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REVIEW

Spreading the word: non-autonomous effects of apoptosis during development, regeneration and disease

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ABSTRACT

Apoptosis, in contrast to other forms of cell death such as necrosis, was originally regarded as a 'silent' mechanism of cell elimination designed to degrade the contents of doomed cells. However, during the past decade it has become clear that apoptotic cells can produce diverse signals that have a profound impact on neighboring cells and tissues. For example, apoptotic cells can release factors that influence the proliferation and survival of adjacent tissues. Apoptosis can also affect tissue movement and morphogenesis by modifying tissue tension in surrounding cells. As we review here, these findings reveal unexpected roles for apoptosis in tissue remodeling during development, as well as in regeneration and cancer.

KEY WORDS: Apoptosis, Morphogenesis, Signaling, Tissue remodeling, Silent mechanism of cell elimination

Introduction

When confronted with death, cells have several different options to choose from. These cell death mechanisms were originally described based on their morphological features (Fuchs and Steller, 2015) and can be classified into three major forms. First, there is a form of cell death, historically considered passive and deleterious, termed necrosis, which involves cell swelling, membrane rupture and the release of cellular contents into the extracellular space. It is thus not surprising that, in this situation, the cells surrounding necrotic cells are greatly impacted by their dying neighbors, and various responses such as inflammation and additional cell death can result. Another classical form is autophagic cell death, in which the cell digests its own contents; this form of cell death is also called self-cannibalism. However, in general, autophagy does not lead to cell elimination but instead constitutes a survival mechanism after starvation or other forms of cellular stress. The most prominent form of programmed cell death during animal development and tissue homeostasis is apoptosis. Apoptosis is characterized by progressive nuclear and cytoplasmic shrinkage, chromatin condensation and the shedding of apoptotic bodies, which are rapidly cleared by phagocytes or adjacent cells. Molecularly, apoptosis is executed by specific types of cysteine proteases called caspases that, once activated, can cleave cellular substrates and lead to the demise of the cell (Fuchs and Steller, 2015).

Apoptosis has essential functions during animal development and homeostasis, and its key role in driving morphogenesis and tissue remodeling has long been recognized (Fuchs and Steller, 2011). However, its function has always been regarded as autonomous: only the cells that die by apoptosis were thought to be important for sculpting tissues and organs, or to control cell number. Apoptosis is also associated with numerous pathologies. For example, excessive apoptosis has been linked to neurodegenerative diseases and organ failure after infarction or toxic insult, while defective apoptosis has been involved in proliferative diseases such as cancer (Favaloro et al., 2012). However, again, research has focused mostly on the dying cells, with efforts being made to induce apoptosis when there is a disproportionate accumulation of cells and to suppress cell death in situations in which there is excessive cell loss.

It is becoming increasingly clear, however, that apoptosis can have profound non-cell-autonomous effects on surrounding tissues by affecting the cell division, cell fate and remodeling of nearby cells. In this Review, we provide an overview of these various non-autonomous effects of apoptosis, which include effects on proliferation, the movement and shape of neighboring cells and non-autonomous cell death. We also discuss what is known about the roles of apoptosis during normal development, and what we can learn from studying the unexpected role of signaling by apoptotic cells in regeneration and in pathological conditions such as cancer.

Apoptosis: a way of shaping tissues without affecting neighboring cells

The significance of apoptosis in vertebrate development was recognized very early (reviewed by Clarke and Clarke, 1996). Long before the term 'apoptosis' was coined or the genetic basis for apoptosis was discovered, embryologists had noticed that extensive cell death was a normal part of early development in many animals, which meant that the death of cells had to be a programmed event in the development of multicellular organisms (Glucksmann, 1951; Saunders, 1966). The purpose of the observed loss of large populations of cells was immediately apparent as a way of sculpting tissues and deleting unwanted structures. Hence, apoptosis as a 'clean and silent' form of cell death appeared well-suited for tissue morphogenesis because it allowed for the deletion of cells with little tissue disruption. Indeed, it is now well accepted that apoptosis is the usual method of eliminating organs and tissues that are useful only during embryonic or larval stages or that are phylogenetic vestiges (Fig. 1A). Examples include the degeneration of the pronephros in amphibians and mammals, the regression of the umbilical arteries after birth, and the elimination of the tadpole tail.

Likewise, apoptosis has been shown to play a major role in sculpting tissues, since elimination of a substantial number of cells can result in the appearance of new shapes. A classical example of this is the individualization of digits that occurs in many animals through the removal of the interdigital webs (Fig. 1B). Indeed, high levels of apoptosis can be detected in the interdigital tissue of many higher vertebrates, including mice and humans. Furthermore, failure to activate cell death in these regions (as in the case of mice mutant for genes involved in the apoptotic cascade) can lead to digit fusion or syndactyly (Lindsten and Thompson, 2006; Zakeri et al., 1994).

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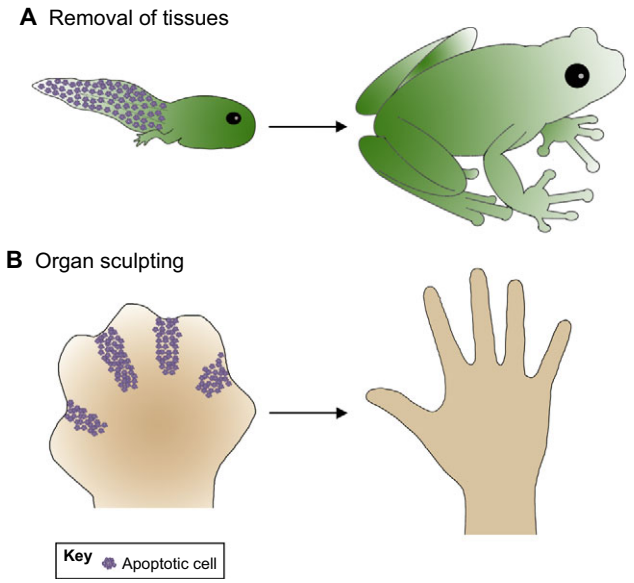

Fig. 1. Roles for apoptosis in tissue and organ sculpting. (A) Apoptosis is known to play a role in eliminating organs and tissues that are useful only during the embryonic or larval stages of development. For example, the tadpole tail is removed by apoptosis at the time of its metamorphosis into a frog. (B) The coordinated elimination of large populations of cells can provide a means to sculpt tissues without affecting neighboring cells, as shown here for the vertebrate limb.

