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.

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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
signalling complex (DISC) and activation of caspases 8 and 10, leading to apoptosis pathway, activation of a ‘death’ pathway is similar to C. elegans (This pathway can be influenced by extrinsic factors including Eiger, upstream of JNK. and lead to activation of effector caspases (Drice, Dcp-1 and Decay) and apoptosis. Inhibition of Diap1. Consequently, initiator caspases (Dronc and Dredd) are activated antagonists of inhibitors of apoptosis (IAP) including Hid, Rpr, Grim or Skl leads to be initiated autonomously or through receptor-mediated pathways. Activation 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) 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 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 Bcl-2 homology 3 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 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

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 (Drice, Dcp-1 and Decay) 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.

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mutants (hypomorphs) have reduced levels of apoptosis during embryonic development. Intriguingly, enhancer screens performed in these hypomorphic ced-3 mutants uncovered mutations in engulfment genes that enhanced cell survival. 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. Interestingly, loss-of-function mutations in engulfment genes alone can increase survival of neuroblast and progenitor daughter cells normally programmed to die by apoptosis. 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. 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. 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. 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). 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. 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. 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. 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. 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-autonomous manner. 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. 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. 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 homoeostasis. In Drosophila, apoptosis is normally prevented by the inhibitor of apoptosis (IAP) protein DIAP1. 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
of effector caspases such as DrICE and DCP-1 to trigger apoptosis (Figure 1b).\textsuperscript{50,51}

The connection between proliferation and apoptosis is well established in \textit{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.\textsuperscript{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.\textsuperscript{50} However, factors have recently been identified that directly influence DIAP1 transcription in novel ways. Dying cells can inhibit apoptosis of surrounding cells through \textit{vps25}, a component of the endosomal sorting complex required for transport, which non-autonomously induces DIAP1 and promotes proliferation.\textsuperscript{59} Notch signalling from \textit{vps25} mutant dying cells activates the Hippo signalling in neighbouring cells, leading to Yorkie-mediated induction of DIAP1.\textsuperscript{60} Furthermore, activation of Notch alone is sufficient to induce Yorkie and DIAP1 in neighbouring cells.\textsuperscript{60} In addition, hyper-activation of hedgehog signalling also makes neighbouring cells resistant to apoptosis through induction of DIAP1.\textsuperscript{61} Thus, in \textit{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 \textit{Drosophila} abdominal epithelial replacement in the pupal stage, larval epidermal cells (LECs) undergo apoptosis and are replaced by abdominal histoblasts that proliferate and migrate.\textsuperscript{66} Interactions between proliferating abdominal histoblasts and LECs were recently shown to be critical for induction of LEC apoptosis.\textsuperscript{63} These histoblasts are normally arrested at G2 before pupariation, but enter the cell cycle once the pupal stage begins.\textsuperscript{54,65} When histoblasts are forced to arrest at S/G2 phase as they are migrating, the adjacent LECs do not undergo apoptosis.\textsuperscript{63} Their transition into the cell cycle is necessary for coordinating apoptosis of neighbouring LECs.\textsuperscript{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 \textit{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).\textsuperscript{66–71} The mechanisms that govern this are best understood in \textit{Drosophila}, where signals emanating from dying cells are at least partially sufficient to stimulate apoptosis of their neighbours. Expression of the viral caspase inhibitor \textit{p35} leads to survival of cells programmed to undergo apoptosis.\textsuperscript{72} When \textit{p35} and the pro-apoptotic gene \textit{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).\textsuperscript{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.\textsuperscript{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.\textsuperscript{73}

In each of the discs tested, the posterior parts with ectopic apoptosis were enlarged as a consequence of compensatory proliferation.\textsuperscript{73} It is possible that some sort of secondary compensatory effect is responsible for the resulting abnormal apoptosis in the anterior disc. Harbouring a large number of abnormal undead cells for an extended period of time may
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. Only overexpressing hid or rpr (without p35) in posterior disc cells is sufficient to induce apoptosis in anterior disc cells. 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. 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. 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. What is that signal? The Drosophila TNF ortholog, Eiger, is known to activate JNK dependent apoptosis in Drosophila (Figure 3a). 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. 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. 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. Injection of mice with TNF-α antibodies is sufficient to disrupt synchronization of apoptosis, or completely inhibit it, in hair follicles. 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. 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. 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. Interestingly, this is dependent on uptake of the aggregates as inhibition of endocytosis prevented the abnormal apoptosis. 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, antimitotic 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 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 KRI1/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 *Kri1/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 germ cell 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.99

Neuronal factors can also regulate germ cell apoptosis. Hypoxia-inducible factor (HIF) is a key regulator of oxygen homoeostasis 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 tautomerasers, 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-1α 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 neurally secreted TYR-2, it has also been reported that endoplasmic reticulum stress in a set of amphiid 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 homoeostatic 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 homoeostasis 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 *vh1-1* 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-1α 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 homoeostasis. 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.
27. Gumienny T, Hengartner M. How the worm removes corpses: the nematode gene.

