

Review

Your neighbours matter – non-autonomous control of apoptosis in development and disease

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Traditionally, the regulation of apoptosis has been thought of as an autonomous process in which the dying cell dictates its own demise. However, emerging studies in genetically tractable multicellular organisms, such as *Caenorhabditis elegans* and *Drosophila*, have revealed that death is often a communal event. Here, we review the current literature on non-autonomous mechanisms governing apoptosis in multiple cellular contexts. The importance of the cellular community in dictating the funeral arrangements of apoptotic cells has profound implications in development and disease.

Cell Death and Differentiation (2016) 23, 1110–1118; doi:10.1038/cdd.2016.41; published online 13 May 2016

Facts

- Engulfment genes act non-autonomously to enable various forms of programmed cell death during development.
- Cells that have initiated apoptosis can coax surrounding cells to evade or undergo apoptosis.
- Stress-induced apoptosis relies on non-autonomous factors.

Open Questions

- How are the intercellular communication networks that regulate non-autonomous apoptosis organised?
- Relevance of non-autonomous apoptosis regulation to cancer and other diseases.
- Are cell non-autonomous apoptosis signals stimulus-induced or constitutively on?

Development occurs through a series of finely orchestrated events that results in the precise sculpting of tissues and organs of varying shapes and sizes. One of the most important processes in animal development is programmed cell death (PCD), where specific sets of cells are eliminated from the organism under rigorous genetic control. Apoptosis is the best

characterised form of PCD, and is critically important not only for development but also differentiation, immunity, stress response, genome stability and tissue homeostasis in both multicellular and unicellular organisms.^{1–4} In general, errors in the regulation of apoptosis can lead to disastrous consequences, such as developmental abnormalities, degenerative diseases, autoimmunity, susceptibility to infection and cancer.^{1,5}

PCD via apoptosis occurs through distinct cellular signalling events that culminate in morphological changes including nuclear and cellular fragmentation, and eventual engulfment of the dying cell by surrounding healthy cells. Apoptosis has traditionally been viewed as a process in which the dying cell controls its own demise in response to stresses or developmentally programmed cues. Historically, the first indication that apoptosis can be regulated by extrinsic biological factors came from the discovery of pro-apoptotic tumour necrosis factors (TNF) and anti-apoptotic growth factors, both of which are now well-characterised.^{6–10} For this review, we focus on more recently discovered non-autonomous regulators of apoptosis and refer readers to several excellent reviews of Rita Levi-Montalcini's work on growth factors, as well as reviews on the discovery and characterisation of TNF.^{11–14}

In recent years, work in a variety of model organisms has uncovered many novel cell non-autonomous regulators of

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Abbreviations: PCD, programmed cell death; TNF, tumour necrosis factor; Bcl-2, B-cell lymphoma 2; CEP-1, *C. elegans* p53-like 1; EGL-1, egg laying defective 1; CED-9, cell death abnormal 9; CED-3, cell death abnormal 3; CED-4, cell death abnormal 4; IAP, inhibitor of apoptosis protein; Hid, head involution defective; Rpr, reaper; Skl, sickle; Diap1, *Drosophila* inhibitor of apoptosis protein 1; Dronc, *Drosophila* Nedd2-like caspase; Drice, *Drosophila* interleukin-1 β converting enzyme; Dcp-1, *Drosophila* caspase 1; Decay, Death executioner caspase related to Apopain/Yama; JNK, c-Jun N-terminal kinase; BH3, Bcl-2 homology 3; Apaf-1, Apoptotic protease activating factor 1; Xkr8, Xk-related protein 8; CED-8, cell death defective 8; VC, ventral cord; CED-1, cell death defective 1; CED-2, cell death defective 2; CED-5, cell death defective 5; CED-6, cell death defective 6; CED-10, cell death defective 10; CED-12, cell death defective 12; PSR-1, phosphatidyserine receptor 1; SRCM-1, scramblase 1; INA-1, integrin α 1; SRC-1, sarcoma oncogene related 1; Ras, Rat sarcoma oncogene; MAP, mitogen activated protein; vps25, vacuolar protein-sorting-associated protein 25; Hippo, Hippopotamus-like; Yorkie, LEC, larval epidermal cell; RanBP2, Ran-binding Protein 2; *lin-35*, abnormal cell lineage 35; *kri-1*, Krev interaction trapped homologue 1 (KRIT1); CCM1, cerebral cavernous malformation 1; PI3K, phosphatidylinositol-3 kinase; IGF-1, insulin-like growth factor 1; DAF-2, abnormal dauer formation 2; AKT-1/2, RAC- α serine/threonine-protein kinase 1/2; DAF-16, abnormal dauer formation 16; FOXO, forkhead box O; HIF, Hypoxia-inducible factor; VHL, von Hippel-Lindau; *tyr-2/3*, tyrosinase 2/3; TRP2, L-dopachrome tautomerase; HIPK2, homeodomain-interacting protein kinase 2; IRE-1, inositol-requiring protein 1; VAB-1, variable abnormal morphology 1; VEGF, vascular endothelial growth factor; RNAi, RNA interference

Received 18.1.16; revised 14.3.16; accepted 07.4.16; Edited by E Baehrecke; published online 13.5.16

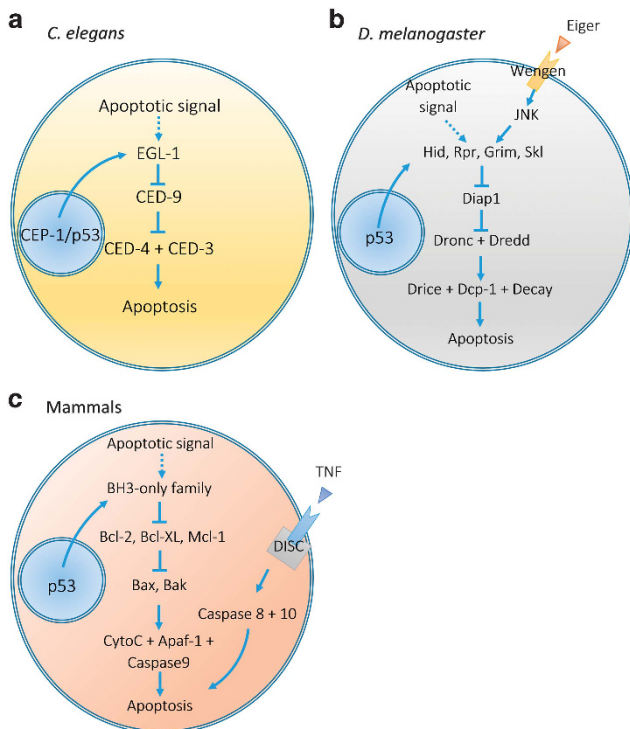


Figure 1 Apoptosis pathways in various organisms. (a) In *C. elegans*, a stimulus (e.g. CEP-1/p53 in response to DNA damage) activates the core apoptosis pathway through transcriptional induction of EGL-1, leading to a suppression of CED-9. Suppression of CED-9 results in the release of CED-3 and formation of a complex with CED-4. This complex leads to apoptosis. (b) Apoptosis in *D. melanogaster* can be initiated autonomously or through receptor-mediated pathways. Activation of antagonists of inhibitors of apoptosis (IAP) including Hid, Rpr, Grim or Skl leads to inhibition of Diap1. Consequently, initiator caspases (Dronc and Dredd) are activated and lead to activation of effector caspases (Drice, Dcp-1 and Decay) and apoptosis. This pathway can be influenced by extrinsic factors including Eiger, upstream of JNK. (c) In mammals, apoptosis can be initiated intrinsically or extrinsically. The intrinsic pathway is similar to *C. elegans* and *D. melanogaster* pathways. In the extrinsic pathway, activation of a 'death' receptor leads to formation of the death-inducing signalling complex (DISC) and activation of caspases 8 and 10, leading to apoptosis

apoptosis, where genetic or biochemical factors in one population of cells can activate and fine-tune the apoptotic program in different populations of cells. Conceptually, new findings demonstrate that even when apoptosis signalling is initiated in a dying cell (and in some cases progressed very far), its progress and eventual completion – which have been regarded as being largely autonomous – depends on regulatory input from neighbouring cells. In this review, we outline several novel non-autonomous regulators of apoptosis, as well as gaps in our understanding of the intercellular communication during this process. Finally, we speculate on the adaptive purpose of these control mechanisms in development, physiology and disease.

