

Review

Killers creating new life: caspases drive apoptosis-induced proliferation in tissue repair and disease

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Apoptosis is a carefully orchestrated and tightly controlled form of cell death, conserved across metazoans. As the executioners of apoptotic cell death, cysteine-dependent aspartate-directed proteases (caspases) are critical drivers of this cellular disassembly. Early studies of genetically programmed cell death demonstrated that the selective activation of caspases induces apoptosis and the precise elimination of excess cells, thereby sculpting structures and refining tissues. However, over the past decade there has been a fundamental shift in our understanding of the roles of caspases during cell death—a shift precipitated by the revelation that apoptotic cells actively engage with their surrounding environment throughout the death process, and caspases can trigger a myriad of signals, some of which drive concurrent cell proliferation regenerating damaged structures and building up lost tissues. This caspase-driven compensatory proliferation is referred to as apoptosis-induced proliferation (AiP). Diverse mechanisms of AiP have been found across species, ranging from planaria to mammals. In this review, we summarize the current knowledge of AiP and we highlight recent advances in the field including the involvement of reactive oxygen species and macrophage-like immune cells in one form of AiP, novel regulatory mechanisms affecting caspases during AiP, and emerging clinical data demonstrating the critical importance of AiP in cancer. *Cell Death and Differentiation* (2017) 24, 1390–1400; doi:10.1038/cdd.2017.47; published online 31 March 2017

Facts

- Activated caspases trigger a form of compensatory proliferation, referred to as apoptosis-induced proliferation (AiP), through secretion of mitogens.
- AiP is best characterized in epithelial cells, and is critical for epithelial wound repair and regeneration across species.
- There are several context- and tissue-specific forms of AiP currently characterized.
- The initiator caspase-dependent AiP mechanism involves JNK signaling, reactive oxygen species (ROS) and recruitment of macrophage-like immune cells.
- Sustained AiP causes tissue overgrowth, and may contribute to tumor growth and treatment resistance.

Open Questions

- Why does activation of caspases in apoptosis sometimes induce AiP and other times not? Conversely, how might caspases be controlled to at times induce only AiP in the absence of cell death?
- Are cell types other than epithelial cells subject to AiP?
- What are the direct targets of caspases in AiP? Are all targets cleaved?
- How general is the involvement of AiP in tumor development?
- Does AiP contribute to other disease processes?

Death-dependent proliferation

Most, if not all, animals have some capacity to regenerate lost tissue, but there is great variability in that capacity, dependent

on the species and developmental context. Some forms of regeneration depend on compensatory proliferation, a process by which the volume of damaged or lost tissue is replaced via additional mitotic divisions within the healthy neighboring tissue (Figure 1a). The first quantitative evidence for compensatory proliferation came from studies in *Drosophila melanogaster*. In 1977, it was demonstrated that irradiated wing precursor tissue (imaginal discs) of the developing fly larva, despite elimination of up to 60% of cells, could yield a normal sized wing with appropriate patterning due to subsequent increase in mitosis among surviving cells.¹ Follow-up studies found that the increased mitosis following apoptosis could consistently and appropriately accommodate for the lost tissue, suggesting the two processes were directly linked.^{2,3}

Several studies in 2004 supported the concept of apoptosis-dependent proliferation. In planarians, caspase-like genes are activated, and apoptotic cells are present throughout the regenerative process, well beyond the original injury.⁴ In newts, apoptosis was found to be required for proper limb regeneration.⁵ Multiple studies in *Drosophila* found that induction of the apoptotic machinery was sufficient to promote non-autonomous proliferation in the surrounding cells, independent of completed cell death.^{6–8} These and follow-up studies established that the *Drosophila* initiator caspase Dronc was specifically responsible for driving compensatory proliferation following initiation of apoptosis.^{6,9,10}

Apoptosis-induced proliferation (AiP) is the designated term for the distinct subtype of compensatory proliferation specifically triggered by the apoptotic machinery.¹¹ AiP is not a single pathway, but encompasses a variety of signaling cascades by

