Tseng et al., 2007; Fan and Bergmann, 2008; Chera et al., 2009; Li et al., 2010). ‘Genuine’ AiP may also be involved in human pathologies such as cancer, and may account for increased cell proliferation and repopulation of tumors following cytotoxic treatments (chemo- or radiotherapy) which induces massive apoptosis (reviewed in [Fogarty and Bergmann, 2017; Ichim and Tait, 2016]). Caspases play significant tumor-promoting roles in these settings (Li et al., 2010; Huang et al., 2011; Donato et al., 2014; Cheng et al., 2015; Zhang et al., 2015; Kurtova et al., 2015).

The second type is ‘undead’ AiP. Here, the apoptosis pathway is induced upstream, but the execution of apoptosis is blocked. In Drosophila, apoptosis inhibition is achieved experimentally by expression of the effector caspase inhibitor p35 which very specifically inhibits DrICE (Hay et al., 1994; Meier et al., 2000; Hawkins et al., 2000). Therefore, because these cells have initiated the apoptotic process and contain active Dronc, but cannot die, they are referred to as ‘undead’. In ‘undead’ cells, non-apoptotic functions of active Dronc can now be examined, one of which is the release of mitogenic signals for induction of AiP which can lead to hyperplastic overgrowth (Wells et al., 2006; Kondo et al., 2006; Huh et al., 2004; Ryoo et al., 2004; Martín et al., 2009; Pérez-Garijo et al., 2009; Pérez-Garijo et al., 2004; Fan et al., 2014; Rudrapatna et al., 2013; Pérez-Garijo et al., 2005). ‘Undead’ states of cells may also be present under pathological conditions. For instance, it has been proposed that RasV12 can maintain apoptotic cells in an ‘undead’-like state promoting tumorigenesis (Hirabayashi et al., 2013).

Mechanistically, we have shown that AiP-mediated hyperplastic overgrowth of ‘undead’ tissue depends on a Dronc-initiated feedback amplification loop which involves reactive oxygen species (ROS) – specifically extracellular ROS produced by the membrane-bound NADPH oxidase Duox –, activation of macrophage-like hemocytes, secretion of Eiger by hemocytes, Eiger-dependent activation of JNK in epithelial disc and further activation of Dronc by JNK (Fogarty et al., 2016) (reviewed by [Diwanji and Bergmann, 2017a; Diwanji and Bergmann, 2017b]). Therefore, similar to the scrib−/− RasV12 case, Eiger and JNK signaling have proliferation- and growth-promoting functions in this ‘undead’ AiP model.

These similarities prompted us to investigate the role of ROS and caspases for tumor growth of scrib−/− RasV12 clones in Drosophila. We report that oncogenic Ras switches the pro-apoptotic activity of caspases into a tumor-promoting one and thereby maintains scrib−/− RasV12 cells in an ‘undead’-like state. Consistently, inhibition of caspases blocks tumor growth and tissue invasion. The tumor-promoting function of apoptotic caspases is dependent on the generation of intra- and extracellular ROS which are required for neoplastic behavior of scrib−/− RasV12 clones. Furthermore, caspase-induced ROS are essential for the recruitment and activation of hemocytes at scrib−/− RasV12 mosaic discs. Hemocytes signal back to tumorous epithelial cells to stimulate JNK signaling which further promotes caspase activity. Thus, these events constitute a feedback amplification loop which is necessary for neoplastic activity of scrib−/− RasV12 cells. This work extends previous models about the conversion of Eiger and JNK signaling from anti-tumor to pro-tumor roles by oncogenic Ras and...
identifies caspases as essential components of this switch. In conclusion, although apoptotic caspases are usually considered to be tumor suppressors, under certain conditions, for example in the presence of oncogenic RasV12 in scrib mutant cells, they can also adopt a tumor-promoting role.

Results

ROS are required for neoplastic characteristics of scrib−/− RasV12 mosaic discs

Recently, in a model of 'undead' AiP, we showed that Duox-generated ROS are important for activation of hemocytes, JNK signaling and hyperplastic overgrowth (Fogarty et al., 2016; Diwanji and Bergmann, 2017a; Diwanji and Bergmann, 2017b). Therefore, we examined if ROS have a similar function in the neoplastic scrib−/− RasV12 tumor model in Drosophila. scrib−/− RasV12 clones were induced by MARCM using ey-FLP in eye/antennal imaginal discs, the traditional tissue for this model (Brumby and Richardson, 2003; Pagliarini and Xu, 2003). The ROS indicator dihydroethidium (DHE) strongly labels scrib−/− RasV12 mutant clones in mosaic discs, while wild-type (wt), scrib−/− and RasV12-expressing clones are not labeled by DHE or very little (Figure 1A–D'; quantified in Figure 1F; see also Figure 2A,A'). Similar results were reported recently (Katheder et al., 2017; Manent et al., 2017). A different ROS indicator, H2DCF-DA, confirms these results (Figure 1—figure supplement 1A–C,G). scrib−/− RasV12 clones display an increased diffuse cytosolic DHE labeling (Figure 1E,E'). At the boundary of scrib−/− RasV12 clones, several cells stain very intensely for DHE (Figure 1E,E'; arrow head).

To examine the function of ROS in this neoplastic tumor model, we reduced their amount either by down-regulating ROS-producing enzymes such as Duox or by overexpressing ROS-removing enzymes such as catalases and superoxide dismutases (SOD). As shown previously, scrib−/− RasV12 occupy a large portion of the eye/antennal imaginal disc and display a strong neoplastic tumor phenotype (Pagliarini and Xu, 2003) (see also Figure 2F). Because expression of the antioxidant enzymes was achieved by the UAS/Gal4 system, we tested first whether increasing the enzymes such as Duox or by overexpressing ROS-removing enzymes was achieved by the UAS/Gal4 system, we tested first whether increasing the enzyme activity results in a reduced ROS content in the tumor cells in mosaic discs (Ku¨ lshammer et al., 2015). To examine the function of ROS in this neoplastic tumor model, we reduced their amount either by down-regulating ROS-producing enzymes such as Duox or by overexpressing ROS-removing enzymes such as catalases and superoxide dismutases (SOD). As shown previously, scrib−/− RasV12 occupy a large portion of the eye/antennal imaginal disc and display a strong neoplastic tumor phenotype (Pagliarini and Xu, 2003) (see also Figure 2F). Because expression of the antioxidant enzymes was achieved by the UAS/Gal4 system, we tested first whether increasing the enzyme activity results in a reduced ROS content in the tumor cells in mosaic discs (Ku¨ lshammer et al., 2015).

In contrast, removing extracellular ROS by UAS-Duox RNAi or overexpression of the UAS-hCatS transgene which encodes a secreted human catalase (Ha et al., 2005b; Ha et al., 2005a), strongly suppressed tumor growth of scrib−/− RasV12 mutant cells (Figure 1J,K) suggesting that extracellular ROS are required for tumor growth. Interestingly and in contrast to the 'undead' AiP model, removing intracellular ROS by misexpression of intracellular Catalase, SOD1 and SOD2 also strongly suppressed tumor growth (Figure 1L–N). These results suggest that both intra- and extracellular ROS are required for tumor growth of scrib−/− RasV12 clones.

Importantly also, reduction of ROS strongly reduces the invasive behavior of scrib−/− RasV12 mutant cells (Figure 1J–N) which significantly improves the survival rate of the affected animals. Compared to ey >MARCM scrib−/− RasV12 mutant larvae, of which only 5% reach pupal stages, between 40% and 70% of the ey >MARCM scrib−/− RasV12 larvae expressing antioxidant enzymes develop into pupae (Figure 1G). Expression of the UAS-lacZ control transgene does not improve pupal survival (Figure 1G). Furthermore, we also recovered viable adult ey >MARCM scrib−/− RasV12 mosaic animals expressing antioxidant genes, although at a low rate (5–15% of the surviving pupae), which was never observed for scrib−/− RasV12 only (Figure 1P–U). Although their eyes and heads are deformed and weakly overgrown often with necrotic patches compared to wt control (Figure 1O,Q–U), these animals live! Previously, only few examples of surviving scrib−/− RasV12 mosaic animals have been reported. In these examples, viable scrib−/− RasV12 mosaic animals were recovered when essential step in tumor development such as oncogenic JNK signaling or the cell cycle were inhibited in scrib−/− RasV12 clones (Brumby and Richardson, 2003; Ku¨ lshammer et al., 2015; Ku¨ lshammer and Uhlirrova, 2013). Therefore, the observation that reduction of ROS suppresses tumor growth and enhances organismal survival strongly suggests that ROS play a very significant role for the neoplastic characteristics of scrib−/− RasV12 animals.
Caspases promote tumors by inducing the generation of ROS

In ‘undead’ cells, the initiator caspase Dronc (caspase-9 ortholog) has been shown to stimulate the production of ROS (Fogarty et al., 2016). Therefore, we examined the role of Dronc as well as the effector caspase DrICE (caspase-3 ortholog) for generation of ROS and tumorous overgrowth in scrib\(^{-/-}\) Ras\(^{V12}\) mosaic eye discs. As an additional assay, we expressed the effector caspase inhibitor p35.