Brief Overview of Apoptosis Pathways

In mammals, there are two distinct apoptosis pathways, intrinsic and extrinsic, that lead to activation of pro-apoptotic caspases (summarised in Figure 1c). In the intrinsic pathway,

intracellular signals (e.g. p53 in response to DNA damage) result in the production of pro-apoptotic Bcl-2 family members that contain single BH3 domains (BH3-only proteins).¹⁵ Interactions between subsets of BH3-only proteins with anti-apoptotic Bcl-2 family members that contain multiple BH domains results in mitochondrial outer membrane permeabilization and cytochrome *c* efflux into the cytosol.^{15–17} This enables the assembly of a complex containing cytochrome *c*, Apaf-1 and caspase 9, termed as the apoptosome holoenzyme, which activates downstream effector caspases that trigger cell execution.^{16,18} In contrast, apoptosis initiated by leukocytes, such as natural killer or cytotoxic T cells, is extrinsic and receptor mediated. Binding of pro-apoptotic ligands (e.g. cytokines of the TNF superfamily) to death receptors leads to the formation of a death-inducing signalling complex, resulting in the activation of caspases 8 and 10.^{19–21} Subsequently, in both pathways, cells that have undergone apoptosis are rapidly engulfed by macrophages or other cells. For comprehensive descriptions of the molecular framework of intrinsic and extrinsic pathways, we recommend several recent reviews.^{19,22–24}

Regulation of Apoptosis by Engulfing Cells

Pioneering studies in the nematode worm *C. elegans* identified the core apoptosis genes and demonstrated that they function in a linear pathway (Figure 1a).^{25,26} The major steps of this pathway are conserved in humans, but with differences in complexity and involvement of mitochondrial proteins. Although in most organisms apoptosis is necessary for viability, *C. elegans* mutants that are unable to eliminate cells by apoptosis during development are viable, making it a convenient model organism to study genetic mechanisms governing this process *in vivo*.^{3,25,26} Although, transcriptional activation of the pro-apoptotic BH3-only gene *egl-1* is sufficient to induce apoptosis, which has been regarded as a cell-autonomous process (Figure 1a)³ it is clear now that there is regulatory input other than *egl-1* induction alone. In fact, in *C. elegans*, there is now clear evidence of non-autonomous regulation of core apoptotic machinery at each of its distinct phases (i.e. specification, execution and engulfment).

In mammals, cells that are undergoing apoptosis are engulfed and degraded by macrophages in order to remove cellular debris that can cause secondary necrosis of surrounding healthy cells. In *C. elegans*, engulfment is carried out by non-specialized cells surrounding the dying cell.^{3,27} In many cases during development, cell death does not have to be initiated or complete before engulfment begins.²⁸ To initiate engulfment, apoptotic cells display surface markers such as phosphatidylserine (PS, the so-called 'eat me' signal) that allow recognition by engulfing cells.^{29,30} These signals are integrated by two genetically distinct pathways in engulfing cells that facilitate engulfment of the dying cell.^{29–33}

In *C. elegans*, engulfment was traditionally viewed as the end stage of apoptosis and dispensable for its activation as cell corpses are readily observed in engulfment defective mutants.^{34,35} However, the first evidence of non-autonomous apoptosis regulation in the worm was shown to be acting during the engulfment phase. The caspase CED-3 is essential for activation of apoptosis, and *ced-3* partial loss-of-function

mutants (hypomorphs) have reduced levels of apoptosis during embryonic development.³⁶ Intriguingly, enhancer screens performed in these hypomorphic *ced-3* mutants uncovered mutations in engulfment genes that enhanced cell survival.³⁴ Engulfment defective and hypomorphic *ced-3* double mutants exhibit a three- to fourfold increase in cell survival compared to *ced-3* single mutants, indicating that elimination of cells by apoptosis is somehow assisted by engulfment genes.^{34,35} Interestingly, loss-of-function mutations in engulfment genes alone can increase survival of neuroblast and progenitor daughter cells normally programmed to die by apoptosis.³⁴ These surviving cells are able to initiate apoptosis and undergo morphological changes associated with CED-3 activation, such as nuclear and cytoplasmic condensation, but can occasionally reverse these effects.³⁴ This does not appear to involve regulation of the anti-apoptotic protein CED-9 or the Xkr8-like protein CED-8; perhaps acting via CED-3 through an unknown mechanism.³⁴ Undead neural progenitors can differentiate into VC motor neurons, although the penetrance and number of surviving cells in engulfment defective mutants is low compared to *ced-3* mutants.

Whereas expression of engulfment genes specifically in engulfing cells is sufficient to rescue apoptosis defects, ablation of engulfing cells promotes survival and differentiation of cells normally programmed to undergo apoptosis.^{34,35} Combined, these observations established that the regulation of apoptosis by engulfment proteins is a cell non-autonomous process (Figure 2a). However, a major question that remains concerns the mechanistic basis by which engulfment genes assist the apoptotic death of their neighbours. Very recently, it was shown that the engulfment receptor CED-1 can stimulate formation of a CED-3 caspase gradient in adjacent dividing cells, resulting in its unequal distribution, and consequently, differential apoptotic potential in the daughter cells (Figure 2b).³⁷ More work needs to be done to determine exactly how CED-1 establishes a CED-3 gradient in the dying cell and whether this is a general phenomenon by which engulfment promotes apoptosis.

In many other organisms, perturbation of engulfment can lead to defects in tissue remodelling and survival of cells normally programmed to die.^{38–40} For instance, genetic ablation of macrophages in the mouse eye or inhibition of macrophages in the tadpole tail results in persistence of tissues that normally should regress.^{38,39} In addition, in the *Drosophila* ovary, engulfment machinery in follicle cells is required for death of nurse cells by a non-apoptotic process during development.⁴⁰ However, in all of these cases it is not entirely clear which factors contribute to communication between engulfing cells and dying cells. Determining these factors is fundamental to understanding PCD as a dynamic cell–cell communication process, and may shed new light on diseases involving its misregulation.

Another stage at which engulfing cells influence apoptosis is during DNA degradation. In mammals, apoptotic cells that are deficient in autonomous caspase-activated DNases are unable to degrade their own DNA.⁴¹ However, once these cells are engulfed by macrophages, DNase II from macrophage lysosomes promotes degradation of engulfed-cell DNA, which can push apoptosis to completion in a non-