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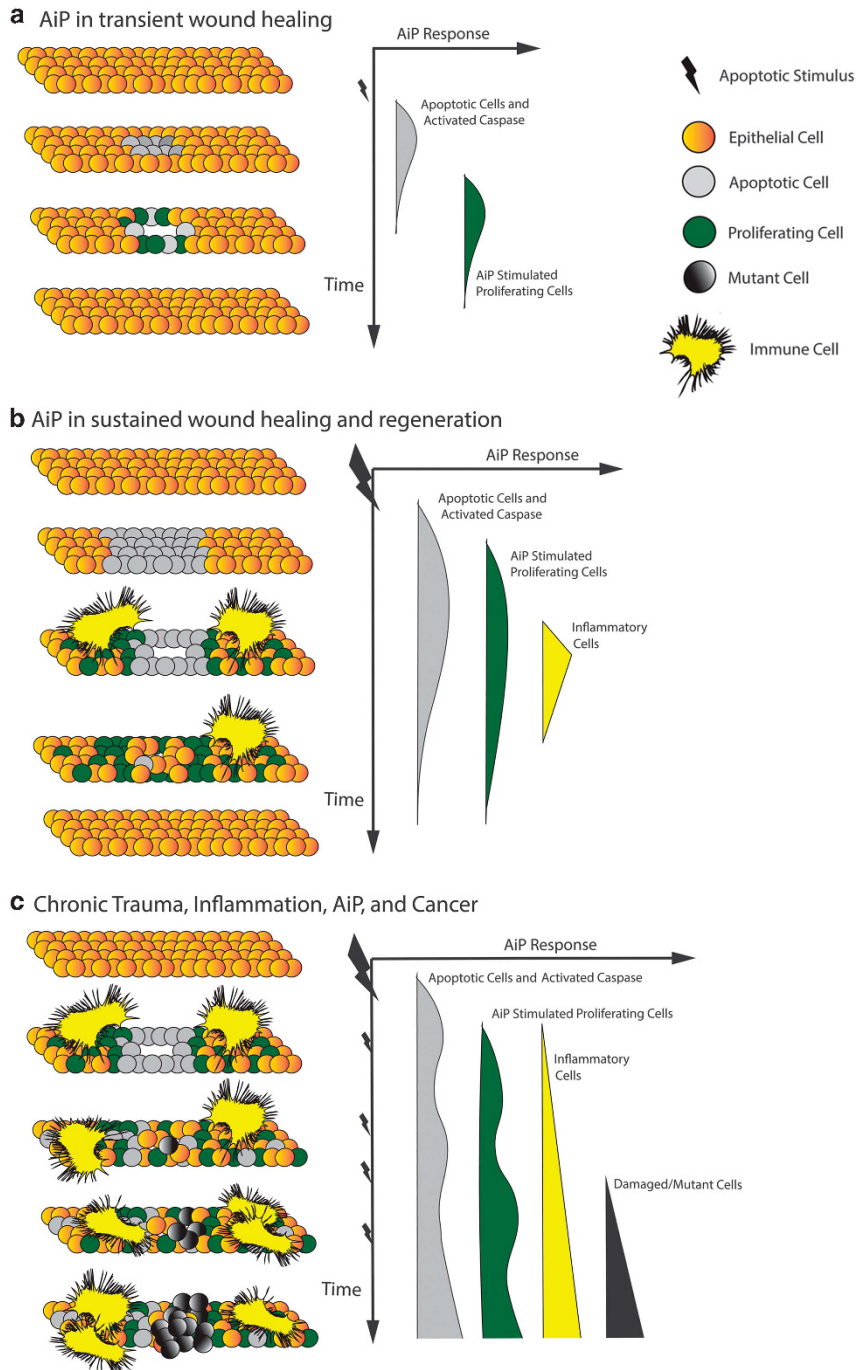


Figure 1 The Spectrum of AiP in wound healing, regeneration, and the development of cancer. On the left, shown are sheets of epithelial cells. On the right, the relative timing of caspase activity, AiP and the recruitment of immune cells as well as cancer cells (c) is illustrated. Based on the variety of caspase-dependent AiP mechanisms, it is clear that the phenomenon of AiP includes a spectrum of functions from simple transient wound healing, to more complex regeneration, to responding to chronic damage and inflammation. (a) Transient AiP is a self-limited proliferation in direct response to caspase activation in some apoptotic cells. (b) AiP associated with more significant or sustained wound healing often requires additional support including from the immune cells recruited by caspase-dependent signals such as extracellular ROS, but eventually resolves upon tissue repair. (c) Caspase-dependent AiP which occurs during ongoing or repeated damage, such as in chronic inflammatory diseases, can lead to an imbalance in cell death versus proliferation, leading to tissue dysplasia, hyperplasia, or possible neoplasia from the AiP stimulation of damaged cells containing new cancer-causing mutations

which caspases promote non-autonomous proliferation through secretion of mitogens, often in parallel to, but independent of the final execution of apoptotic cell death.^{12–18} These caspase-dependent signaling path-

ways play a critical role in wound healing and regeneration (Figure 1a and b). Furthermore, apoptotic caspases may also contribute to tumor initiation, caspase-dependent inflammation, and sustained AiP in tumor initiation. Tumors have been

described as analogous to wounds that don't heal,¹⁹ and chronic inflammatory states, often characterized by repeated cycles of cell death and incomplete wound healing, are known to predispose to the development of certain cancers (Figure 1c).²⁰ Here, we will explore the mechanisms, regulation, and significance of caspase-driven AiP.