In all of these situations it was assumed that the morphogenetic role of apoptosis is simply cell elimination: if enough cells are killed by apoptosis, this alone could lead to the deletion or shaping of structures. However, recent studies indicate that apoptosis may have a more direct role in shaping tissues. For example, in the case of interdigital web apoptosis, mice that are mutant for different components of the retinoic acid pathway exhibit not only reduced interdigital apoptosis, but also reduced expression of tissue remodeling genes such as stromelysin 3 [also known as matrix metallopeptidase 11 (*Mmp11*)], high mobility group nucleosomal binding domain 1 (*Hmgn1*) and fibroblast growth factor 18 (*Fgf18*) (Dupe et al., 1999; Zhao et al., 2010). Therefore, it is possible that apoptosis controls the expression of genes that help carve the contours of the digits.

Another aspect of 'collective cell suicide' that has not been investigated is the mechanism by which the synchronicity of cell death is achieved. Because apoptosis is initiated by some external signal, it was originally thought that all cells respond to this signal simultaneously in a coordinated manner. However, it is now clear that, at least under some circumstances (as discussed in detail below), apoptotic cells can release death signals to orchestrate cohort cell death (Perez-Garijo et al., 2013).

Apoptosis in development and morphogenesis: promoting tissue movement and reshaping

In early studies, embryologists noticed a close association between cell death and tissue remodeling. However, only very recently are we starting to gain mechanistic insight into how apoptosis can influence surrounding cells to promote folding and movement. Below, we review some of the main developmental contexts in which a role for apoptosis has been identified and studied.

Dorsal closure in *Drosophila* embryos

Dorsal closure is a complex morphogenetic process that occurs during *Drosophila* embryogenesis. During this process the lateral epidermis of the embryo spreads dorsally until it covers the embryonic dorsal surface. The amnioserosa, which is the extra-embryonic tissue that was the original occupant of the most dorsal position of the developing embryo, reduces its surface area during closure and eventually disappears inside the embryo. Multiple forces, both from the retracting amnioserosa and from the spreading lateral epidermis, contribute to the movement of the epithelial sheets during dorsal closure (Gorfinkiel et al., 2009; Kiehart et al., 2000). However, apoptosis has also been identified as a major force driving this event (Fig. 2A). In particular, a small subset of amnioserosa cells exhibits the hallmarks of apoptosis (Toyama et al., 2008). Interestingly, cells in the vicinity of these apoptotic cells present a distorted morphology and are pulled towards the apoptotic cells. In this way, a large portion of the amnioserosa cells are impacted by the apoptosis process, and not only those that actively undergo cell death. Importantly, apoptosis in the amnioserosa was shown to influence the dynamics of dorsal closure, identifying this event as one of the first examples in which apoptosis promotes movement by affecting cellular forces. Accordingly, the inhibition of apoptosis led to a delay in dorsal closure, whereas ectopic induction of apoptosis speeded up the process (Toyama et al., 2008).

Some insight into the possible mechanism by which apoptosis affects the progression of closure came from a later study that investigated the spatial, temporal and molecular hierarchies in the relationship between apoptosis and delamination (Muliyil et al., 2011). In this study, it was shown that the apoptotic signal is essential for driving cell delamination, both autonomously and non-autonomously. Furthermore, it was also shown that apoptosis influences the rates of apical constriction, suggesting that apoptosis regulators might regulate cell mechanics by inducing reorganization of the cytoskeleton (Muliyil et al., 2011).

Genital rotation in *Drosophila*

Another example in which apoptosis plays a role in morphogenesis by inducing tissue movement is during development of the *Drosophila* male genitalia (Fig. 2B), which rotate clockwise during development to complete a full 360° loop. A role for apoptosis in this movement was suspected early on because several mutants for apoptosis genes show defects in genital rotation (Abbott and Lengyel, 1991; Grether et al., 1995; Macias et al., 2004). More recently, live imaging studies and a thorough investigation of apoptosis activation led to an important advance in our understanding of the process. First, Suzanne et al. (2010) demonstrated that the full rotation of the genitalia was the sum of two independent 180° rotations, each affecting one distinct ring-shaped domain of cells. Furthermore, they showed that apoptosis was localized at the boundaries of these domains and that it was essential for their movement. Thus, they proposed a model in which apoptosis acts as a mechanism to separate the ring domains from neighboring tissues and allow for their movement (Suzanne et al., 2010). Following on from this, Kuranaga et al. (2011) investigated caspase activation *in vivo* and induced ectopic activation of apoptosis. Based on their findings, they suggested a model in which apoptosis acts as a direct force driving the rotation, similar to its role during dorsal closure (Kuranaga et al., 2011). The exact mechanism underlying this phenomenon remains to be elucidated. Although the directionality of rotation depends on Myosin ID (also known as Myosin 31DF) (Suzanne et al., 2010), the local cellular and molecular changes triggered by apoptosis in adjacent cells remain elusive.

Shaping the vertebrate brain

The vertebrate neural tube is the embryonic precursor of the central nervous system, giving rise to both the brain and the spinal cord. It