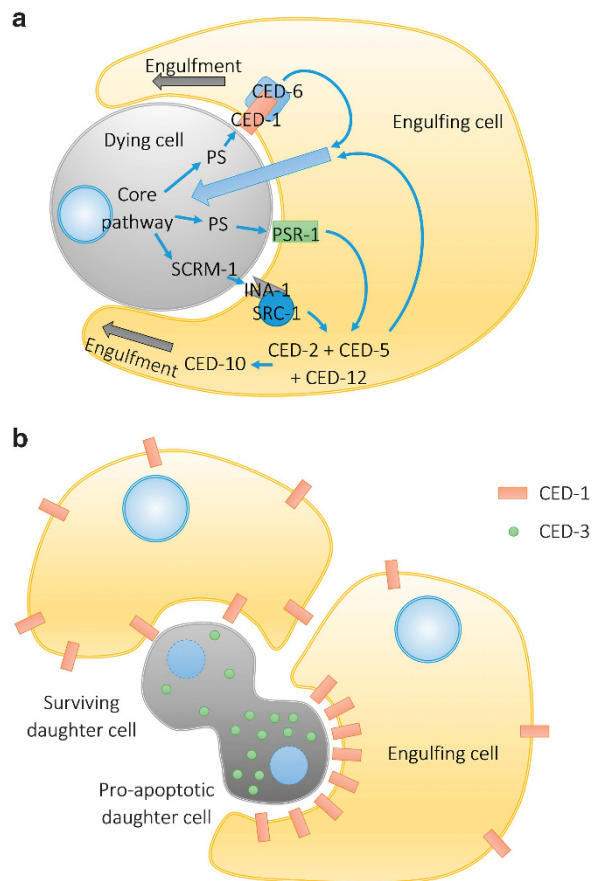


Figure 2 Engulfment pathways regulate core apoptosis machinery in *C. elegans*. (a) ‘Eat-me’ signals from the dying cell signals to phagocytic cell in order to initiate engulfment. Engulfment factors from engulfing cells can act to permit completion of apoptosis during development. (b) CED-1 in engulfing cells can cause CED-3 caspase gradients in dividing cells, which leads to its unequal distribution in daughter cells. This results in differential apoptotic potential

autonomous manner.⁴¹ In fact, caspase-activated DNases-deficient mice are fertile, whereas mice deficient in DNase II die at birth and contain many engulfed cells with undigested DNA.^{41,42} As there is conflicting evidence from *C. elegans* and other model organisms that DNase II may also have cell-autonomous roles, this is still somewhat controversial.^{43–45} It will be interesting to know whether loss of macrophage-specific nucleases allows dying cells to reverse initiation of apoptosis and undergo differentiation in a similar manner to engulfment defective mutants in *C. elegans*. Overall, in many cases engulfing cells that neighbour dying cells appear to have an integral role in the regulation of apoptosis.

Communal Suicide and Herd Mentality in Apoptotic Cells

In multicellular organisms, proper coordination of cell proliferation and apoptosis is a critical determinant of tissue shape, size and homeostasis. In *Drosophila*, apoptosis is normally prevented by the inhibitor of apoptosis (IAP) protein DIAP1.^{46–50} In response to pro-apoptotic signals, DIAP1 antagonists such as Grim, Reaper and Hid, inhibit DIAP1 and relax inhibition of the caspase Dronc, leading to activation

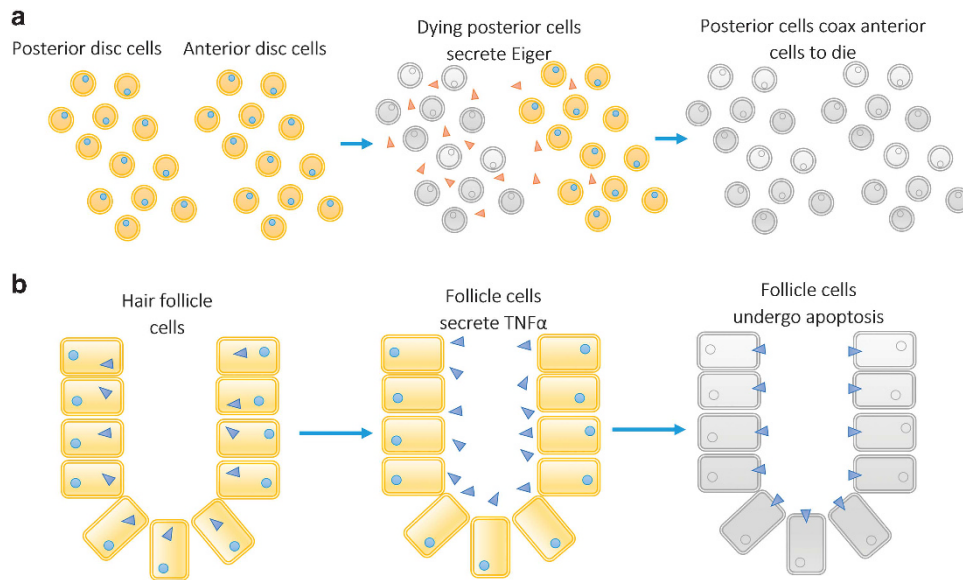


Figure 3 Non-autonomous induction of apoptosis by other apoptotic cells. (a) During *D. melanogaster* development, apoptotic cells secrete Eiger to induce apoptosis of other cells through JNK-mediated pathways. Persistence of apoptotic cells can coax groups of cells that normally survive to undergo apoptosis. (b) Mammalian hair follicle cells undergo coordinated apoptosis through secretion of TNF- α by apoptotic cells. Treatment of follicle with a TNF- α neutralising antibody is sufficient to disrupt apoptosis

of effector caspases such as DrICE and DCP-1 to trigger apoptosis (Figure 1b).^{50,51}

The connection between proliferation and apoptosis is well established in *Drosophila*, where activation of apoptosis in some tissues can trigger a process called compensatory proliferation in nearby cells, a dynamic non-autonomous process required for tissue development, remodelling and response to injury.^{48,52–58} Non-autonomous inhibition of apoptosis by mitogens and survival signals through Ras/MAP kinases has been known to converge at suppression of IAP antagonists (namely Hid) through phosphorylation.⁵⁰ However, factors have recently been identified that directly influence DIAP1 transcription in novel ways. Dying cells can inhibit apoptosis of surrounding cells through *vps25*, a component of the endosomal sorting complex required for transport, which non-autonomously induces DIAP1 and promotes proliferation.⁵⁹ Notch signalling from *vps25* mutant dying cells activates the Hippo signalling in neighbouring cells, leading to Yorkie-mediated induction of DIAP1.⁶⁰ Furthermore, activation of Notch alone is sufficient to induce Yorkie and DIAP1 in neighbouring cells.⁶⁰ In addition, hyperactivation of hedgehog signalling also makes neighbouring cells resistant to apoptosis through induction of DIAP1.⁶¹ Thus, in *Drosophila* different non-autonomous signals that can inhibit apoptosis appear to converge on DIAP1.

The processes that control tissue remodelling – proliferation, migration, and apoptosis – must be coordinated at the multicellular level. During *Drosophila* abdominal epithelial replacement in the pupal stage, larval epidermal cells (LECs) undergo apoptosis and are replaced by abdominal histoblasts that proliferate and migrate.⁶² Interactions between proliferating abdominal histoblasts and LECs were recently shown to be critical for induction of LEC apoptosis.⁶³ These histoblasts are normally arrested at G₂ before pupariation, but enter the cell cycle once the pupal stage begins.^{64,65} When histoblasts

are forced to arrest at S/G₂ phase as they are migrating, the adjacent LECs do not undergo apoptosis.⁶³ Their transition into the cell cycle is necessary for coordinating apoptosis of neighbouring LECs.⁶³ Although proximity between histoblasts and LECs is necessary, the mechanism of apoptosis activation in LECs by cell-cycle transition in histoblasts is not known. The fact that apoptosis can be regulated non-autonomously by such a fundamental process as the cell cycle highlights the importance of coordinating life and death decisions between tissues and cells during development.