28. Ellis H, Horvitz H. Genetic control of programmed cell death in the nematode C. elegans.

24. Lockshin R. Programmed cell death 50 (and beyond).

29. Yang H, Chen YZ, Zhang Y, Wang X, Zhao X, Godfrey J III et al. Engulfment genes nonautonomously promote developmental cell death in human HL-60 cells.

30. Yang H, Chen YZ, Zhang Y, Wang X, Zhao X, Godfrey J III et al. Engulfment genes nonautonomously promote developmental cell death in human HL-60 cells.

31. Gumienny T, Hengartner M. How the worm removes corpses: the nematode gene.

32. Ellis R, Jacobson D, Horvitz H. Genes required for the engulfment of cell corpses during apoptosis in Drosophila.

33. Hoeppner D, Hengartner M, Schnabel R. Engulfment genes cooperate with phagocytosis genes in the recognition and phagocytosis of apoptotic cells.

34. Liambi F, Moldoveanu T, Tait SW, Bouchier-Hayes L, Temirov J, McCormick LL et al. The JNK and the Wingless signaling pathways.

35. Hoeppner D, Hengartner M, Schnabel R. Engulfment genes cooperate with phagocytosis genes in the recognition and phagocytosis of apoptotic cells.

36. Kischkel F, Hellbardt S, Behrmann I, Germer M, Pawlita M, Krammer P et al. A lysine-rich motif in the partial cell-death gene.

37. Kiesler RJ, MacLea KS, Longnecker DS, Fields JL, Fiorenti S, Eastman A. Deoxyribonuclease I is required for the phagocytic phase of apoptosis and its loss causes perinatal lethality. Cell Death Differ 2002; 9: 956–962.

38. Yu H, Hai L, Lin TW, Lo SJ. Autonomous and non-autonomous roles of DNases II during cell death in C. elegans embryos. Biochim Biophys Acta 2015; 1850: e60203.

39. Barry MA, Reynolds JE, Eastman A. Exosome-induced apoptosis in human HL-60 cells is associated with intracellular acidification. Cancer Res 1993; 53: 2349–2357.

40. Barry MA, Eastman A. Identification of deoxyribonuclease II as an endonuclease involved in apoptosis. Arch Biochem Biophys 1993; 300: 440–450.

41. Celli B, McCullagh A, Agapite J, Hartweg E, Steller H. Induction of apoptosis by Drosophila reaper, hid and grim through inhibition of IAP function. EMBO J 2000; 19: 589–597.

42. Krieser RJ, MacLea KS, Longnecker DS, Fields JL, Fiorenti S, Eastman A. Deoxyribonuclease I is required for the phagocytic phase of apoptosis and its loss causes perinatal lethality. Cell Death Differ 2002; 9: 956–962.
76. Kaupila S, Maaty WS, Chen P, Tomar RS, Eby MT, Chapo 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: 2466–2476.

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 TNFAlpha-dependent fashion. Genes Dev 2006; 20: 1363–1364.

80. Cho K, Haque M, Wang J, Yu M, Hao Y, Qi S et al. Distinct and atypical intrinsic and extrinsic cell death pathways between photoreceptor cell types upon specific ablation of Rho-GAP 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. Sendeloe I, 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, Conradi 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 HSF-1: 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. Hainault TPSA mutations in human cancers: origins, consequences, and clinical use. Curr Opin Genet Dev 2010; 20: e001008.

89. Hofmann E, Milstein S, Boulton S, Ye M, Hofmann J, Stergio 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. Conradi 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. Liu S, Greiss S, Gartner A, Derry W. Cell-non-autonomous regulation of C. elegans germ cell death by knl-1. Curr Biol 2010; 20: 333–338.

95. Perrin A, Gunda M, Yu B, Yao 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 Coutelux S, Jang HH, Labauge P, Houtteville JP, Lesca C, Cecillon M et al. Truncating mutations in CCM1, encoding KRIT1, cause hereditary cavernous angiomas. Nat Genet 1999; 23: 189–193.

97. Vivanco I, Sawyer DC. The phosphatidylinositol 3-kinase AKT pathway in human cancer. Nat Rev Cancer 2002; 2: 469–501.

98. Paradis S, Ruksun G. Caenorhabditis elegans Akt/PKB transduces insulin receptor-like signals from AGE-1 P3 kinase to the DAF-16 transcription factor. Genes Dev 1998; 12: 2488-2498.