Development (2015) 142, 3253-3262 doi:10.1242/dev.127878

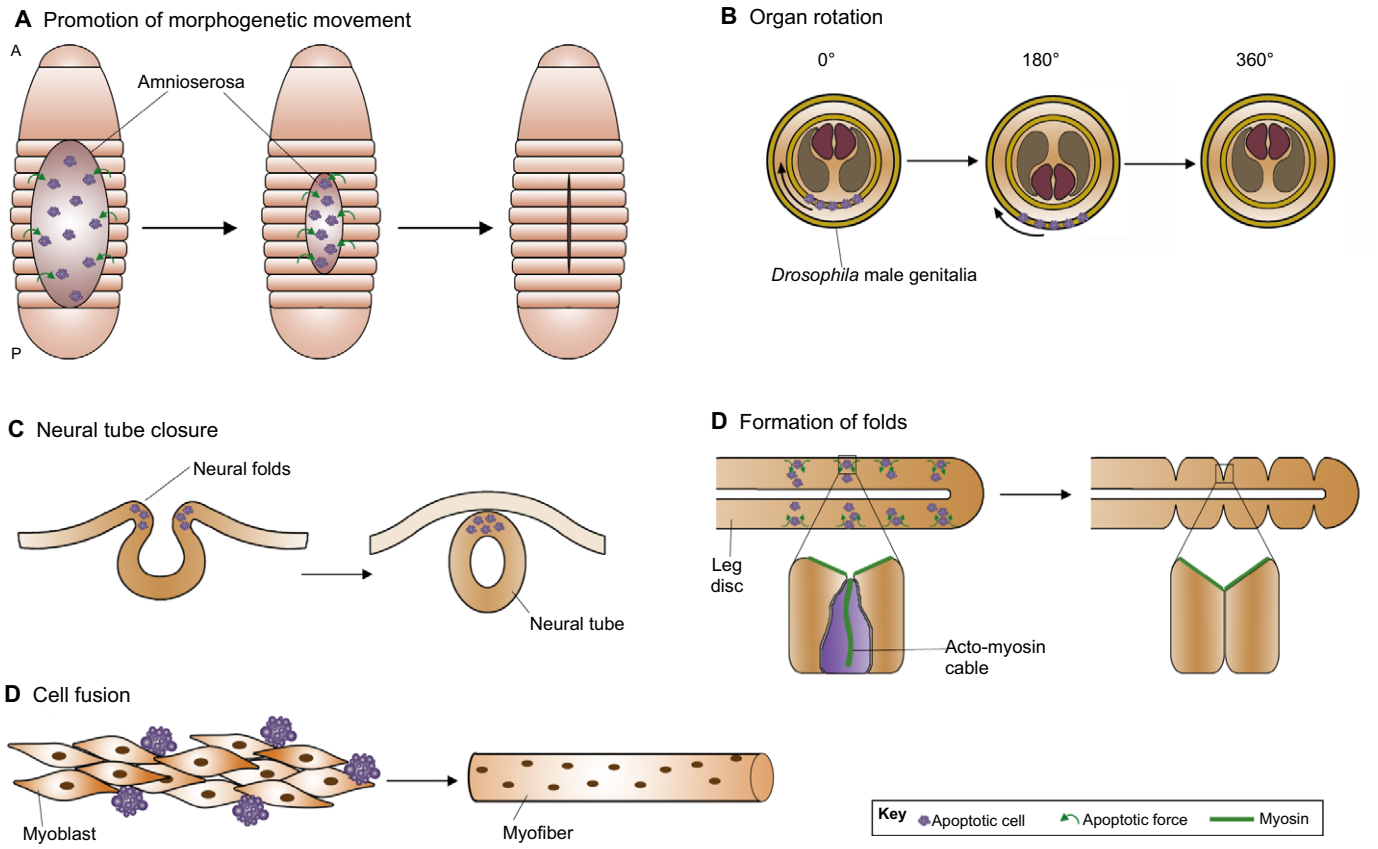

Fig. 2. Apoptosis as a mechanism to promote movement and shape tissues. (A) During *Drosophila* dorsal closure, the apoptosis of amnioserosa cells works as a pulling force to speed up the movement of the epithelial sheets and accelerate the process of closure. (B) During the development of *Drosophila* genitalia, apoptosis within distinct ring-shaped domains of cells is thought to play a crucial role during the two independent 180° rotations that are observed. (C) During the formation of the vertebrate neural tube, extensive apoptosis is observed in the neural folds during neural tube closure and during the remodeling of the dorsal neural tube. (D) Apoptosis, and the subsequent formation of an acto-myosin cable, induces fold formation in the *Drosophila* leg by generating apicobasal forces that propagate to the neighboring tissues. The cable is necessary for generating a pulling force that originates in the dying cell and extends to the neighbors. (E) Apoptosis promotes myoblast fusion in mice. In this context, the apoptotic cells do not fuse with healthy myoblasts but remain in close proximity to them. A, anterior; P, posterior.

develops from the neural plate, and its formation involves an elaborate series of morphogenetic movements. During this period, there is an elevation of both sides of the neural plate (the neural folds), which then contact each other and fuse to form the roof of the neural tube. Extensive apoptosis occurs during neural tube closure (Fig. 2C), especially at the neural folds during bending and fusion, and during the remodeling of the dorsal neural tube after fusion. However, it remains controversial whether cell death is required for these processes. An initial report using explanted chick embryos treated with apoptosis inhibitors suggested that apoptosis plays a functional role in neural tube closure (Weil et al., 1997). Further analysis revealed that apoptosis-deficient mice (*Casp3*$^{-/-}$ and *Apaf1*$^{-/-}$) showed closure defects in the midbrain and hindbrain, whereas closure occurred normally in the forebrain and spinal regions (Massa et al., 2009). However, mouse embryos cultured in the presence of chemical inhibitors of apoptosis displayed normal closure of all regions, including the midbrain and hindbrain, even though apoptosis was effectively blocked (Massa et al., 2009). A possible explanation for this apparent discrepancy is that long-term suppression of apoptosis, as occurs in the case of the mutant mice, might lead to abnormalities that are unrelated to cell death inhibition and that affect neural tube closure. However, another possibility would be that chemical inhibitors fail to fully suppress the initiation of apoptosis and that some of the effects on neighboring cells are still maintained, therefore allowing normal closure in certain regions.

More recent insights into the role of apoptosis in this system have come from the use of live imaging to study caspase activation during neural tube closure and by analyzing the progression of neural tube closure in apoptosis-deficient embryos (Yamaguchi et al., 2011). This study found that caspase-positive cells could be divided into two classes depending on their morphology and behavior. Some apoptotic cells exhibited the typical hallmarks of apoptosis (shrinking morphology and fragmentation into apoptotic bodies) and disappeared very quickly from the tissue. But there were also cells showing caspase activation that maintained their round shape, did not fragment, moved actively (they were named 'dancing' apoptotic cells) and persisted in the tissue for long periods of time. Interestingly, these two types of apoptotic cells showed different distributions and caspase expression patterns. Furthermore, live imaging of apoptosis-impaired embryos (*Apaf1*$^{-/-}$ embryos, or embryos treated with the pan-caspase inhibitor zVAD) revealed that the speed of neural tube closure was reduced, demonstrating a role of apoptosis in the dynamics of this process (Yamaguchi et al., 2011).