Interestingly, in both *Drosophila* and vertebrate development, there are also many instances of communal death, or group suicide behaviour, where a number of adjacent cells undergo apoptosis rapidly and in synchrony (Figure 3).^{66–71} The mechanisms that govern this are best understood in *Drosophila*, where signals emanating from dying cells are at least partially sufficient to stimulate apoptosis of their neighbours. Expression of the viral caspase inhibitor p35 leads to survival of cells programmed to undergo apoptosis.⁷² When p35 and the pro-apoptotic gene *hid* are overexpressed in the posterior wing imaginal discs, the resulting undead cells are able to coax large numbers of neighbouring anterior disc cells to commit suicide (Figure 3a).⁷³ Moreover, the coaxed cells fully undergo apoptosis, whereas the undead cells show no biochemical markers of apoptosis such as caspase activation and fragmented DNA.⁷³ This phenomenon is also observed in the haltere and leg discs, but not in the eye-antennal discs, and is dependent on the amount of apoptotic stimulus.⁷³

In each of the discs tested, the posterior parts with ectopic apoptosis were enlarged as a consequence of compensatory proliferation.⁷³ It is possible that some sort of secondary compensatory effect is responsible for the resulting abnormal apoptosis in the anterior disc. Harboring a large number of abnormal undead cells for an extended period of time may

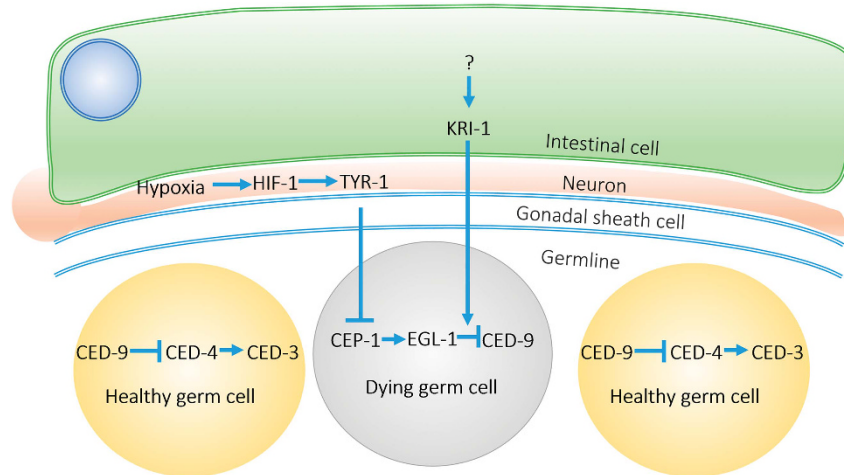


Figure 4 Non-autonomous regulation of germ cell apoptosis in *C. elegans*. CEP-1/p53 is activated in response to DNA damage and initiates apoptosis. Permissive signal from intestinal cells (KRI-1) is required for progression of apoptotic cascade in germ cells. In contrast, accumulation of neuronal HIF-1 results in suppression of the apoptotic cascade, probably through inhibition of CEP-1. Other somatic factors such as insulin/IGF-1 signalling and the RB protein LIN-35 also contribute to apoptosis

result in altered secretion of morphogens that trigger apoptosis. However, correcting for size, morphogen gradients or other developmental effects, does not inhibit activation of non-autonomous apoptosis.⁷³ Only overexpressing *hid* or *rpr* (without p35) in posterior disc cells is sufficient to induce apoptosis in anterior disc cells.⁷³ Altogether, these observations suggest that a secreted factor from bona fide apoptotic cells is sufficient to induce the apoptotic death of their neighbours.

How do apoptotic cells coax non-apoptotic cells to commit suicide? It turns out that the c-Jun N-terminal kinase (JNK) pathway, a MAP kinase pathway that regulates stress response in many organisms, has a role.^{48,74} Flies with undead cells in the posterior compartment, and with upregulated JNK signalling through ablation of its inhibitor *puckered*, show much higher levels of non-autonomous apoptosis.⁷³ Conversely, downregulation of JNK signalling in the anterior disc alone is sufficient to abrogate induction of non-autonomous apoptosis, suggesting that the JNK pathway integrates some pro-apoptotic signal.⁷³ What is that signal? The *Drosophila* TNF ortholog, Eiger, is known to activate JNK dependent apoptosis in *Drosophila* (Figure 3a).^{75–77} In fact, Eiger is upregulated in undead cells, and its depletion in these cells significantly reduces apoptosis in different cells of the anterior disc, consistent with the idea that its secretion activates apoptosis of neighbouring cells.⁷³ This may represent an ancestral developmental mechanism by which tissues sculpt themselves into distinct shapes and sizes.

In mice there is evidence that dying cells can induce death of their neighbouring cells in a manner similar to the *Drosophila* imaginal wing disc (Figure 3b). During hair follicle cycle progression, a number of follicle cells undergo group suicide, which is dependent on TNF signalling.^{67,78,79} Interestingly, only apoptotic cells were found to express TNF- α , which confirmed that the source of the pro-apoptotic signal was dying cells themselves, and not other tissues.⁷³ Injection of mice with TNF- α antibodies is sufficient to disrupt synchronization of apoptosis, or completely inhibit it, in hair follicles.⁷³ In

addition, in the mammalian eye, genetic ablation of the Ran-binding protein (RanBP2) in cone photoreceptors causes them to die by a non-apoptotic mechanism; however, the dying cone photoreceptors stimulate apoptotic death of neighbouring rod photoreceptors.⁸⁰ Together, these observations indicate that dying cells in vertebrates can secrete pro-apoptotic signals that initiate apoptosis of neighbouring cells in a controlled manner. It will be interesting to know the factor(s) responsible for activating the apoptotic death of rod photoreceptors. Perhaps this occurs through TNF or another secreted molecule, such as tyrosinase (see below)? Although secretion of Eiger to activate JNK and subsequently apoptosis in *Drosophila* may explain how an apoptotic signal propagates from one cell to another in other organisms, it is still not clear what establishes the borders of these apoptotic cells. Why are some cells more sensitive than others to the pro-apoptotic signal? Why is it that only anterior wing disc cells undergo non-autonomous apoptosis when undead cells are generated in the posterior? These questions are critical to explaining tissue and organ development, and are currently unanswered.

It is also interesting to note that transmission of protein aggregates between cells are able to induce non-autonomous apoptosis in the developing fly.^{81,82} Aggregates of the huntingtin protein, known for causing Huntington disease, are able to induce widespread apoptosis of nearby neurons when mutant protein is expressed in olfactory neurons.^{81,82} Interestingly, this is dependent on uptake of the aggregates as inhibition of endocytosis prevented the abnormal apoptosis.⁸¹ It will be important to determine exactly how huntingtin aggregates activate the apoptotic machinery in dying cells, which could help in the development of therapies that reverse or slow Huntington disease.

Assisted Suicide From Worms to Humans

Cells in the adult *C. elegans* hermaphrodite germline are competent to undergo apoptosis in response to a variety

of cellular stresses including DNA damage, antimetabolic compounds and pathogenic infection (reviewed in refs 83,84). DNA damage-induced germ cell apoptosis requires the p53-like protein CEP-1 in *C. elegans*.^{85,86} Tumour suppressor p53 has a central role in mediating the cellular response to stress and it is the most frequently mutated gene in human cancer.^{87,88} In *C. elegans*, genotoxic stress creates various forms of DNA damage that are recognised by a set of checkpoint proteins that transduce signals leading to the phosphorylation and stabilisation of CEP-1.^{89,90} CEP-1 activates the core apoptosis pathway in the germline by transcriptionally upregulating the BH3-only gene *egl-1*, which leads to increased EGL-1 protein that binds and inhibits the anti-apoptotic protein CED-9 (Figure 1a).^{89,91–93} Interestingly, apoptosis in response to DNA damage is not regulated entirely by the cells fated to die; communication with the neighbouring somatic cells is also essential. Several recent studies have identified factors produced in somatic cells that assist CEP-1 in promoting apoptosis of *C. elegans* germ cells (Figure 4).^{83,84,94,95}

Somatic Factors Permit *C. elegans* Germ Cell Apoptosis

In addition to CEP-1-dependent transcriptional activation of *egl-1*, germ cells also require input from the soma to promote apoptosis in response to DNA damaging agents, such as ionising radiation. CEP-1-induced apoptosis is at least partially dependent on functional *lin-35*, the *C. elegans* orthologue of the retinoblastoma susceptibility gene, in both the somatic gonad and germline.⁸⁴ Rescuing arrays in *lin-35* loss-of-function mutants fail to restore apoptosis when expressed either in the somatic gonad or the germline, indicating that at least some aspect of *lin-35* dependent apoptosis is non-autonomous.⁸⁴ Furthermore, loss-of-function mutations in the *kri-1* gene, which encodes a scaffold protein orthologous to human *KRIT1/CCM1*, completely prevent ionising radiation-induced germ cell apoptosis despite having no defects in physiological germ cell apoptosis or the DNA damage checkpoint (Figure 4).⁹⁴ Although *kri-1* does not regulate developmental apoptosis in the soma, its expression is required in the soma (intestine) to permit DNA damage-induced apoptosis in the germline by a mechanism that is independent or downstream of *cep-1*.⁹⁴

Since loss of *Krit1/CCM1* is implicated in the formation of cerebral cavernous malformations (CCM) in the human brain, it will be important to determine the mechanism by which *KRI-1* feeds into the core apoptosis pathway in the germline, and which signalling pathways it engages in the soma to assist the suicide of damaged germ cells.⁹⁶ Understanding how *KRI-1/CCM1* modulates cross-tissue signalling should also help understand the pathobiology of CCM in humans, and possibly identify non-invasive ways to treat these patients in the clinic. Many questions remain. For example, does *kri-1* mediate the secretion of a pro-apoptotic factor from intestinal cells that permits apoptosis in the germline or do intestinal cells send anti-death signals to the germline in the absence of *kri-1*? Is the *kri-1* signal constitutive or is it induced in response to genotoxic stress? Are these mechanisms conserved and relevant to CCM disease in humans?