**Figure 2.** Caspases are required for ROS generation and neoplastic overgrowth in scrib\(^{-/-}\) Ras\(^{V12}\). (A–D’) Expression of the effector caspase inhibitor p35 (B), drICE RNAi (C) and dronc RNAi (D) suppresses scrib\(^{-/-}\) Ras\(^{V12}\) clone size (green) and ROS generation in scrib\(^{-/-}\) Ras\(^{V12}\) clones. The (’’) panels indicate the labeling of the ROS indicator DHE (grey). Scale bars: 50 µm. (E) DHE quantification reveals that ROS levels are significantly reduced in scrib\(^{-/-}\) Ras\(^{V12}\) mutant clones with reduced or inhibited caspase activity. Shown is the mean signal intensity ±SD of DHE labelings in clones, analyzed by one-way ANOVA with Holm-Sidak test for multiple comparisons. ****p<0.0001. Multiple clones from five to ten discs of each genotype were analyzed. (F–I) The growth and invasion of cephalic complexes of 11 day old scrib\(^{-/-}\) Ras\(^{V12}\) larvae (F) is strongly suppressed by p35 (G), drICE RNAi (H) and dronc RNAi (I). Clone size (green) in (F–I) is strongly reduced. DAPI labels the outline of the tissue. Scale bars: 200 µm. (J–M) Adult eyes of surviving scrib\(^{-/-}\) Ras\(^{V12}\) animals expressing p35 (K), drICE RNAi (L) and dronc RNAi (M). The percentage number in the top right of each panel indicates the adult survival rate relative to pupal survival. (N) Reduction or inhibition of caspase activity in scrib\(^{-/-}\) Ras\(^{V12}\) mutant clones significantly improves the pupariation rates of animals bearing scrib\(^{-/-}\) Ras\(^{V12}\) mosaic eye imaginal discs. Pupariation rates were determined as the ratio of late stage mutant pupae vs total pupae and were analyzed by one-way ANOVA with Holm-Sidak test for multiple comparisons. Error bars are SD. P values are relative to scrib\(^{-/-}\) Ras\(^{V12}\) results (left column) and are indicated above the experimental columns. ****p<0.0001. At least 100 pupae were counted per genotype. Experiments were performed three times. Genotypes: (A,F,J) yw; ey-FLP+/+; act>Gal4, UAS-GFP\(^{56ST}\)+, FRT82B tub-Gal80/UAS-Ras\(^{V12}\) FRT82B scrib\(^{2}\), (B–D,G–I,K–M) yw; ey-FLP+/+; act>y>Gal4, UAS-GFP\(^{56ST}\)/UAS-X; FRT82B tub-Gal80/UAS-Ras\(^{V12}\) FRT82B scrib\(^{2}\) with UAS-X being UAS-p35 (B,G,K), UAS-drICE RNAi (C,H,L) and UAS-dronc RNAi (D,I,M).

DOI: https://doi.org/10.7554/eLife.26747.006

Caspases promote tumors by inducing the generation of ROS

In ‘undead’ cells, the initiator caspase Dronc (caspase-9 ortholog) has been shown to stimulate the production of ROS (Fogarty et al., 2016). Therefore, we examined the role of Dronc as well as the effector caspase DrICE (caspase-3 ortholog) for generation of ROS and tumorous overgrowth in scrib\(^{-/-}\) Ras\(^{V12}\) mosaic eye discs. As an additional assay, we expressed the effector caspase inhibitor...
p35 inscrib−/−RasV12mutantclones.Removingorinhibitingcaspasesinscrib−/−RasV12mutant clones strongly reduced DHE labeling suggesting suppression of ROS generation (Figure 2A–D; quantified in Figure 2E). Similar results were obtained with a different ROS indicator, H2DCF-DA (Figure 1—figure supplement 1C–G). Consequently, tumor overgrowth and invasion of the VNC is dramatically reduced upon removal or inhibition of caspases inscrib−/−RasV12mutant cells (Figure 2F–I). Reduction of caspase activity also increases pupal survival (Figure 2N) and viable animals with mosaic scrib−/−RasV12heads and eyes were recovered as adults at a rate of 4–9% of the surviving pupae (Figure 2J–M).

The requirement of caspases for generation of ROS and neoplastic behavior suggests that caspases are activated inscrib−/−RasV12mutant cells. To verify this, we labeledscrib−/−RasV12mosaic eye discs with cleaved caspase-3 (CC3) antibody which detects activated (cleaved) effector caspases and an unknown non-apoptotic substrate of Dronc (Fan and Bergmann, 2010; Srinivasan et al., 1998). Indeed, while there is very little CC3 labeling in mosaic control discs (FRT +), scrib−/−RasV12mutant clones label significantly stronger with CC3 antibody (Figure 3A–B′, yellow arrowhead; quantified in Figure 3D). In addition to the CC3 labeling inscrib−/−RasV12clones, there is also staining immediately outside the clones which appears even more intense than the labeling inside the clones (Figure 3B,B′; white arrows). In fact, quantification reveals that this non-autonomous CC3 labeling is 2 to 2.5-fold higher than autonomous CC3 labeling inscrib−/−RasV12clones (Figure 3D). Both, CC3 labeling inside and outside ofscrib−/−RasV12clones are autonomously dependent on the caspases DrICE (Figure 3C) and Dronc as well as on ROS (Figure 3—figure supplement 1).

Labeling with the CC3 antibody indicates active Dronc both in apoptotic and non-apoptotic cells (Fan and Bergmann, 2010; Fan et al., 2014). Therefore, because of the strong tumor growth ofscrib−/−RasV12mosaics, we wondered if the CC3-positive cells inscrib−/−RasV12clones are actually apoptotic and labeledscrib−/−RasV12discs with TUNEL, an apoptotic assay that detects DNA fragmentation, a hallmark of apoptosis downstream of effector caspases (Gavrieli et al., 1992). Interestingly, although a few TUNEL-positive cells are detectable withinscrib−/−RasV12clones, the majority of TUNEL-positive cells are located outside the clones (Figure 3E,E′; arrows). Quantification reveals that almost 90% of all apoptotic cells inscrib−/−RasV12mosaic discs are outside the mutant clones (Figure 3F).

These observations allow us to make an important conclusion. Despite detectable caspase (Dronc) activity inscrib−/−RasV12mutant clones by CC3 labeling, this activity does not appear to trigger a significant amount of apoptosis in these clones. In contrast, our genetic analysis suggest that the strong tumor growth phenotype ofscrib−/−RasV12mosaic eye discs is dependent on caspases (Figure 2) suggesting that they have adopted a tumor-promoting function. This is surprising as caspase activity and apoptosis in general are thought to act as tumor suppressors (Hanahan and Weinberg, 2011; Hanahan and Weinberg, 2000). In fact, caspase activity inscrib−/−single mutant cells does act as a tumor suppressor by killing them (Brumby and Richardson, 2003; Igaki et al., 2006; Igaki et al., 2009; Uhlirva et al., 2005; Chen et al., 2012). In contrast, inscrib−/−RasV12mutant cells, this caspase activity persists, but does not appear to induce a significant amount of apoptosis. Therefore, these data suggest thatRasV12maintains scrib−/−cells in an ‘undead’-like condition, consistent with a previous report (Hirabayashi et al., 2013). Furthermore, RasV12changes the activity of caspases to adopt a tumor-promoting role.