Recently, another role for apoptosis during brain development that has a major impact on neighboring tissues has been revealed (Nonomura et al., 2013). In this case, the non-autonomous effects do not result from the influence of dying cells on their neighbors,

Development (2015) 142, 3253-3262 doi:10.1242/dev.127878

but from the nature of the cells destined to die. Soon after neural tube closure, apoptosis is involved in the timely removal of a cluster of Fgf8-expressing cells. The persistence of these cells in apoptosis-deficient mice led to the accumulation of FGF and to brain malformations (Nonomura et al., 2013). Interestingly, this mechanism could explain some of the early phenotypes observed in apoptosis-deficient mice, which were originally attributed to an excessive number of neural cells that fail to die by apoptosis (Kuan et al., 2000). Thus, an intriguing possibility would be that the main function of apoptosis in the central nervous system is not to simply control cell number, as has been generally thought, but to regulate its morphogenesis by modulating morphogen signaling and gradients (Yamaguchi and Miura, 2015).

Creating folds in the *Drosophila* leg

In all of the examples discussed above, the role of apoptosis in morphogenesis is well established but the mechanisms behind this apoptosis-induced remodeling remain unclear. However, a recent study of the *Drosophila* leg has provided some mechanistic insight into how apoptosis can drive tissue movement and reshaping by affecting neighboring tissues (Monier et al., 2015).

The leg of *Drosophila* is divided into segments that are separated by flexible joints. Previous studies had shown that local cell death is required for the formation of the distal joints and for imaginal disc folding in the regions of presumptive joint formation (Manjon et al., 2007). Recently, the mechanism of this folding has been uncovered (Monier et al., 2015), showing that apoptotic cells exert apicobasal forces in the epithelium via the generation of myosin cables and the regulation of tissue tension (Fig. 2D). Prior studies in monolayer cell cultures had suggested that the extrusion of apoptotic cells depends on the formation of acto-myosin rings that induced contraction, both in apoptotic cells and in their neighbors (Kuipers et al., 2014; Rosenblatt et al., 2001). Monier et al. (2015) built upon these findings by revealing the formation of an acto-myosin cable in apoptotic cells *in vivo*. This cable was necessary for the generation of a pulling force that originates in the dying cell and extends to the neighbors. The transmission of this force relies on apical stabilization of actin/myosin and leads to an increase in tissue tension and cell shape changes in adjacent cells. It was also demonstrated, both *in vivo* and through an *in silico* biophysical model, that both apoptosis and the transmission of apoptotic forces to neighbors are necessary for efficient tissue folding. Furthermore, it was shown that ectopic apoptosis is sufficient to induce folding in a flat tissue as long as apoptosis is locally concentrated in a certain region; dispersed apoptosis is unable to modify tissue shape (Monier et al., 2015). Overall, this study constitutes an important step forward in our understanding of the mechanism behind apoptosis-induced remodeling and will, hopefully, guide future work on the subject.

Inducing cell fusion

As well as influencing tissue remodeling and reshaping, apoptosis has been shown to play a role in cell-cell fusion (Fig. 2E). For instance, apoptotic cells can regulate the fusion of undifferentiated muscle precursors, known as myoblasts, into the long multinucleated myofibers that make up skeletal muscle (Hochreiter-Hufford et al., 2013). In this context, apoptosis promotes myoblast fusion, acting through the phosphatidylserine receptor BAI1 (also known as Adgrb1) (Hochreiter-Hufford et al., 2013). Using an *in vitro* model for myogenesis, it was shown that a significant amount of cell death, presenting all the hallmarks of apoptosis, could be detected in myoblasts. Blocking apoptosis with pan-caspase inhibitors led to a dramatic suppression of myoblast fusion. Interestingly, adding apoptotic cells back to these apoptosis-inhibited cultures rescued myoblast fusion. The addition of dying cells could also stimulate the fusion of human myoblasts in culture; the apoptotic myoblasts did not fuse with the healthy ones but remained in close proximity to the fusing myoblasts. *In vivo*, *Bai1* mutant mice showed defects both in normal myogenesis and in muscle regeneration after injury, suggesting an important role for apoptosis during muscle development and repair (Hochreiter-Hufford et al., 2013). Interestingly, phosphatidylserine receptor 1 (PSR-1) has recently been shown to play a crucial role in regenerative axonal fusion in *C. elegans*, suggesting that this mechanism of apoptosis-induced fusion is not restricted to muscle (Neumann et al., 2015).

Signaling from apoptotic cells: modulating the proliferation and survival of surrounding cells

One of the most striking examples of how apoptotic cells can affect neighboring tissues came from the discovery that apoptotic cells can produce and secrete diffusible mitogenic signals (Morata et al., 2011) (Fig. 3A). This phenomenon was first revealed in studies of 'undead' cells (Box 1), but subsequent studies indicated that the same signals can also be produced by normal apoptotic cells. The nature of these signaling molecules varies depending on the tissue and organism, although some of these signals appear to have maintained their function throughout evolution. Likewise, the effect of apoptotic signaling on neighboring cells can differ depending on the context. Below, we discuss the two key ways in which signals from apoptotic cells can affect neighboring cells.

Apoptosis-induced proliferation

The first evidence that apoptotic cells can release signals that affect their neighboring cells came from studies of *Drosophila* wing imaginal discs. The key to making this unexpected discovery was the use of an approach that enabled apoptotic cells to stay alive and harbor intact signaling capabilities for a long period of time (Box 1). Under these conditions, apoptotic cells were shown to activate ectopic expression of the mitogenic signals Wingless (Wg; the ortholog of mammalian Wnt) and Decapentaplegic [Dpp; the ortholog of mammalian bone morphogenetic protein (BMP)], which in turn gave rise to overgrowths in neighboring tissues (Huh et al., 2004; Perez-Garijo et al., 2004; Ryoo et al., 2004). Mechanistically, the production of these signals depends on activation of the JNK pathway, which is activated downstream of the apical caspase Dronc (Kondo et al., 2006; Perez-Garijo et al., 2009; Ryoo et al., 2004) (Fig. 3C). The activation of p53 has also been shown to play a crucial role in apoptosis-induced proliferation but, interestingly, only one of the two *Drosophila* p53 isoforms seems to be responsible for this effect (Dichtel-Danjoy et al., 2013; Wells and Johnston, 2012; Wells et al., 2006).