Secreted Factors Regulate *C. elegans* Germ Cell Apoptosis

Recently, several secreted factors have been identified that promote apoptosis in the *C. elegans* germline. Across many species, activated phosphatidylinositol 3-kinase (PI3K) signalling is antagonistic to apoptosis.⁹⁷ In *C. elegans*, PI3K can be activated by the insulin/insulin-like growth factor 1 (IGF-1) receptor DAF-2. This normally leads to activation of AKT-1 and AKT-2, which are partially redundant for inhibition of the DAF-16/FOXO transcription factor.^{98,99} Curiously, whereas AKT-1 autonomously antagonises CEP-1 dependent apoptosis, DAF-2 selectively engages AKT-2 to promote apoptosis in response to DNA damage, which is independent or downstream of CEP-1.⁹⁵ Selective knockdown of DAF-2 in either the soma or germline is not sufficient to suppress DNA damage-induced germline apoptosis, which indicates that DAF-2 is likely functions in both tissues through a combination of cell-autonomous and non-autonomous mechanisms.⁹⁵ More work is needed to fully understand the mechanism by which DAF-2 regulates stress-induced apoptosis, which appears to converge on the MAPK pathway in the germline.⁹⁵

Neuronal factors can also regulate germ cell apoptosis. Hypoxia-inducible factor (HIF) is a key regulator of oxygen homeostasis that is conserved in all animals, including *C. elegans*.^{100,101} HIF is normally hydroxylated and targeted for degradation through ubiquitylation by the von Hippel-Lindau tumour suppressor (VHL) under normal physiological oxygen levels.^{102–104} Mainly two factors lead to accumulation of HIF: reduced oxygen (hypoxia) and loss-of-function in VHL, which occurs in some forms of cancer.^{105–107} Tumours with accumulated HIF generally have poor prognoses and are resistant to standard therapies.¹⁰⁸ In *C. elegans*, accumulation of neuronal HIF-1 (the alpha subunit of mammalian HIF) through loss-of-function mutations in the VHL gene (*vhl-1*) or hypoxia treatment, causes resistance of germ cells to DNA damage-induced apoptosis (Figure 4).⁸³ There is evidence that HIF-1 regulates the core apoptotic pathway at the level of (or downstream of) CEP-1 through post-translational modifications including phosphorylation, which is known to modulate its stability and activation.⁸³ An RNAi screen of HIF-1 transcriptional targets revealed that the tyrosinase genes *tyr-2* and *tyr-3* were responsible for conferring resistance to germline apoptosis in *vhl-1* mutants.⁸³ Intriguingly, loss-of-function in *vhl-1* leads to increased HIF-1-dependent expression of TYR-2 in neurons, which is secreted into the germline to inhibit CEP-1-dependent apoptosis.⁸³ TYR-2 is homologous to human TRP2, which both seem to function as L-dopachrome tautomerase, and knockdown of TRP2 sensitises cancer cells to p53-dependent apoptosis.⁸³ As this strongly suggests conservation from *C. elegans* to human, it is not clear whether TRP2 functions through a non-autonomous mechanism or how it stabilizes p53 in human cells. HIF-1 α can also induce degradation of HIPK2, a homeodomain interacting protein kinase that can phosphorylate p53, but the relevance of this *in vivo* is not clear.¹⁰⁹

In addition to the non-autonomous effects of neuronally secreted TYR-2, it has also been reported that endoplasmic reticulum stress in a set of amphid sensory neurons causes increased germline apoptosis.¹¹⁰ This appears to be mediated by

the ribonuclease inositol requiring protein-1 (IRE-1), and independently of KRI-1, but whether it functions in a similar manner to TYR-2 remains to be determined.¹¹⁰ As TYR-2 is also secreted from amphid sensory neurons, it appears that the nervous system may have numerous influences on the health of the germline. Perhaps this is the worm version of the mind–body connection?

Looking Forward: Death, Disease and New Life

In this review, we have outlined recently reported cases of non-autonomous mechanisms governing apoptosis in animals. It has become clear now that many diverse mechanisms exist to control apoptosis at many of its stages as opposed to the few that have been historically recognised as required for its initiation. Furthermore, not only do extracellular signals regulate apoptosis, dying cells can also influence developmental decisions of surrounding cells, which is reviewed in detail in ref. 111. Together, these phenomena emphasise the importance of the multicellular community in making life and death decisions of individual or groups of cells. This is not entirely surprising given that cell death exerts constant homeostatic pressures on tissues.^{112,113} To speculate, non-autonomous regulation of apoptosis may have evolved to ensure that cell death is properly orchestrated during development and in response to stress. The importance of maintaining tissue homeostasis may explain why it arose in multicellular organisms, particularly during phases of rapid tissue growth and remodelling.

Because proper regulation of apoptosis is critical for suppressing many diseases, understanding the mechanisms by which surrounding cells assist the suicide of their neighbours may have critical implications in the treatment of pathologies such as cancer, where cells eventually become resistant to apoptosis-inducing therapies. Aside from stress-induced and developmental apoptosis in *C. elegans*, there is also evidence that the ephrin receptor VAB-1 in the gonadal sheath cells regulates physiological germ cell apoptosis, suggesting that non-autonomous mechanisms may be a general principle of apoptosis control in this organism.¹¹⁴ An important question is whether signals generated by genes such as *kri-1* act constitutively to permit apoptosis, similar to what was observed for VAB-1 in physiological germ cell apoptosis, or are the signals induced by stress to fine-tune apoptotic thresholds?

Insights from tractable model organisms such as *C. elegans* and *Drosophila* provide testable hypotheses to address whether abnormal non-autonomous apoptosis regulation is a major contributing factor in human disease. For instance, it is known that evasion of apoptosis is a hallmark feature of cancer cells.¹¹⁵ Hypothetically, loss of apoptotic regulators in adjacent tissues may act to increase resistance to apoptosis by similar non-autonomous mechanisms observed in *kri-1* or *vhl-1* mutant worms. In humans, endothelial cells of the stroma have strong effects on the survival of irradiated tumour cells, conceivably through hypoxia effects or secretion of factors such as VEGF.¹¹⁶ In addition, as upregulated hedgehog signalling is a common hallmark of many human cancers, it may be that secretion of anti-apoptotic factors contributes to the aggressiveness and growth of these tumours, similar to what has been observed in *Drosophila*. In humans, mutations

in *CCM1* (the human orthologue of *C. elegans kri-1*) lead to cerebral cavernous malformations, which are abnormal vascular structures, which frequently involve loss of surrounding smooth muscle. As the only therapeutic option currently available for CCM patients is invasive neurosurgery, elucidating the *kri-1* pathway in *C. elegans* may uncover druggable targets that are conserved in humans. Another important clinical problem is the development of CCM lesions in brain cancer patients who have undergone radiotherapy.¹¹⁷ Do these radiation-induced lesions arise through known CCM signalling pathways? Going forward, identifying the secreted factors that transduce pro- and/or anti-apoptosis signals across tissue boundaries, and defining the molecular mechanisms by which they engage core apoptotic machinery, is likely to yield profound insights into the understanding and treatment of many diseases.