Hemocyte recruitment to scrib−/−RasV12tumors depends on caspase-generated ROS

Next, we examined the role of ROS for neoplastic growth ofscrib−/−RasV12mosaic discs. One known function of extracellular ROS is the recruitment and activation of Drosophila macrophages (hemocytes) in the ‘undead’ AiP model (Diwanji and Bergmann, 2017a; Fogarty et al., 2016; Diwanji and Bergmann, 2017b). Hemocytes have been shown to be associated withscrib−/−RasV12mosaic discs (Cordero et al., 2010; Pastor-Pareja et al., 2008; Kühlhammer and Uhlirva, 2013). Therefore, we tested if ROS contribute to the recruitment and activation of hemocytes toscrib−/−RasV12mosaic eye imaginal discs. At control discs, hemocytes adhere in small cellular aggregates, mostly at the antennal disc (Figure 4A,A′,L). In contrast, they are recruited to neoplasticscrib−/−RasV12tumor sites in large numbers where they cover the eye portion of the disc (Figure 4B,B′; quantified in Figure 4K) consistent with previous reports (Cordero et al., 2010; Pastor-Pareja et al., 2008; Kühlhammer and Uhlirva, 2013). They also change their morphological...
appearance at neoplastic scrib<sup>−/−</sup> Ras<sup>V12</sup> discs. They single out from the cellular clusters and develop cellular protrusions, similar to cytonemes (Figure 4M, arrows). This change in cellular behavior and morphology of hemocytes was also observed in the ‘undead’ AiP model (Fogarty et al., 2016).
Figure 4. Caspase-generated ROS are required of recruitment and activation of hemocytes to scrib\(^{-/}\) Ras\(^{V12}\) mosaic eye/antennal imaginal discs. Hemocytes were labeled with the NimC antibody (Kurucz et al., 2007) (red in top panels; grey in (') panels and in (L–R)). Scale bars in (A–J): 50 µm; in (L–R): 100 µm. (A,A') Control mosaic discs (FRT +) carry small hemocyte clusters mostly at the antennal portion of the disc. (B,B') scrib\(^{-/}\) Ras\(^{V12}\) mosaic discs are covered by large quantities of hemocytes. + indicates scrib\(^{-/}\) Ras\(^{V12}\) in otherwise wt background. (C–I) Hemocyte recruitment to scrib\(^{-/}\) Ras\(^{V12}\) eye/antennal imaginal discs is strongly impaired upon loss of ROS (C–F') and caspase activity (G–I'). (J,J') Expression of a dominant negative JNK transgene (JNK\(^{DN}\)) in scrib\(^{-/}\) Ras\(^{V12}\) mutant clones blocks hemocyte recruitment. (K) Quantification of NimC labelings reveals that the number of hemocytes attached to scrib\(^{-/}\) Ras\(^{V12}\) mosaic discs is significantly lower when ROS levels or caspase activity are reduced in scrib\(^{-/}\) Ras\(^{V12}\) clones. To facilitate the quantification, the mean intensity of NimC labelings across the entire disc was determined, normalized to GFP (to account for the reduced size of ROS-depleted or caspase-inhibited scrib\(^{-/}\) Ras\(^{V12}\) clones) and analyzed by one-way ANOVA with Holm-Sidak test for multiple comparisons. Error bars are SD. P values are referenced to scrib\(^{-/}\) Ras\(^{V12}\) and are indicated by asterisks above each column. ****p<0.0001. Ten discs per genotype were analyzed. + indicates scrib\(^{-/}\) Ras\(^{V12}\) in otherwise wt background. (L–R) High magnification images of hemocytes attached to the discs of indicated genotype. Note that in (M) hemocytes attached to scrib\(^{-/}\) Ras\(^{V12}\) discs extend cellular protrusions (arrows), similar to cytonemes. These protrusions are absent in hemocytes attached to control (L) and caspase-inhibited or ROS-depleted discs (N–R). + in (M) indicates scrib\(^{-/}\) Ras\(^{V12}\) in otherwise wt background. Genotypes: (A,L) yw;ey-FLP/+;act>\(\gamma\)--Gal4, UAS-GFP\(^{65ST}\)/+; FRT82B tub-Gal80\(^{L}\). FRT82B w\(^{56ST}\}; (B,M) yw;ey-FLP/+; act>\(\gamma\)--Gal4, UAS-GFP\(^{65ST}\)/+; FRT82B tub-Gal80/UAS-Ras\(^{V12}\) FRT82B scrb\(^2\); (C–I, N–R) yw;ey-FLP/+; act>\(\gamma\)--Gal4, UAS-GFP\(^{65ST}\)/UAS-X; FRT82B tub-Gal80/UAS-Ras\(^{V12}\) FRT82B scrb\(^2\) with UAS-X being UAS-Duox\(^{RNAi}\) (C), UAS-hCatS (D,N), UAS-SOD1 (E), UAS-Catalase (F,O), UAS-SOD2 (P), UAS-dronc\(^{RNAi}\) (G,Q), UAS-driCE\(^{RNAi}\) (H,R) and UAS-p35 (I). (J) yw;ey-FLP/UAS-JNK\(^{DN}\), act>\(\gamma\)--Gal4, UAS-GFP\(^{65ST}\)/+; FRT82B tub-Gal80/UAS-Ras\(^{V12}\) FRT82B scrb\(^2\). DOI: https://doi.org/10.7554/eLife.26747.009

We examined if ROS and caspases are required for hemocyte recruitment to scrib\(^{-/}\) Ras\(^{V12}\) mosaic discs. Indeed, reduction of ROS strongly reduces the recruitment of hemocytes to scrib\(^{-/}\) Ras\(^{V12}\) mosaic discs (Figure 4C–F'). Likewise, the recruitment of hemocytes to scrib\(^{-/}\) Ras\(^{V12}\) tumors is impaired by loss of caspase activity (Figure 4G–I'). Quantification of hemocyte recruitment to ROS-depleted or caspase-inhibited scrib\(^{-/}\) Ras\(^{V12}\) discs normalized to GFP (to account for the
reduced size of ROS-depleted or caspase-inhibited scrib−/− RasV12 clones), revealed a significant loss of hemocytes compared to scrib−/− RasV12 mosaic discs (Figure 4K). For example, more than 90% of scrib−/− RasV12 discs expressing Duox RNAi are not attached by any hemocyte despite the presence of many small clones (Figure 4C). Furthermore, in addition to the significant loss of hemocytes, the few hemocytes that are attached to ROS- and caspase-depleted scrib RasV12 discs (Figure 4D–I’), display the naive morphology seen at control discs (Figure 4N–R). These observations provide strong evidence that caspase-dependent generation of ROS is essential for recruitment and activation of hemocytes to scrib−/− RasV12 tumors.

Caspase activation and ROS generation depends on JNK signaling

As reported previously, JNK activity is strongly induced in scrib−/− RasV12 clones (Figure 5C) and is essential for the neoplastic phenotype of scrib−/− RasV12 mosaic animals (Brumby and Richardson, 2003; Igaki et al., 2006; Igaki et al., 2009; Uhlirova et al., 2005; Leong et al., 2009; Cordero et al., 2010). In fact, activation of JNK in oncogenic RasV12 background is sufficient to trigger a neoplastic tumor phenotype similar to the scrib−/− RasV12 condition (Uhlirova et al., 2005; Uhlirova and Bohmann, 2006). Therefore, we examined the relation between ROS, caspases and JNK signaling. In a first set of experiments, we blocked JNK signaling by expressing a dominant negative JNK construct (JNKDN) in scrib−/− RasV12 mutant clones. In JNKDN-expressing scrib−/− RasV12 mutant clones, caspase activity (CC3) and ROS production are strongly reduced (Figure 5A–B’). Likewise, the recruitment and activation of hemocytes is strongly impaired at JNKDN, scrib−/− RasV12 discs (Figure 4J) consistent with a previous report (Külshammer and Uhlirova, 2013). These findings suggest that caspase activation, ROS generation and hemocyte activation are dependent on JNK signaling.

In the second set of experiments, we examined if there is a dependence of JNK signaling on caspases and ROS using anti-phosphoJNK (pJNK) antibody as a JNK activity marker. These labelings revealed a significant loss of JNK activity in caspase-inhibited or ROS-depleted scrib−/− RasV12 clones (Figure 5D–K’; quantified in Figure 5L). Similar results were obtained using MMP1 antibody labeling as an additional JNK marker (Figure 5—figure supplement 1). These observations suggest that the maintenance of JNK activity requires caspases and ROS. Combined, these results imply that JNK is acting both upstream (Figure 5A,B) and downstream (Figure 5E–L) of caspases and ROS. The easiest way to explain such a behavior is that caspases, ROS, hemocytes and JNK signaling constitute an amplification loop in scrib−/− RasV12 mutant clones similar to the ‘undead’ AIP model (Diwanji and Bergmann, 2017a; Fogarty et al., 2016).

Discussion

The traditional view of caspases holds that they counter tumorigenesis by eliminating tumor cells and thus mediate a tumor suppressor function (Hanahan and Weinberg, 2000; Hanahan and Weinberg, 2011). This tumor-suppressing function of caspases has been reported in mammalian systems (Asselin-Labat et al., 2011; Ho et al., 2009) and in Drosophila, where, for example, scrib mutant cells undergo caspase-dependent apoptosis in a JNK- and Eiger-dependent manner (Brumby and Richardson, 2003; Igaki et al., 2006; Igaki et al., 2009; Uhlirova et al., 2005). However, more recent work has suggested that caspases and apoptosis in general can also have the opposite, tumor-promoting function, both in flies and in mammals (Cheng et al., 2015; Donato et al., 2014; Huang et al., 2011; Kurtova et al., 2015; Li et al., 2010; Zhang et al., 2015); reviewed by (Ryoo and Bergmann, 2012; Ichim and Tait, 2016; Fogarty and Bergmann, 2017). Furthermore, in Drosophila it was previously shown that oncogenic Ras switches the tumor-suppressing function of JNK and Eiger in scrib−/− RasV12 mutant cells to a tumor-promoting one in scrib−/− RasV12 cells (Igaki et al., 2006; Cordero et al., 2010; Uhlirova et al., 2005; Enomoto et al., 2015). Mechanistic details about this oncogenic switch have been largely elusive. Our data presented here imply that a critical step for this oncogenic switch is the conversion of caspase activity by oncogenic Ras.