More recent studies have shown that other signaling pathways are also implicated in apoptosis-induced proliferation (Fig. 3A). For example, the *Drosophila* EGF ligand Spitz has been identified as a key signal regulating the overgrowths produced by undead cells and the tissue recovery after induction of cell death in the eye imaginal disc (Fan et al., 2014). In addition, Simon et al. (2014) uncovered a role for Notch as a target of p53 involved in apoptosis-induced proliferation in *Drosophila* wing discs. Genes controlling apicobasal polarity and the Hippo pathway have also been implicated in the proliferative response triggered by dying cells (Grusche et al., 2011; Sun and Irvine, 2011; Warner et al., 2010; Sun and Irvine, 2013). In this regard, the upregulation of Yorkie activity in cells that surround apoptotic cells was found to be important for

Development (2015) 142, 3253-3262 doi:10.1242/dev.127878

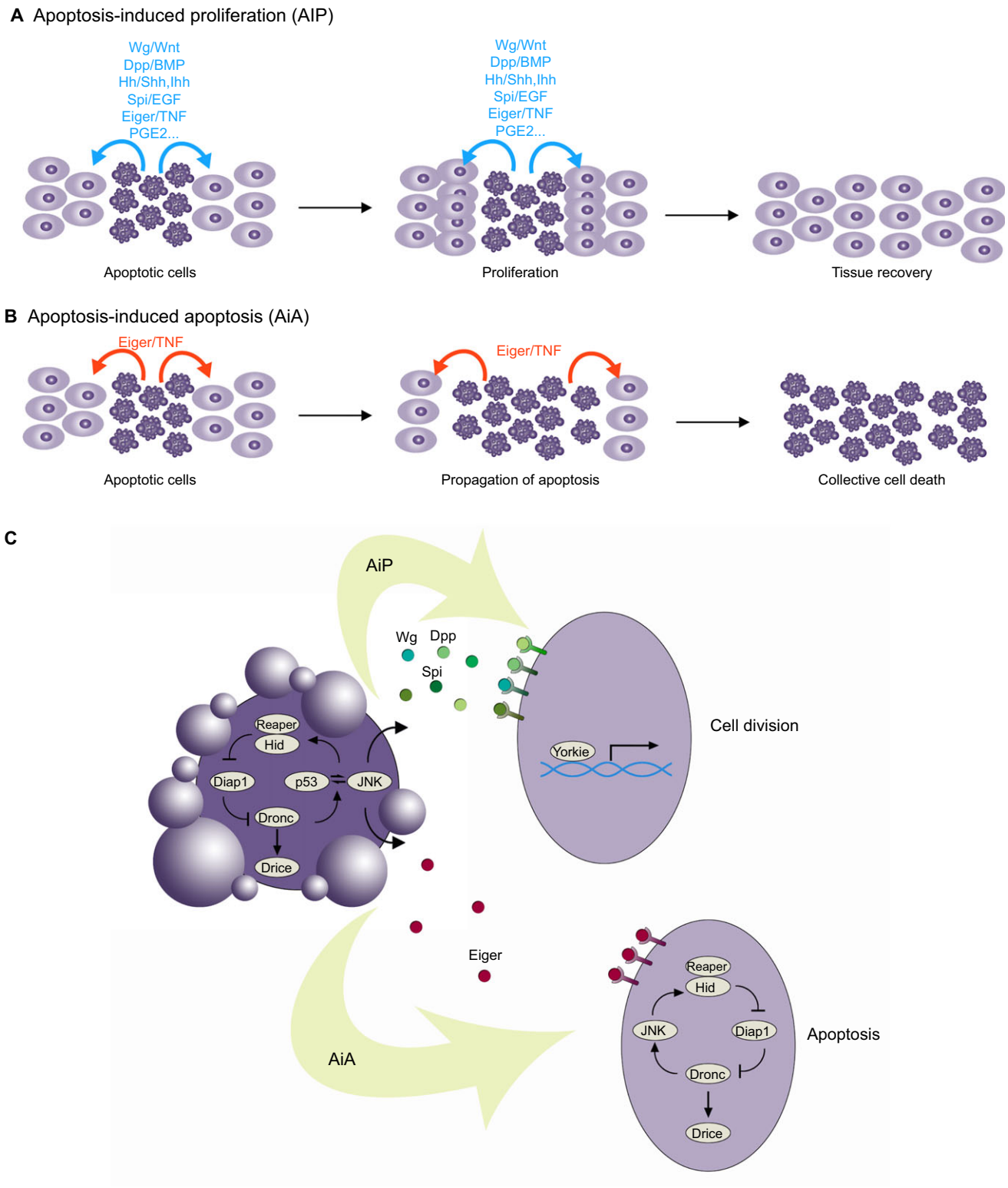

Fig. 3. Signaling by apoptotic cells. (A) Apoptosis-induced proliferation. Dying cells can release mitogenic signals (such as Wnt, Dpp, Shh, EGF, TNF) and induce proliferation in neighboring cells. This mechanism can promote tissue homeostasis and regeneration. (B) Apoptosis-induced apoptosis. Apoptotic cells can also produce pro-death signals (such as Eiger/TNF) as a mechanism to spread apoptosis throughout tissues and achieve collective cell death. (C) Schematic representation of the molecular mechanisms underlying apoptosis-induced proliferation (AiP) and apoptosis-induced apoptosis (AiA).

regeneration after the genetic ablation of wing disc tissue (Fig. 3C). Interestingly, a recent study has proposed that tissue tensions generated by the extrusion of apoptotic cells may trigger the activation of Ajuba, an inhibitor of the Hippo pathway, suggesting a link between apoptotic forces and Yorkie activation (Meserve and Duronio, 2015; Rauskolb et al., 2014). Furthermore, entirely distinct pathways for the activation of apoptosis-induced proliferation have been identified in some differentiating tissues,

Development (2015) 142, 3253-3262 doi:10.1242/dev.127878

Box 1. Undead cells

The term 'undead' has been used in the literature to refer to cells that were destined to die but have been rescued from death and, hence, can still be found in tissues. However, this meaning is different to the definition of undead cells that we use here: in the former case the cells 'forgot' they were once doomed, whereas in the definition that we use the cells still 'think' that they have to die. Thus, they keep continuously activating the apoptosis program and retain all the molecular features of apoptotic cells, except they are unable to execute death. In this situation, such cells produce high levels of signaling molecules that induce overgrowths in neighboring tissues (Huh et al., 2004; Perez-Garijo et al., 2004; Ryoo et al., 2004).