Although many novel examples of non-autonomous apoptosis regulation have been identified, more work needs to be done to define the mechanistic basis of intercellular communication between dying cells and their neighbours. It was shown recently that ablation of RNAi processing gene *Dicer* in mouse astroglia leads to widespread non-autonomous neuronal apoptosis and neurodegeneration.¹¹⁸ Whether this modulation of p53, through HIF-1 α and Ranbp2-induced apoptosis, all involve the TNF-regulated extrinsic apoptosis pathway or feed into the intrinsic pathway through some other mechanism, remains to be determined. Regardless, whether apoptosis is initiated intrinsically or extrinsically its completion often relies on signalling input from neighbouring cells.

As non-autonomous regulation of apoptosis has been shown to be important in many different organisms, this is likely not a specialised process specific to a small subset of tissues and organisms but a general phenomenon of animal development, systemic stress response and maintenance of tissue homeostasis. Looking ahead, the power of genetically tractable model organisms holds great promise for gaining a comprehensive understanding of how communities of cells and tissues regulate apoptosis of their neighbours. For humans, understanding the spatiotemporal patterns by which pro- and anti-apoptotic factors are secreted and learning how to manipulate them will not only help in the development of new treatments for a variety of diseases, but perhaps also aid in the effort to synthesise artificial tissues and organs in the lab.

Conflict of Interest

The authors declare no conflict of interest.

1. Fuchs E, Steller H. Programmed cell death in animal development and disease. *Cell* 2011; **147**: 742–758.
2. Madeo F, Herker E, Wissing S, Jungwirth H, Eisenberg T, Frohlich K. Apoptosis in yeast. *Curr Opin Microbiol* 2004; **7**: 655–660.
3. Lettre G, Hengartner M. Developmental apoptosis in *C. elegans*: a complex CEDnario. *Nat Rev Mol Cell Biol* 2006; **7**: 97–108.
4. Ciara T, McCarthy J. Pathways of apoptosis and importance in development. *J Cell Mol Med* 2005; **9**: 345–359.
5. Hipfner D, Cohen S. Connecting proliferation and apoptosis in development and disease. *Nat Rev Mol Cell Biol* 2004; **5**: 805–815.
6. Kolb WP, Granger GA. Lymphocyte in vitro cytotoxicity: characterization of human lymphotoxin. *Proc Natl Acad Sci U S A* 1968; **61**: 1250–1255.