Consistent with a previous report in different context (Hirabayashi et al., 2013), our results demonstrated that oncogenic Ras can suppress the apoptotic activity of caspases and keeps scrib−/− RasV12 cells in an ‘undead’-like condition. While caspases may still induce apoptosis in a few tumor cells, they now largely promote generation of intra- and extracellular ROS which are required for malignant growth and tissue invasion of surviving neoplastic cells. Evidence that caspases are indeed
Figure 5. JNK acts upstream and downstream of caspase activation and ROS generation. (A–B’) Expression of JNK\textsuperscript{DN} in \textit{scrib} \textsuperscript{-/-} \textit{Ras} \textsuperscript{V12} clones inhibits caspase activity (A, A’; CC3) and ROS generation (B, B’; DHE). Scale bars: 50 µm. (C–K’) \textit{pJNK} labeling (red in (C–K); grey in (C’–K’)) as JNK marker in \textit{scrib} \textsuperscript{-/-} \textit{Ras} \textsuperscript{V12} (C,C’), JNK\textsuperscript{DN}-expressing \textit{scrib} \textsuperscript{-/-} \textit{Ras} \textsuperscript{V12} (D,D’), and ROS-depleted or caspase-inhibited \textit{scrib} \textsuperscript{-/-} \textit{Ras} \textsuperscript{V12} mosaic discs (E–K’). The strong \textit{pJNK} labeling in (C,C’) is significantly reduced in (D–K’). Scale bars: 50 µm. (L) The mean intensity of \textit{pJNK} labelings in \textit{scrib} \textsuperscript{-/-} \textit{Ras} \textsuperscript{V12} clones in panels Figure 5 continued on next page.
(C–K) is significantly reduced upon ROS-depletion or reduction of caspase activity. Analysis of JNK labelings was done by one-way ANOVA with Holm-Sidak test for multiple comparisons. Error bars are SD. P values are referenced to *scr*<sup>–/–</sup> Ras<sup>V12</sup> and are indicated by asterisks above each column (****p<0.0001). At least ten discs per genotype were analyzed.

**Supplementary Figure 1.** MMP1 labeling is reduced in *scr*<sup>–/–</sup> Ras<sup>V12</sup> clones with reduced ROS or caspase activity.

DOI: https://doi.org/10.7554/eLife.26747.011

The following figure supplement is available for figure 5:

**Figure supplement 1.** MMP1 labeling is reduced in *scr*<sup>–/–</sup> Ras<sup>V12</sup> clones with reduced ROS or caspase activity.

DOI: https://doi.org/10.7554/eLife.26747.011

activated in *scr*<sup>–/–</sup> Ras<sup>V12</sup> clones is not only provided in this study, but also in a report that showed that small malignant clones (igl<sup>–/–</sup> Ras<sup>V12</sup>) undergo caspase-mediated apoptosis and elimination in a similar way as *scr* clones (Menéndez et al., 2010). Only when mutant clones have reached a certain size, can they develop malignant tumors despite intrinsic caspase activation (Menéndez et al., 2010; Ballesteros-Arias et al., 2014).

Our data suggest that JNK activity acts both upstream and downstream of caspases and ROS generation in *scr*<sup>–/–</sup> Ras<sup>V12</sup> mutant clones (Figure 5). It is possible that initially, when the *scr*<sup>–/–</sup> Ras<sup>V12</sup> mutant cells form, a cell competition signal triggers JNK activation in *scr*<sup>–/–</sup> Ras<sup>V12</sup> cells, similar to the events in *scr*<sup>–/–</sup>-only mutant cells (Figure 6). In both *scr*<sup>–/–</sup> and *scr*<sup>–/–</sup> Ras<sup>V12</sup> mutant cells, this JNK activity results in caspase activation (Figures 3 and 5A). However, due to the anti-apoptotic activity of Ras<sup>V12</sup>, caspases induce only very little apoptosis in *scr* Ras<sup>V12</sup> cells (Figure 3). Therefore, although caspases are active, most *scr*<sup>–/–</sup> Ras<sup>V12</sup> cells do not die and thus are in an ‘undead’-like state. Caspases now promote the generation of extracellular ROS through activation of NADPH oxidase Duox. These ROS recruit and activate hemocytes (Figure 4). It is known that hemocytes can release Eiger (Cordero et al., 2010; Parisi et al., 2014; Fogarty et al., 2016) which signals through its receptor Grindelwald (Andersen et al., 2015) for further stimulation of JNK activity in *scr*<sup>–/–</sup> Ras<sup>V12</sup> cells. Thus, we postulate that JNK, caspases, ROS, hemocytes and Eiger constitute an amplification loop (Figure 6) which may be necessary for tumor initiation.

Evidence of an amplification loop is provided by the mutual dependence of caspases, ROS and JNK (Figure 5; Figure 3—figure supplement 1). Similar amplification loops have been described in apoptotic and ‘undead’ cells (Wells et al., 2006; Shlekov and Morata, 2012; Fogarty et al., 2016). This amplification loop ensures persistent oncogenic signaling in *scr*<sup>–/–</sup> Ras<sup>V12</sup> cells. This is in striking contrast to *scr*<sup>–/–</sup> mutant cells alone in which JNK signaling triggers linear caspase activation and apoptosis (Brumby and Richardson, 2003; Igaki et al., 2006; Igaki et al., 2009; Uhlirova et al., 2005). In later stages of tumorigenesis, the amplification loop may have reached full strength and promotes malignant growth and invasion of *scr*<sup>–/–</sup> Ras<sup>V12</sup> cells (Figure 6). Amplification loops have also been observed in other neoplastic tumor models in Drosophila. For example, intestinal stem cell tumors form in response to an amplification loop (Chen et al., 2016). In a glycolytic tumor model, ROS are also part of an amplification loop that facilitates metabolic reprogramming (Wang et al., 2016). Thus, it is possible that tumorigenesis in general depends on amplification loops to sustain oncogenic signaling.

There are similarities and differences in the amplification loops of the ‘undead’ hyperplastic AiP model and the neoplastic *scr*<sup>–/–</sup> Ras<sup>V12</sup> tumor model. In both models, caspases, ROS, hemocytes and JNK are required for growth. However, regarding caspases, the ‘undead’ AiP model only involves the initiator caspase Dronc for growth (in fact, effector caspases are inhibited by P35 in this model) (Fan et al., 2014; Huh et al., 2004; Pérez-Garíjo et al., 2004; Pérez-Garíjo et al., 2009; Ryoo et al., 2004). In contrast, the neoplastic *scr*<sup>–/–</sup> Ras<sup>V12</sup> model requires both initiator (Dronc) and effector (DrICE) caspases. Inhibition of either suppresses malignant growth and invasion. Another interesting difference is the differential involvement of ROS. While only extracellular ROS are essential in the ‘undead’ AiP model, neoplastic growth of *scr*<sup>–/–</sup> Ras<sup>V12</sup> cells requires both intra- and extracellular ROS. Mitochondria are the likely source of intracellular ROS because expression of mitochondrial SOD2 can suppress tumor growth and invasion of *scr*<sup>–/–</sup> Ras<sup>V12</sup> tumors (Figure 1N). It is unclear if these two populations of ROS are dependent or independent of each
other. Recently, it was shown in endothelial cells that Nox-derived ROS can trigger an increase in mitochondrial-derived ROS and loss of mitochondrial membrane potential suggesting a dependence of mitochondrial ROS from Nox-generated ROS (Shafique et al., 2017). However, alternatively, it is also possible that intra- and extracellular ROS are produced independently by caspase activity, and both are separately required for neoplastic transformation.

Furthermore, while we mostly focused from the point-of-view of ‘undead’ AiP, it is also possible that ‘genuine’ AiP contributes to the tumor growth in scrib<sup>−/−</sup> Ras<sup>V12</sup> mutant cells (Figure 3E’,F). Genuinely apoptotic cells can also produce ROS in Drosophila imaginal discs (Santabárbara-Ruiz et al., 2015). Therefore, it is tempting to speculate that some of the differences between the ‘undead’ AiP model and the scrib<sup>−/−</sup> Ras<sup>V12</sup> model are due to a combination of ‘undead’ and

**Figure 6.** Mechanistic view about the conversion of caspases from tumor suppressors to tumor promoters in scrib Ras<sup>V12</sup> mutant cells. (A) After scrib<sup>−/−</sup> mutant cells have formed in mosaic discs, a cell competition signal mediated by Eiger triggers JNK and caspase activity which induces apoptosis of scrib mutant cells (dashed outline of the cell). (B) After scrib<sup>−/−</sup> Ras<sup>V12</sup> cells have formed, JNK activity may be induced by the same cell competition signal as in scrib<sup>−/−</sup> mutant cells (early). However, despite activation of JNK and caspases in scrib Ras<sup>V12</sup> cells, Ras<sup>V12</sup> keeps these cells in an ‘undead’-like condition and enables caspases to initiate a feedback amplification loop involving ROS generation and recruitment of hemocytes which amplifies JNK and caspase activity (late). This amplification loop is necessary for malignant growth and invasion. Neighboring wild-type cells undergo apoptosis (dashed outline of the cell). The mechanism of non-autonomous apoptosis is not clear.