The many mechanisms of caspase-driven AiP

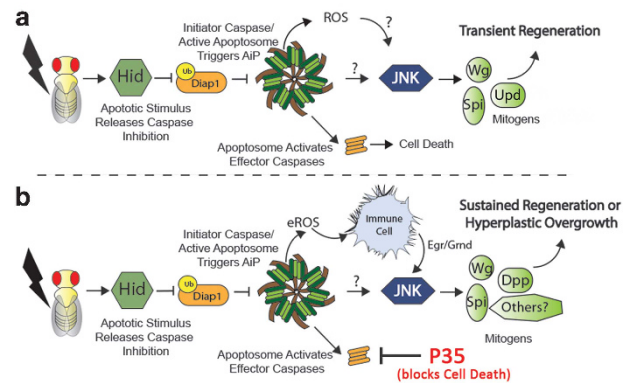
The mitochondrial apoptosis pathway is well conserved among species. The major players include: (1) the death proteases—initiator and effector caspases, synthesized as inactive zymogens and activated through multimerization and proteolytic cleavage. Seven caspases in humans are involved in control of apoptosis, of which caspase-2,-8,-9 and -10 function as initiators of apoptosis, while caspase-3,-6 and -7 are the effectors of apoptosis.²¹ *Drosophila* have three caspases known to be involved in apoptosis: the initiator caspase Dronc (homolog of caspase-2,-9) and the effector caspases DrICE and Dcp-1 (homologs of caspase-3,-7).²² (2) Apaf-1/Dark, scaffolding proteins that complex with the initiator caspase (caspase-9/Dronc) to form the active apoptosome. (3) Multiple upstream pro-apoptotic and anti-apoptotic factors whose opposing actions control where and when initiation of apoptosis occurs.^{22–24} In mammals, Bcl-2 family proteins regulate activation via the mitochondria,²⁵ whereas in flies, the pro-apoptotic genes *reaper*, *hid* and *grim* activate apoptosis by antagonizing the E3-ligase *Drosophila* Inhibitor of Apoptosis Protein1 (DIAP1), a negative regulator of caspase activation (Figure 2a).^{26–30} DIAP1 mono-ubiquitylates the initiator caspase Dronc to inhibit its apoptotic activity.^{31–36} These regulatory mechanisms affect both apoptosis and caspase-driven AiP, but potentially to different degrees.^{17,37,38}

In mammals, to date only the effector caspase-3 and -7 have been shown to be involved in AiP.^{15,39–44} In *Drosophila*, Dronc as well as DrICE and Dcp-1 have been implicated in distinct forms of AiP.^{6,9,10,13,17,45,46} Here, we will discuss the roles of caspases in AiP and their effects on downstream signaling events in flies and mammals.

The *Drosophila* initiator caspase Dronc triggers a JNK-dependent proliferative stress response. Most of the foundational work characterizing caspase-dependent proliferation has utilized genetic models of AiP in the *Drosophila* imaginal discs, developing epithelial tissues with high regenerative capacity.^{46,47} The 'undead' model, so called as pro-apoptotic genes (such as *reaper* or *hid*) are over-expressed while execution of cell death is blocked by co-expressing the effector caspase inhibitor *p35*,^{48,49} allows for uncoupling of the functions of Dronc from the effector caspases and the actual execution of apoptosis (Figure 2b).^{6–9,17,50–52} This uncoupling was key to revealing the role of activated Dronc as a necessary and sufficient driver of mitogen production and AiP in developing epithelial tissues. Dronc-mediated AiP depends on activation of JNK,^{8,17,53} and the production of mitogens such as the Wnt homolog Wingless (Wg), the EGF homolog Spitz (Spi), the interleukin-6 homolog Unpaired (Upd), and the BMP/TGF β homolog Decapentaplegic (Dpp) (Figure 2a and b).^{7,8,17,51,54}

Interestingly, heterozygosity of *dronc* substantially suppressed JNK-dependent AiP, but not apoptosis,^{9,10,17,53,55} suggesting that there is a higher caspase threshold for activation of AiP as compared to apoptosis, when pro-apoptotic stimuli are present.