7. Ruddle NH, Byron HW. Cytotoxicity mediated by soluble antigen and lymphocytes in delayed hypersensitivity. *J Exp Med* 1968; **128**: 1267–1279.
8. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci U S A* 1975; **72**: 3666–3670.
9. Aggarwal BB, Kohr WJ, Hass PE, Moffat B, Spencer SA, Henzel WJ *et al*. Human tumor necrosis factor. Production, purification and characterization. *J Biol Chem* 1985; **260**: 2345–2354.
10. Cohen S, Levi-Montalcini R, Hamburger V. A nerve growth-stimulating factor isolated from sarcomas 37 and 180. *Proc Natl Acad Sci U S A* 1954; **40**: 1014–1018.
11. Levi-Montalcini R. The nerve growth factor 35 years later. *Science* 1987; **237**: 1154.
12. Aloe L, Levi-Montalcini R. The discovery of nerve growth factor and modern neurobiology. *Trends Cell Biol* 2004; **14**: 395–399.
13. Aloe L, Rocco ML, Patrizia B, Manni L. Nerve growth factor: from the early discoveries to the potential clinical use. *J Transl Med* 2012; **10**: 239.
14. Aggarwal BB, Gupta SC, Kim JH. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. *Blood* 2012; **119**: 651–665.
15. Ashkenazi A. Directing cancer cells to self-destruct with pro-apoptotic receptor agonists. *Nat Rev* 2008; **7**: 1001–1012.
16. Tait S, Green D. Mitochondrial regulation of cell death. *Cold Spring Harb Perspect Biol* 2013; **5**: a008706.
17. Liambi F, Moldoveanu T, Tait SW, Bouchier-Hayes L, Temirov J, McCormick LL *et al*. a unified model of mammalian BCL-2 protein family interactions at the mitochondria. *Mol Cell* 2011; **44**: 517–531.
18. Zou H, Li Y, Liu X, Wang X. An APAF-1, cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. *J Biol Chem* 1999; **274**: 11549–11556.
19. Nair P, Lu M, Petersen S, Ashkenazi A. Apoptosis initiation through the cell-extrinsic pathway. *Methods Enzymol* 2014; **544**: 99–128.
20. Kischkel F, Hellbardt S, Behrmann I, Germer M, Pawlita M, Krammer P *et al*. Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. *EMBO J* 1995; **14**: 5579–5588.
21. Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J *et al*. FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. *Cell* 1996; **85**: 817–827.
22. Green D, Liambi F. Cell death signaling. *Cold Spring Harb Perspect Biol* 2015; **7**. doi:10.1101/cshperspect.a006080.
23. Ouyang L, Shi Z, Zhao S, Wang F, Zhou T, Liu B *et al*. Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis. *Cell Prolif* 2012; **45**: 487–498.
24. Lockshin R. Programmed cell death 50 (and beyond). *Cell Death Differ* 2016; **23**: 10–17.
25. Ellis H, Horvitz H. Genetic control of programmed cell death in the nematode *C. elegans*. *Cell* 1986; **44**: 817–829.
26. Horvitz H. Worms, life, and death. *ChemBiochem* 2003; **4**: 697–711.
27. Sulston JE, Horvitz HR. Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev Biol* 1977; **56**: 110–156.
28. Robertson A, Thomson J. Morphology of programmed cell death in the ventral nerve cord of *C. elegans* larvae. *J Embryol Exp Morphol* 1982; **67**: 89–100.
29. Grimsley C, Ravichandran K. Cues for apoptotic cell engulfment: eat-me and come-get-me signals. *Trends Cell Biol* 2003; **13**: 648–656.
30. Yang H, Chen YZ, Zhang Y, Wang X, Zhao X, Godfrey JJ III *et al*. A lysine-rich motif in the phosphatidyserine receptor PSR-1 mediates recognition and removal of apoptotic cells. *Nat Commun* 2015; **6**: 5717.
31. Gumienny T, Hengartner M. How the worm removes corpses: the nematode *C. elegans* as a model system to study engulfment. *Cell Death Differ* 2001; **8**: 564–568.
32. Ellis R, Jacobson D, Horvitz H. Genes required for the engulfment of cell corpses during programmed cell death in *Caenorhabditis elegans*. *Genetics* 1991; **129**: 79–94.
33. Hsu T, Wu Y. Engulfment of apoptotic cells in *C. elegans* is mediated by integrin alpha/SRC signaling. *Curr Biol* 2010; **20**: 477–486.
34. Reddien P, Cameron S, Horvitz H. Phagocytosis promotes programmed cell death in *C. elegans*. *Nature* 2001; **412**: 198–202.
35. Hoepfner D, Hengartner M, Schnabel R. Engulfment genes cooperate with *ced-3* to promote cell death in *Caenorhabditis elegans*. *Nature* 2001; **412**: 02–206.
36. Shaham S, Reddien P, Davies B, Horvitz H. Mutational analysis of the *Caenorhabditis elegans* cell-death gene *ced-3*. *Genetics* 1999; **153**: 1655–1671.
37. Chakraborty S, Lambie E, Bindu S, Mikeladze-Dvali T, Conradt B. Engulfment pathways promote programmed cell death by enhancing the unequal segregation of apoptotic potential. *Nat Commun* 2015; **6**: 10126.
38. Lang R, Bishop J. Macrophages are required for cell death and tissue remodeling in the developing mouse eye. *Cell* 1993; **74**: 453–462.
39. Little G, Flores A. Inhibition of programmed cell death by catalase and phenylalanine methyl ester. *Comp Biochem Physiol Physiol* 1993; **105**: 79–83.
40. Timmons AK, Mondragon AA, Schenkel CE, Yalonskaya A, Taylor JD, Moynihan KE *et al*. Phagocytosis genes nonautonomously promote developmental cell death in the *Drosophila* ovary. *Proc Natl Acad Sci U S A* 2016; **113**: 1246–1255.
41. Kawane K, Fukuyama H, Yoshida H, Nagase H, Ohsawa Y, Uchiyama Y *et al*. Impaired thymic development in mouse embryos deficient in apoptotic DNA degradation. *Nat Immunol* 2003; **4**: 138–144.
42. Krieser RJ, MacLea KS, Longnecker DS, Fields JL, Fiering S, Eastman A. Deoxyribonuclease Ialpha is required during the phagocytic phase of apoptosis and its loss causes perinatal lethality. *Cell Death Differ* 2002; **9**: 956–962.
43. Yu H, Lai HJ, Lin TW, Lo SJ. Autonomous and non-autonomous roles of DNase II during cell death in *C. elegans* embryos. *Biosci Rep* 2015; **35**: e00203.
44. Barry MA, Reynolds JE, Eastman A. Etoposide-induced apoptosis in human HL-60 cells is associated with intracellular acidification. *Cancer Res* 1993; **53**: 2349–2357.
45. Barry MA, Eastman A. Identification of deoxyribonuclease II as an endonuclease involved in apoptosis. *Arch Biochem Biophys* 1993; **300**: 440–450.
46. Goyal L, McCall K, Agapite J, Hartwig E, Steller H. Induction of apoptosis by *Drosophila* reaper, hid and grim through inhibition of IAP function. *EMBO J* 2000; **19**: 589–597.
47. Lisi S, Mazzon I, White K. Diverse domains of THREAD/DIAP1 are required to inhibit apoptosis induced by REAPER and HID in *Drosophila*. *Genetics* 2000; **154**: 669–678.
48. Ryoo H, 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.
49. Wang S, Hawkins C, Yoo S, Muller H, Hay B. The *Drosophila* caspase inhibitor DIAP1 is essential for cell survival and is negatively regulated by HID. *Cell* 1999; **98**: 453–463.
50. Steller H. Regulation of apoptosis in *Drosophila*. *Cell Death Differ* 2008; **15**: 1132–1138.
51. White K, Grether ME, Abrams JM, Young L, Farrell K, Steller H. Genetic control of programmed cell death in *Drosophila*. *Science* 1994; **264**: 677–683.
52. Bergmann A, Steller H. Apoptosis, stem cells, and tissue regeneration. *Sci Signal* 2010; **3**: re8.
53. Morata G, Shlevkov E, Perez-Garijo A. Mitogenic signaling from apoptotic cells in *Drosophila*. *Dev Growth Differ* 2011; **53**: 168–176.
54. Greco V. The death and growth connection. *Nat Rev Mol Cell Biol* 2013; **14**: 6.
55. Huh J, Guo M, Hay B. Compensatory proliferation induced by cell death in the *Drosophila* wing disc requires activity of the apical cell death caspase Dronc in a nonapoptotic role. *Curr Biol* 2004; **14**: 1262–1266.
56. Perez-Garijo A, Martin F, Morata G. Caspase inhibition during apoptosis causes abnormal signalling and developmental aberrations in *Drosophila*. *Development* 2004; **131**: 5591–5598.
57. Fan Y, Bergmann A. Distinct mechanisms of apoptosis-induced compensatory proliferation and differentiating tissues in the *Drosophila* eye. *Dev Cell* 2008; **14**: 399–410.
58. Perez-Garijo A, Shlevkov E, Morata G. The role of Dpp and Wg in compensatory proliferation and in the formation of hyperplastic overgrowths caused by apoptotic cells in the *Drosophila* wing disc. *Development* 2009; **136**: 1167–1177.
59. Herz H, Chen Z, Scherr H, Lackey M, Bolduc C, Bergmann A. *vps25* mosaics display non-autonomous cell survival and overgrowth, and autonomous apoptosis. *Development* 2006; **133**: 1871–1880.
60. Graves H, Woodfield S, Yang C, Halder G, Bergman A. Notch signaling activates Yorkie non-cell autonomously in *Drosophila*. *PLoS ONE* 2012; **7**: e37615.
61. Christiansen A, Ding T, Fan Y, Graves H, Herz HM, Lindblad J *et al*. Non-cell autonomous control of apoptosis by ligand-independent Hedgehog signaling in *Drosophila*. *Cell Death Differ* 2013; **20**: 302–311.
62. Madhavan M, Madhavan K. Morphogenesis of the epidermis of adult abdomen of *Drosophila*. *J Embryol Exp Morphol* 1980; **60**: 1–31.
63. Nakajima Y, Kuranaga E, Sugimura K, Miyawaki A, Miura M. Nonautonomous apoptosis is triggered by local cell cycle progression during epithelial replacement in *Drosophila*. *Mol Cell Biol* 2011; **31**: 2499–2512.
64. Klysten P, Saint R. Imaginal tissues of *Drosophila melanogaster* exhibit different modes of cell proliferation control. *Dev Biol* 1997; **192**: 509–522.
65. Ninov N, Marjon C, Martin-Blanco E. Dynamic control of cell cycle and growth coupling by ecdysone, EGFR, and PI3K signaling in *Drosophila* histoblasts. *PLoS Biol* 2009; **7**: e1000079.
66. Marjon C, Sanchez-Herrero E, Suzanne M. Sharp boundaries of Dpp signalling trigger local cell death required for *Drosophila* leg morphogenesis. *Nat Cell Biol* 2007; **9**: 57–63.
67. Botchkareva N, Ahluwalia G, Shaender D. Apoptosis in the hair follicle. *J Invest Dermatol* 2006; **126**: 258–264.
68. Lohmann I, McGinnis N, Bodmer M, McGinnis W. The *Drosophila* Hox gene deformed sculpts head morphology via direct regulation of the apoptosis activator reaper. *Cell* 2002; **110**: 457–466.
69. Link N, Chen P, Lu W, Pogue K, Chuong A, Mata M *et al*. A collective form of cell death requires homeodomain interacting protein kinase. *J Cell Biol* 2007; **178**: 567–574.
70. Suzanne M, Petzoldt A, Speder P, Coutelis J, Steller H, Noselli S. Coupling of apoptosis and L/R patterning controls stepwise organ looping. *Curr Biol* 2010; **20**: 1773–1778.
71. Suzanne M, Steller H. Shaping organisms with apoptosis. *Cell Death Differ* 2013; **20**: 669–675.
72. Hay B, Wolff T, Rubin G. Expression of baculovirus P35 prevents cell death in *Drosophila*. *Development* 1994; **120**: 2121–2129.
73. Perez-Garijo A, Fuchs Y, Steller H. Apoptotic cells can induce non-autonomous apoptosis through the TNF pathway. *eLife* 2013; **2**: e01004.
74. Leppa S, Bohmann D. Diverse functions of JNK signaling and c-Jun in stress response and apoptosis. *Oncogene* 1999; **18**: 6158–6162.
75. Igaki T, Kanda H, Yamamoto-Goto Y, Kanuka H, Kuranaga E, Aigaki T *et al*. Eiger, a TNF superfamily ligand that triggers the *Drosophila* JNK pathway. *EMBO J* 2002; **21**: 3009–3018.