DOI: https://doi.org/10.7554/eLife.26747.012
‘genuine’ AiP in scrib\(^{-/-}\) Ras\(^{V12}\). This may explain the differences in caspase requirement, the differences in ROS production and the different outcomes of growth – hyperplastic vs. neoplastic – between the ‘undead’ AiP and the scrib\(^{-/-}\) Ras\(^{V12}\) tumor models. Future work will address this important question.

Another important question for future studies will be to address how Ras\(^{V12}\) switches the activity of caspases from tumor-suppressors to tumor-promoters. A known target of survival signaling by Ras\(^{V12}\) is the pro-apoptotic gene Hid which acts upstream of caspase activation (Bergmann et al., 1998; Kurada and White, 1998). Because Eiger and JNK can induce expression of hid (Moreno et al., 2002), it is possible that Hid activity is inhibited by oncogenic Ras. However, while we do not exclude this possibility, it alone may not be sufficient to explain the altered caspase activity because inhibition of hid would result in loss of caspase activity. However, caspases are still active in scrib\(^{-/-}\) Ras\(^{V12}\) mutant cells (Figure 3B,D) and they are also able to induce apoptosis at least in a small amount of mutant cells (Figure 3E,F). Therefore, it is possible that Ras\(^{V12}\) modifies caspase activity in a different manner – directly or indirectly – for non-apoptotic ROS generation.

Oncogenic Ras is mediating many steps in the tumorigenic process of scrib\(^{-/-}\) Ras\(^{V12}\) tissue. It changes the transcriptome of these cells and modifies the downstream activities of JNK (Atkins et al., 2016; Külshammer et al., 2015). However, as shown in this work, a critical step mediated by Ras\(^{V12}\) is the modification of caspase activity – directly or indirectly – in the early stage of tumorigenesis in a way that the cells survive. At present it is unclear if the caspase-modulating activity of Ras\(^{V12}\) is dependent on transcription. There may not be enough time for a transcriptional response by the cell to escape the apoptotic activity of caspases. Consistently, work by others has suggested that changes in the transcriptome alone does not fully explain the neoplastic phenotype of scrib\(^{-/-}\) Ras\(^{V12}\) cells and that other potentially non-transcriptional processes are involved (Atkins et al., 2016). Modification of caspase activity may be one of these non-transcriptional processes.

Non-apoptotic functions of caspases have been reported (Shalini et al., 2015; Fogarty and Bergmann, 2017; Mukherjee and Williams, 2017; Nakajima and Kuranaga, 2017). However, it is largely unknown how cells survive in the presence of activated caspases during non-apoptotic processes. It is possible that a reduction of caspase activity below a certain apoptotic threshold is sufficient for survival. Other models include changes in the subcellular localization of caspases or interaction with modifying factors such as Tango7 (D’Brot et al., 2013). Interesting in this respect is a recent study which showed that mitochondrially-derived SCS\(\beta\) restricts caspase activity for spermatid maturation in the Drosophila testis (Aram et al., 2016). Since mitochondria release apoptotic signaling molecules such as cytochrome c and Smac during apoptosis (Fuchs and Steller, 2011), it may also be possible that they release signals such as SCS\(\beta\) or related factors which modulate the activity of caspases for non-apoptotic functions. More work is necessary to address these essential questions for understanding of tumor initiation and progression.

Materials and methods

**Drosophila genetics**

The scrib allele used is scrib\(^2\) (also known as scrib\(^{673}\)) (Bilder and Perrimon, 2000). The recombinant UAS-Ras\(^{V12}\) FRT82B scrib\(^2\) (Chen et al., 2012; Pérez et al., 2015) line was a kind gift of Madhuri Kango-Singh (U Dayton, OH, USA). The MARCM system (Lee and Luo, 1999) with ey-FLP (Newson et al., 2000) was used to generate mosaics of eye/antennal imaginal discs and experimental clones were marked by GFP. In (Figure 1—figure supplement 1), we used a modified MARCM system that marks clones with myrRFP (Chabu and Xu, 2014). The wt control line (FRT +) is FRT82B (Xu and Rubin, 1993).

The following transgenes are all inserted on chromosome 2 and were crossed into the scrib\(^{-/-}\) Ras\(^{V12}\) background for analysis: UAS-lacZ (Bloomington, BL3955), UAS-Duox RNAi and UAS-hCatS (a kind gift of Won-Jae Lee) (Ha et al., 2005a; Ha et al., 2005b2005), UAS-Catalase (BL24621), UAS-SOD1 (BL24754), UAS-SOD2 (BL24494), UAS-p35 (BL5072), UAS-drone RNAi and UAS-drICE RNAi (a kind gift of Pascal Meier) (Leulier et al., 2006). UAS-JNK\(^{DN}\) (aka UAS-bsk\(^{DN}\)) (BL6409) is an insertion on X chromosome. Crosses were incubated at either 22° or 25°C.
Imaging and quantification

DHE and H$_2$DCF-DA (both from Invitrogen/Molecular Probes) labeling of unfixed tissue was performed as described (Owusu-Ansah et al., 2008). TUNEL labeling (Roche) was done according to the manufacturer’s instructions. Antibody labelings were done on fixed tissue following standard procedures (Fan and Bergmann, 2014; Fogarty and Bergmann, 2014). The following antibodies were used: cleaved caspase-3 (CC3; Cell Signaling Technology); NimC (kind gift of I. Andó) (Kurucz et al., 2007); MMP1 (Developmental Studies Hybridoma Bank (DSHB)) and pJNK (Promega). Secondary antibodies were donkey Fab fragments from Jackson Immunoresearch. Eye/antennal cephalic complexes were counterlabeled with the nuclear dye DAPI to visualize tissue outline. Images were taken with a Zeiss LSM700 confocal microscope. For quantification of confocal images, the ‘Record Measurement’ function of Photoshop was used. Clones were outlined and signal intensity determined. Multiple clones of five to ten imaginal discs per genotype obtained in three independent experiments were measured. Analysis and graph generation was done using GraphPad Prism 7.03. The statistical method and the P values are indicated in the figure legends.

Acknowledgements

We would like to thank István Andó, Madhuri Kango-Singh, Won Jae Lee, Pascal Meier, Tian Xu, the Bloomington and Vienna stock centers for fly stocks and reagents; Eric Baehrecke, Yun Fan and Caitlin E. Fogarty for stimulating discussions. This work was supported by MIRA award R35 GM118330 from the NIH/NIGMS. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Additional information

Funding

| Funder                          | Grant reference number | Author       |
|---------------------------------|------------------------|--------------|
| National Institute of General   | R35GM118330            | Andreas Bergmann |
| Medical Sciences                |                        |              |
| National Institute of General   | R01GM107789            | Andreas Bergmann |
| Medical Sciences                |                        |              |

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Author contributions

Ernesto Pérez, Conceptualization, Formal analysis, Supervision, Funding acquisition, Writing—original draft, Writing—review and editing; Jillian L Lindblad, Conceptualization, Data curation, Formal analysis, Investigation, Writing—original draft; Andreas Bergmann, Data curation, Formal analysis, Investigation

Author ORCIDs

Andreas Bergmann, http://orcid.org/0000-0002-9134-871X

Decision letter and Author response

Decision letter https://doi.org/10.7554/eLife.26747.014
Author response https://doi.org/10.7554/eLife.26747.015

Additional files

Supplementary files

• Transparent reporting form

DOI: https://doi.org/10.7554/eLife.26747.013
References

Andersen DS, Colombani J, Palmerini V, Chakrabandhu K, Boone E, Röthlisberger M, Toggweiler J, Basler K, Mapelli M, Hueber AO, Léopold P. 2015. The Drosophila TNF receptor Grindelwald couples loss of cell polarity and neoplastic growth. Nature 522:482–486. DOI: https://doi.org/10.1038/nature14298, PMID: 25874673

Arum L, Braun T, Braverman C, Kaplan Y, Ravid L, Levin-Zaidman S, Arama E. 2016. A krebs cycle component limits caspase activation rate through mitochondrial surface restriction of CRL activation. Developmental Cell 37:15–33. DOI: https://doi.org/10.1016/j.devcel.2016.02.025, PMID: 27052834