Undead cells can be generated in *Drosophila* imaginal discs by expressing the baculoviral caspase inhibitor p35, which blocks effector caspases without affecting initiator caspases (Hay et al., 1994). By contrast, blocking apoptosis at an earlier step in the pathway leads to survival of the cell but without maintaining its apoptotic nature (Martin et al., 2009). It remains to be determined which types of apoptosis inhibition would elicit an undead state in other contexts, but it is tempting to speculate that this mechanism could be the underlying cause of certain types of tumors. Actually, a recent study revealed that certain metastatic tumors generated in insulin-resistant flies exhibit features of undead cells: they show high levels of caspase activity and Wg production. Given that Wnt inhibition, both genetically and pharmacologically (in combination therapy), can efficiently suppress tumor growth (Hirabayashi et al., 2013), this finding highlights the importance of understanding 'undead cell' biology and how these cells might affect tumor development.

such as the region of the *Drosophila* eye posterior to the morphogenetic furrow. In this tissue, the proliferative effect of apoptosis relies on the activation of effector caspases (as opposed to initiator caspases, which play a major role in proliferating tissues) and Hedgehog signaling (Fan and Bergmann, 2008; Kondo et al., 2006). Finally, a role for apoptosis-induced proliferation has been described in the adult *Drosophila* brain. In this context, neuronal cell death has been shown to trigger the division of neighboring glial cells, both in physiological conditions and upon injury, and this effect is mediated by the TNF homolog Eiger (Kato et al., 2009).

Despite intensive efforts, very little is known about the role of apoptosis-induced proliferation under physiological conditions. Likewise, its role as a compensatory mechanism is still unresolved. It was first proposed that mitogenic signals emitted by apoptotic cells could play a role in fueling the compensatory proliferation that occurs after cell loss. This would explain, for example, the case of imaginal discs that have been subjected to irradiation, where damage results in the elimination of a large number of cells yet these discs still develop into adult structures of normal size and shape, implying that the remaining surviving cells must undergo additional rounds of proliferation to compensate for cell loss (Haynie and Bryant, 1977). The exact requirement for apoptotic signals is still unclear; even though Wg and Dpp are required for the overgrowths produced by undead cells, discs can recover from irradiation when apoptotic cells are mutant for *wg*, *dpp* or both (Perez-Garijo et al., 2009). Screens aimed to identify genes involved in this process (e.g. by screening for impaired compensatory proliferation) might provide better insights into this question (Gerhold et al., 2011). Nevertheless, it is clear that the pioneering work on apoptotic signaling in *Drosophila* discussed above has greatly transformed our view of cellular suicide. Furthermore, major roles for apoptosis-induced proliferation have since been demonstrated in processes such as regeneration and cancer (as discussed in more detail below).

Apoptosis-induced apoptosis

Recently, an unexpected mode of signaling from apoptotic cells has been uncovered: it was shown that apoptotic cells can also induce non-autonomous cell death in neighboring cells and tissues (Perez-Garijo et al., 2013). This process, which has been termed apoptosis-induced apoptosis (Fig. 3B), was observed by activating apoptosis in one *Drosophila* imaginal disc compartment, which, in turn, led to the induction of cell death in the neighboring compartment. It was further shown that this process relies on the production of the TNF ortholog Eiger by apoptotic cells, which in turn activates the JNK pathway in neighboring cells, inducing them to die (Fig. 3C). Accordingly, inhibition of Eiger in the initial dying cells or of JNK in the secondary dying cells efficiently suppressed apoptosis-induced apoptosis. This phenomenon of apoptosis-induced apoptosis was also shown to function in coordinating collective cell death in the mouse hair follicle during a phase of degeneration known as catagen, in which a large portion of the hair follicle cells are eliminated by apoptosis. In this physiological context, TNFα was produced by apoptotic cells and its inhibition led to a loss of synchronicity in the cell death process (Perez-Garijo et al., 2013). Overall, this study provides insights into a novel mechanism by which cellular suicide can be used to achieve the synchronized communal death that often occurs during normal development or under pathological conditions.

It should be pointed out, however, that apoptotic cells have also been shown to induce increased survival of adjacent cells (Bilak et al., 2014). This process depends on activation of the anti-apoptosis microRNA Bantam and the receptor tyrosine kinase Tie. In addition, the *Drosophila* PDGF/VGF-like protein Pvf1 was identified as a potential signal produced by apoptotic cells and is involved in Tie activation (Bilak et al., 2014). The exact mechanism by which apoptotic signaling modulates cell survival remains to be elucidated. Interestingly, this mechanism could explain why the apoptosis-induced apoptosis observed in *Drosophila* imaginal discs was seen at a distance from the initial focus of apoptosis.

The role of apoptosis in regeneration

Regeneration is the fascinating process by which some organisms are able to restore damaged or lost tissues and organs. It can involve a variety of mechanisms, such as remodeling, dedifferentiation/differentiation, migration and, very importantly, proliferation. Since cell death often occurs at the site of injury, and given the recently discovered mitogenic properties of apoptotic cells, it was intriguing to consider whether signals produced by dying cells could fuel regeneration. Indeed, apoptosis has recently been identified as a key source of signals required for wound healing and regeneration in multiple organisms (Bergmann and Steller, 2010; Vriz et al., 2014).

One of the model systems that has provided the most compelling evidence for the role of apoptosis in regeneration is the freshwater polyp *Hydra* (Fig. 4A). *Hydra* has a remarkable regenerative capacity: when its body is cut transversally in half, the upper part is able to grow a new foot and the lower part will produce a new head. However, the mechanisms behind these two regenerative programs seem to differ, and apoptosis is only detected in the lower half that will regrow a head. Importantly, inhibition of apoptosis significantly abrogated head, but not foot, regeneration (Chera et al., 2009). Moreover, the ectopic induction of apoptosis in the foot-regenerating half triggered ectopic head regeneration, giving rise to a doubled-headed *Hydra*. Wnt3 was identified as a signal that is produced by apoptotic cells and that is responsible for the regeneration, acting via the activation of β-catenin signaling in