76. Kauppila S, Maaty WS, Chen P, Tomar RS, Eby MT, Chappo J *et al.* Eiger and its receptor, Wengen, comprise a TNF-like system in *Drosophila*. *Oncogene* 2003; **22**: 4860–4867.
77. Moreno E, Yan M, Basler K. Evolution of TNF signaling mechanisms: JNK-dependent apoptosis triggered by Eiger, the *Drosophila* homolog of the TNF superfamily. *Curr Biol* 2002; **12**: 1263–1268.
78. Lindner G, Botchkarev V, Botchkareva N, Ling G, van der Veen C, Paus R. Analysis of apoptosis during hair follicle regression (catagen). *Am J Pathol* 1997; **151**: 1601–1617.
79. Tong X, Coulombe PA. Keratin 17 modulates hair follicle cycling in a TNF α -dependent fashion. *Genes Dev* 2006; **20**: 1353–1364.
80. Cho K, Hague M, Wang J, Yu M, Hao Y, Qiu S *et al.* Distinct and atypical intrinsic and extrinsic cell death pathways between photoreceptor cell types upon specific ablation of Ranbp2 in cone photoreceptors. *PLoS Genet* 2013; **9**: e1003555.
81. Babcock D, Ganetzky B. Transcellular spreading of huntingtin aggregates in the *Drosophila* brain. *Proc Natl Acad Sci USA* 2015; **112**: E5417–E5433.
82. Babcock D, Ganetzky B. Non-cell autonomous cell death caused by transmission of Huntingtin aggregates in *Drosophila*. *Fly* 2015; **9**: 107–109.
83. Sandoel A, Kohler I, Fellmann C, Lowe S, Hengartner M. HIF-1 antagonizes p53-mediated apoptosis through a secreted neuronal tyrosinase. *Nature* 2010; **465**: 577–583.
84. Schertel C, Conradt B. *C. elegans* orthologs of components of the RB tumor suppressor complex have distinct pro-apoptotic functions. *Development* 2007; **134**: 3691–3701.
85. Derry W, Putzke A, Rothman J. *Caenorhabditis elegans* p53: role in apoptosis, meiosis, and stress resistance. *Science* 2001; **294**: 591–595.
86. Schumacher B, Hofmann K, Boulton S, Gartner A. The *C. elegans* homolog of the p53 tumor suppressor is required for DNA damage-induced apoptosis. *Curr Biol* 2001; **11**: 1722–1727.
87. Hollstein M, Sidransky D, Vogelstein B, Harris C. p53 mutations in human cancers. *Science* 1991; **253**: 49–53.
88. Olivier M, Hollstein M. Hainaut TP53 mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harb Perspect Biol* 2010; **2**: a001008.
89. Hofmann E, Milstein S, Boulton S, Ye M, Hofmann J, Stergiou L *et al.* *Caenorhabditis elegans* HUS-1 is a DNA damage checkpoint protein required for genome stability and EGL-1-mediated apoptosis. *Curr Biol* 2002; **12**: 1908–1918.
90. Quevedo C, Kaplan D, Derry W. AKT-1 regulates DNA-damage-induced germline apoptosis in *C. elegans*. *Curr Biol* 2007; **17**: 286–292.
91. Conradt B, Horvitz H. The *C. elegans* protein EGL-1 is required for programmed cell death and interacts with the Bcl-2-like protein CED-9. *Cell* 1998; **93**: 519–529.
92. del Peso L, Gonzalez V, Inohara N, Ellis R, Nunez G. Disruption of the CED-9: CED-4 complex by EGL-1 is a critical step for programmed cell death in *Caenorhabditis elegans*. *J Biol Chem* 2000; **275**: 27205–27211.
93. Yan N, Chai J, Lee ES, Gu L, Liu Q, He J *et al.* Structure of the CED-4: CED-9 complex provides insights into programmed cell death in *Caenorhabditis elegans*. *Nature* 2005; **437**: 831–837.
94. Ito S, Greiss S, Gartner A, Derry W. Cell-nonautonomous regulation of *C. elegans* germ cell death by *kri-1*. *Curr Biol* 2010; **20**: 333–338.
95. Perrin A, Gunda M, Yu B, Yen K, Ito S, Forster S *et al.* Noncanonical control of *C. elegans* germline apoptosis by the insulin/IGF-1 and Ras/MAPK signaling pathways. *Cell Death Differ* 2013; **20**: 97–107.
96. Laberge-le Couteulx S, Jung HH, Labauge P, Houtteville JP, Lescoat C, Cecillion M *et al.* Truncating mutations in CCM1, encoding KRIT1, cause hereditary cavernous angiomas. *Nat Genet* 1999; **23**: 189–193.
97. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat Rev Cancer* 2002; **2**: 489–501.
98. Paradis S, Ruvkun G. *Caenorhabditis elegans* Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. *Genes Dev* 1998; **12**: 2488–2498.
99. Paradis S, Ailion M, Toker A, Thomas J, Ruvkun G. A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in *Caenorhabditis elegans*. *Genes Dev* 1999; **13**: 1438–1452.
100. Semenza G. Hypoxia-inducible factor 1 (HIF-1) pathway. *Sci STKE* 2007; **407**: cm8.
101. Kaelin W. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell* 2008; **23**: 393–402.
102. Epstein A, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR *et al.* *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* 2001; **107**: 43–54.
103. Bruckner R, McKnight S. A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* 2001; **294**: 1337–1340.
104. Ivan M, Haberberger T, Gervasi DC, Michelson KS, Gunzler V, Kondo K *et al.* Biochemical purification and pharmacological inhibition of a mammalian prolyl hydroxylase acting on hypoxia-inducible factor. *Proc Natl Acad Sci USA* 2002; **99**: 13459–13464.
105. Yu F, White S, Zhao Q, Lee F. HIF-1 α binding to VHL is regulated by stimulus-sensitive proline hydroxylation. *Proc Natl Acad Sci USA* 2001; **98**: 9630–9635.
106. Latif F, Tory K, Gnarr J, Yao M, Duh FM, Orcutt ML *et al.* Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science* 1993; **260**: 1317–1320.
107. Hockel M, Vaupel Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. *J Natl Cancer Inst* 2001; **93**: 266–276.
108. Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D *et al.* Overexpression of hypoxia-inducible factor 1 α in common human cancers and their metastases. *Cancer Res* 1999; **59**: 5830–5835.
109. Nardinocchi L, Puca R, D'Orazi G. HIF-1 α antagonizes p53-mediated apoptosis by triggering HIPK2 degradation. *Aging* 2011; **3**: 33–43.
110. Levi-Ferber M, Salzberg Y, Safra M, Haviv-Chesner A, Bulow HE, Henis-Korenblit S. It's all in your mind: determining germ cell fate by neuronal IRE-1 in *C. elegans*. *PLoS Genet* 2014; **10**: e1004747.
111. Perez-Garjao A, Steller H. Spreading the word: non-autonomous effects of apoptosis during development, regeneration and disease. *Development* 2015; **142**: 3253–3262.
112. Guillot C, Lecuit T. Mechanics of epithelial tissue homeostasis and morphogenesis. *Science* 2013; **340**: 1185–1189.
113. Saelens X, Festjens N, Vande Walle L, van Gurp M, van Loo G, Vandenabeele. Toxic proteins released from mitochondria in cell death. *Oncogene* 2004; **23**: 2861–2874.
114. Li X, Johnson R, Park D, Chin-Sang I, Chamberlin H. Somatic gonad sheath cells and Eph receptor signaling promote germ-cell death in *C. elegans*. *Cell Death Differ* 2012; **19**: 1080–1089.
115. Hanahan D, Weinberg R. The hallmarks of cancer. *Cell* 2000; **100**: 57–70.
116. Hellevik T, Martinez-Zubiaurre I. Radiotherapy and the tumor stroma: the importance of dose and fractionation. *Front Oncol* 2014; **4**: 1–12.
117. Cutsforth-Gregory J, Lanzino G, Link M, Brown RJ, Flemming K. Characterization of radiation-induced cavernous malformations and comparison with a nonradiation cavernous malformation cohort. *J Neurosurg* 2015; **122**: 1214–1222.
118. Tao J, Wu H, Lin Q, Wei W, Lu XH, Cantle JP *et al.* Deletion of astroglial Dicer causes non-cell autonomous neuronal dysfunction and degeneration. *J Neurosci* 2012; **31**: 8306–8319.



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