Asselin-Labat ML, Sutherland KD, Vaillant F, Gyorki DE, Wu D, Holroyd S, Breslin K, Ward T, Shi W, Bath ML, Deb S, Fox SB, Smyth GK, Lindeman GJ, Visvader JE. 2011. Gata-3 negatively regulates the tumor-initiating capacity of mammary luminal progenitor cells and targets the putative tumor suppressor caspase-14. Molecular and Cellular Biology 31:4609–4622. DOI: https://doi.org/10.1128/MCB.05766-11, PMID: 21930782

Atkins M, Potier D, Romanelli L, Jacobs J, Mach J, Haramestouglu F, Aerts S, Haider G. 2016. An ectopic network of transcription factors regulates growth by hippoc signaling drives growth and invasion of a malignant tumor model. Current Biology 26:2101–2113. DOI: https://doi.org/10.1016/j.cub.2016.06.035, PMID: 27476594

Ballesteros-Arias L, Saavedra V, Morata G. 2014. Cell competition may function either as tumour-suppressing or as tumour-stimulating factor in Drosophila. Oncogene 33:4377–4384. DOI: https://doi.org/10.1038/onc.2013.407, PMID: 24096487

Bergmann A, Agapite J, McCall K, Steller H. 1998. The Drosophila gene hid is a direct molecular target of Ras-dependent survival signaling. Cell 95:331–341. DOI: https://doi.org/10.1016/S0092-8674(00)81765-1, PMID: 9814704

Bergstrahl DT, St Johnston D. 2012. Epithelial cell polarity: what flies can teach us about cancer. Essays In Biochemistry 53:129–140. DOI: https://doi.org/10.1042/bse0530129, PMID: 22928513

Bilder D, Li M, Perrimon N. 2000. Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. Science 289:113–116. DOI: https://doi.org/10.1126/science.289.5476.113, PMID: 10844224

Bilder D, Perrimon N. 2000. Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. Nature 403:676–680. DOI: https://doi.org/10.1038/35001108, PMID: 10688207

Brumby AM, Richardson HE. 2003. scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. The EMBO Journal 22:5769–5779. DOI: https://doi.org/10.1093/emboj/cdg548, PMID: 14592797

Brumby AM, Goulding KR, Schlosser T, Loi S, Galea R, Khoob P, Bolden JE, Aigaki T, Humbert PO, Richardson HE. 2011. Identification of novel Ras-cooperating oncogenes in Drosophila melanogaster: a RhoGEF/Rho-family/JNK pathway is a central driver of tumorigenesis. Genetics 188:105–125. DOI: https://doi.org/10.1534/genetics.111.127910, PMID: 21368274

Chabu C, Xu T. 2014. Oncogenic Ras stimulates Eiger/TNF exocytosis to promote growth. Development 141:4729–4739. DOI: https://doi.org/10.1242/dev.108092, PMID: 25411211

Chen CL, Schroeder MC, Kango-Singh M, Tao C, Halder G. 2012. Tumor suppression by cell competition through regulation of the Hippo pathway. Proceedings of the National Academy of Sciences 109:484–489. DOI: https://doi.org/10.1073/pnas.1113882109, PMID: 22190496

Chen J, Xu N, Huang H, Cai T, Xi R. 2016. A feedback amplification loop between stem cells and their progeny promotes tissue regeneration and tumorigenesis. eLife 5:e14330. DOI: https://doi.org/10.7554/eLife.14330, PMID: 27187149

Cheng J, Tian L, Ma J, Gong Y, Zhang Z, Chen Z, Xu B, Xiong H, Li C, Huang Q. 2015. Dying tumor cells stimulate proliferation of living tumor cells via caspase-dependent protein kinase Cα activation in pancreatic ductal adenocarcinoma. Molecular Oncology 9:105–114. DOI: https://doi.org/10.1038/молонкол.2014.07.024, PMID: 25156550

Chera S, Ghila L, Dobretz K, Wenger Y, Bauer C, Buzgariu W, Martinou JC, Galliot B. 2009. Apoptotic cells provide an unexpected source of Wnt3 signaling to drive hydra head regeneration. Developmental Cell 17:279–289. DOI: https://doi.org/10.1016/j.devcel.2009.07.014, PMID: 19686688

Cordero JB, Macagno JP, Stefanatos RK, Strathdee KE, Cagan RL, Vidal M. 2010. Oncogenic Ras diverts a host TNF tumor suppressor activity into tumor promoter. Developmental Cell 18:999–1011. DOI: https://doi.org/10.1016/j.devcel.2010.05.014, PMID: 20627081

D’Brot A, Chen P, Vaishnav M, Yuan S, Akey CW, Abrams JM. 2013. Tango7 directs cellular remodeling by the Drosophila apotosome. Genes & Development 27:1650–1655. DOI: https://doi.org/10.1101/gad.219287.113, PMID: 23913920

Diwanji N, Bergmann A. 2017a. The beneficial role of extracellular reactive oxygen species in apoptosis-induced compensatory proliferation. Fly 11:1–7. DOI: https://doi.org/10.1080/19336934.2016.1222997, PMID: 27575697

Diwanji N, Bergmann A. 2017b. An unexpected friend - ROS in apoptosis-induced compensatory proliferation: Implications for regeneration and cancer. Seminars in Cell & Developmental Biology. DOI: https://doi.org/10.1016/j.semcdb.2017.07.004, PMID: 28688927

Donato AL, Huang Q, Liu X, Li F, Zimmerman MA, Li CY. 2014. Caspase 3 promotes surviving melanoma tumor cell growth after cytotoxic therapy. Journal of Investigative Dermatology 134:1686–1692. DOI: https://doi.org/10.1038/jid.2014.18, PMID: 24434746
Elsum I, Yates L, Humbert PO, Richardson HE. 2012. The Scribble-Dlg-Lgl polarity module in development and cancer: from flies to man. Essays In Biochemistry 53:141–168. DOI: https://doi.org/10.1042/bse0530141,

PMID: 22928514

Enomoto M, Kizawa D, Ohsawa S, Igaki T. 2015. JNK signaling is converted from anti- to pro-tumor pathway by Ras-mediated switch of Warts activity. Developmental Biology 403:162–171. DOI: https://doi.org/10.1016/j.devbio.2015.05.001, PMID: 25967126

Fan Y, Bergmann A. 2008. Distinct mechanisms of apoptosis-induced compensatory proliferation in proliferating and differentiating tissues in the Drosophila eye. Developmental Cell 14:399–410. DOI: https://doi.org/10.1016/j.devcel.2008.01.003, PMID: 18331718

Fan Y, Bergmann A. 2010. The cleaved-Caspase-3 antibody is a marker of Caspase-9-like DRONC activity in Drosophila. Cell Death and Differentiation 17:534–539. DOI: https://doi.org/10.1038/cdd.2009.185, PMID: 19960024

Fan Y, Bergmann A. 2014. Multiple mechanisms modulate distinct cellular susceptibilities toward apoptosis in the developing Drosophila eye. Developmental Cell 30:48–60. DOI: https://doi.org/10.1016/j.devcel.2014.05.007, PMID: 24981611

Fan Y, Wang S, Hernandez J, Yenigun VB, Hertlein G, Fogarty CE, Lindblad JL, Bergmann A. 2014. Genetic models of apoptosis-induced proliferation decipher activation of JNK and identify a requirement of EGFR signaling for tissue regenerative responses in Drosophila. PLoS Genetics 10:e1004131. DOI: https://doi.org/10.1371/journal.pgen.1004131, PMID: 24497843

Fogarty CE, Bergmann A. 2013. Detecting caspase activity in Drosophila larval imaginal discs. Methods in Molecular Biology 1133:109–117. DOI: https://doi.org/10.1007/978-1-4939-0357-3_7, PMID: 24567098

Fogarty CE, Diwanji N, Lindblad JL, Tare M, Amcheslavsky A, Makhijani K, Brückner K, Fan Y, Bergmann A. 2016. Exocellular reactive oxygen species drive apoptosis-induced proliferation via drosophila macrophages. Current Biology 26:575–584. DOI: https://doi.org/10.1016/j.cub.2015.12.064, PMID: 26898463

Fogarty CE, Bergmann A. 2017. Killers creating new life: caspases drive apoptosis-induced proliferation in tissue repair and disease. Cell Death and Differentiation 24:1390–1400. DOI: https://doi.org/10.1038/cdd.2017.47, PMID: 28362431

Fuchs Y, Steller H. 2011. Programmed cell death in animal development and disease. Cell 147:742–758. DOI: https://doi.org/10.1016/j.cell.2011.10.033, PMID: 22078876

Gateff E. 1978. Malignant neoplasms of genetic origin in Drosophila melanogaster. Science 200:1448–1459.