Development (2015) 142, 3253-3262 doi:10.1242/dev.127878

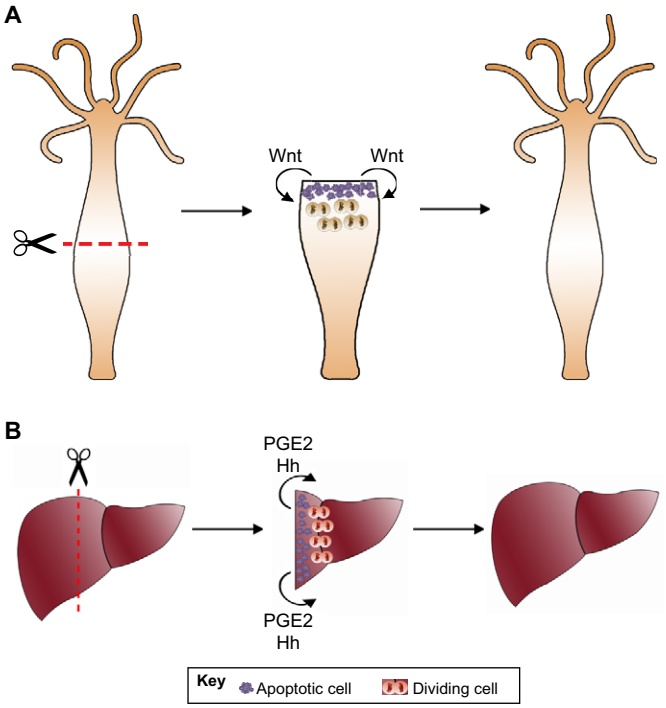

Fig. 4. The role of apoptosis in regeneration. (A) Head regeneration in the freshwater polyp *Hydra*. When the *Hydra* body is cut transversally in half, the lower part is able to produce a new head. Apoptotic cells (purple) are found in the lower part and are the source of Wnt ligands, which induce the proliferation of neighboring tissues and are required for regeneration of the head to proceed. (B) Apoptosis also plays a role in vertebrate liver regeneration; the signals (e.g. PGE2, Hh) produced by dying hepatocytes help to fuel liver regeneration.

neighboring cells. Consistently, inhibition of apoptosis blocked Wnt3 activation, whereas Wnt3 treatment rescued head regeneration in apoptosis-inhibited *Hydra* (Chera et al., 2009).

Apoptosis has also been shown to play a role in regeneration in vertebrates; for example, in the case of *Xenopus* tadpoles, which are able to regenerate their tails after amputation (Tseng et al., 2007). However, in this model system, the mechanism underlying the role of cell death, and whether apoptotic cells can produce signals that trigger proliferation, remain to be determined. Apoptosis has also been shown to play a role in wound healing and liver regeneration in mice (Fig. 4B) (Jung et al., 2010; Li et al., 2010b); apoptosis-deficient mice (*Casp3* and *Casp7* mutants) exhibited delayed healing after excisional wounds in the skin and showed impaired liver regeneration after partial hepatectomy. Based on their findings, Li et al. (2010b) proposed a model in which caspase activation leads to the production of prostaglandin E2 (PGE2), which in turn promotes stem cell proliferation and tissue regeneration. Interestingly, PGE2 has also been identified as a signal produced by apoptotic tumor cells and is involved in the repopulation of tumors following radiotherapy and chemotherapy (Huang et al., 2011; Kurtova et al., 2014) (Box 2). Another signal that may be involved in apoptosis-induced regeneration is Hedgehog, which was identified as the signal produced by dying hepatocytes and is responsible for liver compensatory growth, and which is also involved in apoptosis-induced proliferation in *Drosophila* (Fan and Bergmann, 2008; Jung et al., 2010).

Surprisingly, the regeneration of *Drosophila* imaginal discs, the model system in which the signaling capabilities of apoptotic cells was first discovered, does not seem to rely on apoptosis-induced proliferation, although the different models used to study regeneration (e.g. cutting versus genetic ablation) have led to somewhat differing conclusions (Bergantinos et al., 2010; Bosch et al., 2005; Herrera et al., 2013; Herrera and Morata, 2014; Smith-Bolton et al., 2009). For example, whereas Herrera et al. (2013) showed that Wg and Dpp are not required for imaginal disc regeneration, Smith-Bolton et al. (2009) showed an involvement of Wg in the regenerative process. However, even in this last scenario, the source of Wg was not the dying cells but the non-apoptotic surviving cells. Thus, it still remains to be determined whether apoptosis functions as a driving force to effectively complete regeneration in *Drosophila* imaginal discs. There are, nonetheless, other settings in which apoptosis-induced proliferation does seem to play a role in the renewal of *Drosophila* tissues. This is the case for the adult midgut, where numerous studies have shown that the signals produced by damaged enterocytes are able to drive the intestinal stem cell proliferation needed for homeostasis and regeneration (Jiang et al., 2011, 2009; Shaw et al., 2010; Staley and Irvine, 2010). Recently, mitogenic signaling from dying enterocytes has also been shown to promote growth of stem cell tumors in the fly midgut (Patel et al., 2015).

Although it is apoptosis-induced proliferation that seems best-suited to aid regeneration, apoptosis-induced apoptosis might also play an important role. Recently, two waves of apoptosis have been observed during planarian regeneration that are dependent on JNK signaling and have possible roles in remodeling and rescaling (Almuedo-Castillo et al., 2014; Pellettieri et al., 2010). In addition, two rounds of apoptosis have been described during zebrafish caudal fin regeneration and, importantly, the second wave of cell death is essential for regeneration (Gauron et al., 2013). Since regeneration involves extensive remodeling and morphogenesis, it would be interesting to investigate the effects of apoptosis in the

Box 2. Apoptotic signaling in tumor development

One commonly accepted hallmark of cancer cells is resistance to cell death (Hanahan and Weinberg, 2000). However, apoptosis is a common feature of tumor development, although this is usually regarded as a desirable outcome (Wyllie, 1985). Cell death is in fact induced during tumor initiation in an attempt to eliminate tumor cells (Menendez et al., 2010), and is the main goal of cancer therapy. Thus, it might be counterintuitive that high levels of apoptosis are linked to poor prognosis in cancer (Jalalinadoushan et al., 2004; Leoncini et al., 1993; Nakopoulou et al., 2001; Naresh et al., 2001; Ohbu et al., 1995; Sun et al., 2006). The mechanism behind this unexpected observation is only now beginning to be understood: it has been shown that apoptotic signaling can drive tumor proliferation and invasion in different tumor models (Ballesteros-Arias et al., 2014; Gdynia et al., 2007; Liu et al., 2013; Maeda et al., 2005; Rudrapatna et al., 2013). Likewise, the treatment of tumors by chemotherapy or radiotherapy has recently been shown to induce tumor repopulation by increasing the release of PGE2 by apoptotic cells, which stimulates the growth of surviving tumor cells (Huang et al., 2011; Kurtova et al., 2014).