Gateff E. 1994. Tumor suppressor and overgrowth suppressor genes of Drosophila melanogaster: developmental aspects. The International journal of developmental biology 38:565–590. PMID: 7779680

Gavrieli Y, Sherman Y, Ben-Sasson SA. 1992. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. The Journal of Cell Biology 119:493–501. DOI: https://doi.org/10.1083/jcb.119.3.493, PMID: 1400587

Ha EM, Oh CT, Bae YS, Lee WJ. 2005a. A direct role for dual oxidase in Drosophila gut immunity. Science 310:847–850. DOI: https://doi.org/10.1126/science.1117311, PMID: 16272120

Ha EM, Oh CT, Ryu JH, Bae YS, Kang SW, Jang IH, Brey PT, Lee WJ. 2005. An antioxidant system required for host protection against gut infection in Drosophila. Developmental Cell 8:125–132. DOI: https://doi.org/10.1016/j.devcel.2004.11.007, PMID: 15621536

Hanahan D, Weinberg RA. 2000. The hallmarks of cancer. Cell 100:57–70. DOI: https://doi.org/10.1016/S0092-8674(00)01683-9, PMID: 10647931

Hanahan D, Weinberg RA. 2011. Hallmarks of cancer: the next generation. Cell 144:664–674. DOI: https://doi.org/10.1016/j.cell.2011.02.013, PMID: 21376230

Hawkins CJ, Yoo SJ, Peterson EP, Wang SL, Vernoy SY, Hay BA. 2000. The Drosophila caspase DRONC cleaves following glutamate or aspartate and is regulated by DIA1P1, Hid, and GRIM. The Journal of Biological Chemistry 275:27084–27093. DOI: https://doi.org/10.1074/jbc.M000869200, PMID: 10825159

Hay BA, Wolff T, Rubin GM. 1994. Expression of baculovirus P35 prevents cell death in Drosophila. Development 120:2121–2129. PMID: 7925015

Hirabayashi S, Baranski TJ, Cagan RL. 2013. Transformed Drosophila cells evade diet-mediated insulin resistance through wingless signaling. Cell 154:664–675. DOI: https://doi.org/10.1016/j.cell.2013.06.030, PMID: 23911328

Ho LH, Taylor R, Dorstyn L, Cakouros D, Bouillet P, Kumar S. 2009. A tumor suppressor function for caspase-2. Proceedings of the National Academy of Sciences of the United States of America 106:5336–5341. DOI: https://doi.org/10.1073/pnas.0811928106, PMID: 19279217

Huang Q, Li F, Liu X, Li W, Shi W, Peng Y, Tan AC, Zhou L, Shen J, Han G, Wang XJ, Thorburn J, Thorburn A, Jimeno A, Raben D, Bedford JS, Li CY. 2011. Caspase 3-mediated stimulation of nuclear DNA fragmentation. Nature Medicine 17:860–866. DOI: https://doi.org/10.1038/nm.2385, PMID: 21725296

Huh JR, Guo M, Hay BA. 2004. Compensatory proliferation induced by cell death in the Drosophila wing disc requires activity of the apical cell death caspase Drong in a nonapoptotic role. Current Biology 14:1262–1266. DOI: https://doi.org/10.1016/j.cub.2004.06.015, PMID: 15268856

Ichiyama G, Tait SW. 2016. A fate worse than death: apoptosis as an oncogenic process. Nature Reviews Cancer 16:539–548. DOI: https://doi.org/10.1038/nrc.2016.58, PMID: 27364482

Igaki T, Pagliarini RA, Xu T. 2006. Loss of cell polarity drives tumor growth and invasion through JNK activation in Drosophila. Current Biology 16:1139–1146. DOI: https://doi.org/10.1016/j.cub.2006.04.042, PMID: 16753569
Igaki T, Pastor-Pareja JC, Aonuma H, Miura M, Xu T. 2009. Intrinsic tumor suppression and epithelial maintenance by endocytic activation of Eiger/TNF signaling in Drosophila. Developmental Cell 16:458–465.

DOI: https://doi.org/10.1016/j.devcel.2009.01.002, PMID: 19289090

Katheder NS, Khezri R, O’Farrell F, Schultz SW, Jain A, Rahman MM, Schink KO, Theodossiou TA, Johansen T, Juhász G, Bilder D, Brech A, Stemmark H, Rusten TE. 2017. Microenvironmental autophagy promotes tumour growth. Nature 541:417–420. DOI: https://doi.org/10.1038/nature20815, PMID: 28077876

Kondo S, Seno-Matsuda N, Hiromi Y, Miura M. 2006. DRONC coordinates cell death and compensatory proliferation. Molecular and Cellular Biology 26:7258–7268. DOI: https://doi.org/10.1128/MCB.00183-06, PMID: 16980627

Kurada P, White K. 1998. Ras promotes cell survival in Drosophila by downregulating hid expression. Cell 95:319–329. DOI: https://doi.org/10.1016/S0092-8674(00)81764-X, PMID: 9814703

Kurtova AV, Xiao J, Mo Q, Pazhanisamy S, Krasnov R, Lerner SP, Chen F, Roh TT, Lay E, Ho PL, Chan KS. 2015. Blocking PGE2-induced tumour repopulation abrogates bladder cancer chemoresistance. Nature 517:209–213. DOI: https://doi.org/10.1038/nature14034, PMID: 25470039

Kurucz E, Váčzi M, Márkus R, Laurinycz B, Vilmos P, Zsámboki J, Csorba K, Gateff E, Hultmark D, André I. 2007. Definition of Drosophila hemocyte subsets by cell-type specific antigens. Acta Biologica Hungarica 58:95–111. DOI: https://doi.org/10.1556/ABiol.58.2007.Suppl.8, PMID: 18297977

Külshammer E, Uhlirová M. 2013. The actin cross-linker Filamin/Cheerio mediates tumor malignancy downstream of JNK signaling. Journal of Cell Science 126:927–938. DOI: https://doi.org/10.1242/jcs.114462, PMID: 23239028

Külshammer E, Mundorf J, Klinic M, Frommolt P, Wagle P, Uhlirová M. 2015. Interplay among Drosophila transcription factors Ets21c, Fos and Ftz-F1 drives JNK-mediated tumor malignancy. Disease Models & Mechanisms 8:1279–1293. DOI: https://doi.org/10.1242/dmm.020719, PMID: 26398940

Lee T, Luo L. 1999. Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron 22:451–461. DOI: https://doi.org/10.1016/S0896-6273(00)80701-1, PMID: 10197526

Leong GR, Goulding KR, Amin N, Richardson HE, Brumby AM. 2009. Scribble mutants promote αPKC and JNK-dependent epithelial neoplasia independently of Crumbs. BMC Biology 7:62. DOI: https://doi.org/10.1186/1741-7007-7-62, PMID: 19778415

Leulier F, Ribeiro PS, Palmer E, Tenev T, Takahashi K, Robertson D, Zachariou A, Ueda R, Meier P. 2006. Systematic in vivo RNAi analysis of putative components of the Drosophila cell death machinery. Cell Death and Differentiation 13:1663–1674. DOI: https://doi.org/10.1038/sj.cdd.4400868, PMID: 16485033

Li F, Huang Q, Chen J, Peng Y, Roop DR, Bedford JS, Li CY. 2010. Apoptotic cells activate the “phoenix rising” pathway to promote wound healing and tissue regeneration. Science Signaling 3:a13. DOI: https://doi.org/10.1126/sci signal.2000634

Manent J, Banerjee S, de Matos Simoes R, Zoranovic T, Mitsiades C, Penninger JM, Simpson KJ, Humbert PO, Richmond HE. 2017. Autophagy suppresses Ras-driven epithelial tumourigenesis by limiting the accumulation of reactive oxygen species. Oncogene. DOI: https://doi.org/10.1038/onc.2017.175, PMID: 28581519

Martin-Belmonte F, Perez-Moreno M. 2011. Epithelial cell polarity, stem cells and cancer. Nature Reviews Cancer 12:23–38. DOI: https://doi.org/10.1038/nrc3169, PMID: 22169974

Martin FA, Pérez-Garrio A, Morata G. 2009. Apoptosis in Drosophila: compensatory proliferation and undead cells. The International Journal of Developmental Biology 53:1341–1347. DOI: https://doi.org/10.1387/ijdb.072447fm, PMID: 19247932

Meier P, Silke J, Leeser SJ, Evan GI. 2000. The Drosophila caspase DRONC is regulated by DIAP1. The EMBO Journal 19:598–611. DOI: https://doi.org/10.1093/emboj/19.4.598, PMID: 10675329

Menéndez J, Pérez-Garrio A, Calleja M, Morata G. 2010. A tumor-suppressing mechanism in Drosophila involving cell competition and the Hippo pathway. Developmental Cell 17:799–810. DOI: https://doi.org/10.1016/j.devcel.2009.09.017, PMID: 2006343