Such apoptosis-induced proliferation is probably not the only non-autonomous effect of apoptosis on tumors. For example, apoptosis-induced apoptosis might be involved in the phenomenon of radiation-induced bystander effect, a process by which cell death appears in non-irradiated regions after radiation therapy (Prise and O'Sullivan, 2009). Interestingly, a recent study reported multiple pro-oncogenic effects of apoptosis on lymphoma: the presence of apoptotic tumor cells in this system stimulated tumor growth but also macrophage accumulation, angiogenesis and remodeling (via the upregulation of metalloproteinases) (Ford et al., 2015). Collectively, these observations might profoundly affect our views of cancer development and treatment.

Development (2015) 142, 3253-3262 doi:10.1242/dev.127878

generation of tissue forces and their effects on the migration and shaping of the newly generated tissues.

Conclusions

During the last decade it has become clear that apoptotic cells can profoundly influence their environment. There are two key mechanisms by which apoptosis can exert its effects on neighboring cells: (1) by modifying the tension and remodeling of nearby tissues; and (2) by the release of signals by dying cells. An important question that has not been resolved is whether there is a connection between these mechanical changes and signal secretion. It is possible that one leads to the other, or that they are totally independent processes. Interestingly, Wnt, one of the signals proven to be consistently produced by apoptotic cells in different contexts and organisms, can regulate cytoskeletal reorganization by inducing the stabilization of microtubules (Ciani et al., 2004). Furthermore, shear stress, which rapidly promotes actin cytoskeleton reorganization, has recently been found to modify the Wnt signaling pathway through different mechanisms (Kuo et al., 2015). These studies suggest a possible connection between the two major effects of apoptosis. Even if they turn out to be unrelated, the two mechanisms can co-exist and cooperate. For instance, in aggressive B cell lymphomas, apoptotic tumor cells can simultaneously promote tumor growth, angiogenesis and remodeling (Ford et al., 2015).

Another very interesting recent observation is the existence of different types of apoptotic cells: the 'conventional' apoptotic cells, which are fragmented into apoptotic bodies and are cleared very quickly from the tissues, and the 'dancing' apoptotic cells, which are not fragmented and remain in tissues for longer periods of time, even though they contain active caspases (Yamaguchi et al., 2011). It would be interesting to investigate whether these two types of apoptotic cells are present in other contexts and whether they have distinct signaling roles or produce different types of signaling molecules.

Recent advances in the field of apoptotic signaling have given rise to a very complex picture: the signals produced by apoptotic cells can be of very different natures and sometimes their effects can be contradictory. It would be expected that, depending on the context, apoptotic cells would need to provide only certain instructions to neighboring cells. For instance, it would seem desirable that when apoptosis is used to trim cell numbers or eliminate whole structures, apoptosis-induced proliferation is not triggered, as that could delay or compromise the role that apoptosis has to play. This selectivity could be achieved by generating different signals in different circumstances, by neighboring cells being receptive to only certain signals or by producing a signal that has different roles depending on the context. This is seen, for example, in the case of Eiger/TNF, which can promote proliferation and survival in the *Drosophila* brain but works as a pro-death factor in the mouse hair follicle (Kato et al., 2009; Perez-Garijo et al., 2013). The main challenge will be to elucidate the relevant physiological settings in which these different modes of signaling operate.

In this regard, it is interesting that most of the apoptotic signaling properties that have been described so far depend on JNK signaling, which triggers apoptosis under stress conditions but is often not involved in developmentally programmed cell death. Thus, the signaling pathways described so far will likely play an important role in stress situations, as is the case in regeneration and cancer, but might not function during normal development. It remains to be determined whether signaling by apoptotic cells is completely absent during development. Given the enormous potential of apoptotic signaling, it is tempting to speculate that organisms might use apoptotic events as signaling centers to induce a desirable outcome at an appropriate time. On the other hand, it is possible that different pathways, perhaps independent of JNK signaling, underlie apoptotic signaling in developmental situations.

Studies of the non-autonomous effects of apoptosis have tremendous potential to guide regenerative and cancer therapies (Box 2). The phenomenon of apoptosis-induced proliferation, which might have evolved as an advantageous tissue regeneration mechanism, is exploited by tumor cells to avoid eradication (Zimmerman et al., 2013). This suggests that strategies countering apoptosis-induced proliferation might improve cancer treatment. For example, caspase inhibition has been shown to increase radiosensitivity in lung and breast cancer models (Kim et al., 2008; Moretti et al., 2009). Additionally, approaches to study global cellular responses and the secretomes of apoptotic tumor cells might provide novel strategies for cancer treatment (Obenauf et al., 2015; Wiita et al., 2013). Furthermore, given the importance of apoptosis in multiple disease conditions, it would be interesting to study the non-autonomous effects of cell death not only in cancer, but also in other pathologies. For example, a role for apoptosis has recently been described in cardiac hypertrophy (Putinski et al., 2013). Moreover, caspases have been shown to play a crucial role in the reprogramming of induced pluripotent stem cells (iPSCs), which opens up exciting new opportunities for exploring the relevance of apoptotic signaling in stem cell biology (Li et al., 2010a).

Finally, it would be interesting to investigate the impact of other forms of cell death in neighboring tissues. Even though apoptosis, necrosis and autophagy were originally considered independent and exclusive death routes, recent findings have revealed a connection between these forms of cell death, with some of the main apoptosis mediators being shared by the necrotic and autophagic pathways. Interestingly, there is growing evidence to suggest secretion-dependent roles for autophagy (Deretic et al., 2012). Indeed, the production of secreted factors by autophagic cells has recently been shown to modify the tumor microenvironment and tumor invasion (Kraya et al., 2014; Lock et al., 2014). Effects of necrosis on surrounding cells might be expected because necrosis involves cell rupture and the release of cellular contents. It is surprising that, under these circumstances, some of the same players have been identified as relevant signals producing non-autonomous effects. For example, neuronal necrosis can result in the spreading of cell death through the JNK and TNF pathways, in striking similarity to the phenomenon of apoptosis-induced apoptosis, suggesting a mechanistic link between the two processes (Yang et al., 2013).

Since the initial description of apoptosis, we have gained tremendous knowledge about the molecular and genetic bases of this process, and numerous roles for apoptosis have been described during normal ontogeny and in human pathologies. Recently, we have learnt that apoptotic cells are definitely not silent – actually, they are quite loquacious – and listening to them will provide important insights for furthering our understanding of development, regeneration and disease.

Competing interests

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