Mollereau B, Perez-Garrio A, Bergmann A, Miura M, Gerlitz O, Ryoo HD, Steller H, Morata G. 2013. Compensatory proliferation and apoptosis-induced proliferation: a need for clarification. Cell Death and Differentiation 20:181. DOI: https://doi.org/10.1038/cdd.2012.82, PMID: 22722336

Moreno E, Yan M, Basler K. 2002. Evolution of TNF signaling mechanisms: JNK-dependent apoptosis triggered by Eiger, the Drosophila homolog of the TNF superfamily. Current Biology : CB 12:1263–1268. DOI: https://doi.org/10.1016/S0960-9822(02)00954-5, PMID: 12176339

Mukherjee A, Williams DW. 2017. More alive than dead: non-apoptotic roles for caspases in neuronal development, plasticity and disease. Cell Death and Differentiation 24:1411–1421. DOI: https://doi.org/10.1038/cdd.2017.64, PMID: 2864437

Nakajima YI, Kuranaga E. 2017. Caspase-dependent non-apoptotic processes in development. Cell Death and Differentiation 24:1422–1430. DOI: https://doi.org/10.1038/cdd.2017.36, PMID: 28524858

Newcombe TP, Asling B, Dickson BJ. 2000. Analysis of Drosophila photoreceptor axon guidance in eye-specific mosaics. Development 127:851–860. PMID: 10648243

Ohsawa S, Sugimura K, Takino K, Xu T, Miyawaki A, Igaki T. 2011. Elimination of oncogenic neighbors by JNK-mediated engulfment in Drosophila. Developmental Cell 20:315–328. DOI: https://doi.org/10.1016/j.devcel.2011.02.007, PMID: 21397843

Owusu-Ansah E, Yavari A, Banerjee U. 2008. A protocol for in vivo detection of reactive oxygen species. Protocol Exchange. DOI: https://doi.org/10.1038/nprot.2008.23

Pagliarini RA, Xu T. 2003. A genetic screen in Drosophila for metastatic behavior. Science 302:1227–1231. DOI: https://doi.org/10.1126/science.1088474, PMID: 14551319

Pérez et al. eLife 2017;6:e26747. DOI: https://doi.org/10.7554/eLife.26747
Parisi F, Stefanatos RK, Strathdee K, Yu Y, Vidal M. 2014. Transformed epithelia trigger non-tissue-autonomous tumor suppressor response by adipocytes via activation of Toll and Eiger/TNF signaling. Cell Reports 6:855–867. DOI: https://doi.org/10.1016/j.celrep.2014.01.039, PMID: 24582964

Pastor-Pareja JC, Wu M, Xu T. 2008. An innate immune response of blood cells to tumors and tissue damage in Drosophila. Disease Models and Mechanisms 1:144–154. DOI: https://doi.org/10.1242/dmm.000950, PMID: 19048777

Pérez E, Das G, Bergmann A, Baehrecke EH. 2015. Autophagy regulates tissue overgrowth in a context-dependent manner. Oncogene 34:3369–3376. DOI: https://doi.org/10.1038/onc.2014.285, PMID: 25174403

Pérez-Garíjo A, Martin FA, Morata G. 2004. Caspase inhibition during apoptosis causes abnormal signalling and developmental aberrations in Drosophila. Development 131:5591–5598. DOI: https://doi.org/10.1242/dev.01432, PMID: 15496444

Pérez-Garíjo A, Martin FA, Struhl G, Morata G. 2005. Dpp signaling and the induction of neoplastic tumors by caspase-inhibited apoptotic cells in Drosophila. Proceedings of the National Academy of Sciences 102:17664–17669. DOI: https://doi.org/10.1073/pnas.0508966102, PMID: 16314564

Pérez-Garíjo A, Shlekovk E, Morata G. 2009. The role of Dpp and Wg in compensatory proliferation and in the formation of hyperplastic overgrowth caused by apoptotic cells in the Drosophila wing disc. Development 136:1169–1177. DOI: https://doi.org/10.1242/dev.034017, PMID: 19424279

Rudrapatna VA, Banj E, Cagan RL. 2013. Caspase signalling in the absence of apoptosis drives Jnk-dependent invasion. EMBO Reports 14:172–177. DOI: https://doi.org/10.1002/emb.2012.217, PMID: 23306653

Ryoo HD, Gorenc T, Steller H. 2004. Apoptotic cells can induce compensatory cell proliferation through the JNK and the Wingless signaling pathways. Developmental Cell 7:491–501. DOI: https://doi.org/10.1016/j.devcel.2004.08.019, PMID: 15469938

Ryoo HD, Bergmann A. 2012. The role of apoptosis-induced proliferation for regeneration and cancer. Cold Spring Harbor Perspectives in Biology 4:a008797. DOI: https://doi.org/10.1101/cshperspect.a008797, PMID: 22855725

Salvesen GS, Hempel A, Coll NS. 2016. Protease signaling in animal and plant-regulated cell death. The FEBS Journal 283:2577–2598. DOI: https://doi.org/10.1111/febs.13613, PMID: 26648190

Santabárbara-Ruiz P, López-Santillán M, Martínez-Rodríguez I, Binaquí-Casas A, Pérez L, Milán M, Corominas M, Serras F. 2015. ROS-Induced JNK and p38 Signaling Required for Unpaired Cytokine Activation during Drosophila Regeneration. PLOS Genetics 11:e1005595. DOI: https://doi.org/10.1371/journal.pgen.1005595, PMID: 26496642

Shafique E, Torina A, Reichert K, Colantuono B, Nur N, Zeeshan K, Ravichandran V, Liu Y, Feng J, Zeeshan K, Benjamin LE, Irani K, Harrington EO, Sellke FW, Abid MR. 2017. Mitochondrial redox plays a critical role in the paradoxic effects of NAPDH oxidase-derived ROS on coronary endothelium. eLife 6:e234–246. DOI: https://doi.org/10.7554/eLife.2016.01133, PMID: 25174403

Shalini S, Dorstyn L, Dawar S, Kumar S. 2015. Old, new and emerging functions of caspases. Cell Death and Differentiation 22:526–539. DOI: https://doi.org/10.1038/cdd.2014.216, PMID: 25174403

Shlekovk E, Morata G. 2012. A dp53/JNK-dependant feedback amplification loop is essential for the apoptotic response to stress in Drosophila. Cell Death and Differentiation 19:451–460. DOI: https://doi.org/10.1038/cdd.2011.113, PMID: 21886179

Srinivasan A, Roth KA, Sayers RO, Shindler KS, Wong AM, Fritz LC, Tomaselli KJ. 1998. In situ immunodetection of activated caspase-3 in apoptotic neurons in the developing nervous system. Cell Death and Differentiation 5:1004–1016. DOI: https://doi.org/10.1038/sj.cdd.4400449, PMID: 9894607

Tseng AS, Adams DS, Qiu D, Koustubhan P, Levin M. 2007. Apoptosis is required during early stages of tail regeneration in Xenopus laevis. Developmental Biology 301:62–69. DOI: https://doi.org/10.1016/j.ydbio.2006.10.048, PMID: 17150209

Uhlirrova M, Jasper H, Bohmann D. 2005. Non-cell-autonomous induction of tissue overgrowth by JNK/Ras cooperation in a Drosophila tumor model. Proceedings of the National Academy of Sciences 102:13123–13128. DOI: https://doi.org/10.1073/pnas.0504170102, PMID: 16150723

Uhlirrova M, Bohmann D. 2006. JNK- and Fos-regulated Mmp1 expression cooperates with Ras to induce invasive tumors in Drosophila. The EMBO Journal 25:5294–5304. DOI: https://doi.org/10.1038/sj.emboj.7601401, PMID: 17082773

Vaughan J, Igaki T. 2016. Slit-Robo Repulsive Signaling Extrudes Tumorigenic Cells from Epithelia. Developmental Cell 39:683–695. DOI: https://doi.org/10.1016/j.devcel.2016.11.015, PMID: 27997825

Wang CW, Purkayastha A, Jones KT, Thaker SK, Banerjee U. 2016. In vivo genetic dissection of tumor growth and the Warburg effect. eLife 5:e18126. DOI: https://doi.org/10.7554/eLife.18126, PMID: 27585295

Wells BS, Yoshida E, Johnston LA. 2006. Compensatory proliferation in Drosophila imaginal discs requires Dronc-receptor dependent p53 activity. Development 133:13123–13128. DOI: https://doi.org/10.1242/dev.01432, PMID: 16150723

Zhang Z, Wang M, Zhou L, Feng X, Cheng J, Yu Y, Gong Y, Zhu Y, Li C, Tian L, Huang Q. 2015. Increased HMGB1 and cleaved caspase-3 stimulate the proliferation of tumor cells and are correlated with the poor...