Initiator Caspase-Dependent AiP in *Drosophila*



Effector Caspase-Dependent AiP Across Species

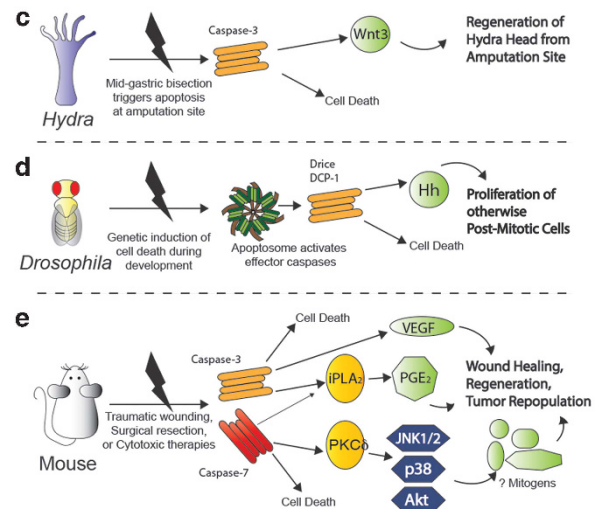


Figure 2 Mechanisms of caspase-dependent AiP. Apoptosis-induced proliferation may be induced by either initiator (a and b) or effector (c–e) caspases. Activation of these caspases under certain conditions results in a variety of signaling cascades, producing a variety of growth factors and other mitogenic signals. (a and b) The *Drosophila* caspase Dronc is the only initiator caspase to date characterized as independently driving AiP. In transient regeneration (a), the active apoptosome including Dronc, triggers production of extracellular ROS and activates JNK, leading to production of the secreted mitogens Wingless (Wg) and Spitz (Spi). More sustained regeneration (b) requires additional input from recruited immune cells, signaling through the TNF homolog Eiger (Egr) and its receptor Grindelwald (Grnd), and possibly involves additional mitogens. (c–e) Effector caspases are implicated in AiP across the species, but examples shown here have confirmed the requirement of effector caspase activity. (c) In hydra, regeneration of an amputated head is dependent on caspase activity in the apoptotic cells at the regeneration site. These cells release the mitogen Wnt3. (d) The effector caspases DrICE and DCP-1 are required for Hedgehog (Hh) production in *Drosophila* during AiP in maturing eye tissues. (e) In mice and human cell lines, following any number of injuries, including surgical resection, radiation or cytotoxic chemotherapies, caspase-3 cleaves a phospholipase A₂ (iPLA₂) triggering production of prostaglandin E₂ (PGE₂) which stimulates AiP. Caspase-3 also is required for VEGF-A production. Caspase-7 activates the kinase PKC δ , activating similar stress response pathways as seen in *Drosophila*

Upstream of JNK, a number of kinases and adaptor proteins contribute to the context-dependent initiation of JNK activity.^{56,57} Selective activation of subsets of these upstream factors directs whether JNK activity is pro-apoptotic or pro-survival.⁵⁸ How activation of the same JNK (flies only have one JNK homolog) by distinct upstream factors can trigger different outcomes as diverse as death or survival remains largely enigmatic. In undead models, only *dTraf2* (JNKKKK), *dTAK1* (JNKKK), and both *Hep* and *dMKK4* (JNKs) are required upstream of JNK to drive AiP.¹⁷ However, despite the identification of upstream JNK pathway components, how Dronc activates JNK during AiP was less clear—until recently.

Dronc-dependent recruitment of immune cells promotes JNK activation and enhances AiP through a positive feedback amplification loop. A prominent feature of wound healing and regeneration is the infiltration of immune cells to the site of injury, which likely contributes to efficient healing under typical conditions (Figure 1b), but they are not fundamentally required for effective regeneration. For example, mice lacking inflammatory cell lineages are still able to repair of damage to their skin.^{59,60} However, we have demonstrated recently that under certain circumstances caspases can signal to immune cells to contribute to sustained proliferation during AiP (Figure 2b). In epithelial tissues, Dronc activation triggers the production of extracellular reactive oxygen species (eROS) through the NADPH-oxidase Duox.¹⁸ In many systems, eROS gradients are known to recruit immune cells to sites of wound healing,^{61,62} and in the undead model, eROS are required to recruit macrophage-like hemocytes to sites of apoptotic activity.^{18,63} Hemocytes in turn contribute to proliferation via amplification of JNK-dependent signaling through production of the TNF homolog Eiger (Figure 2b).¹⁸ Therefore, Dronc promotes JNK activation through an inflammatory response that involves eROS, hemocytes and TNF/Eiger signaling.

Effector caspases induce production of secreted mitogens from dying cells. Genetic screening using the undead (*p35*-dependent) AiP model has provided a lot of intriguing insight into the mechanisms of AiP, but excludes the contributions of effector caspases, the enzymes most directly associated with executing cell death, to AiP. Therefore, several 'genuine' or regenerative (*p35*-independent) models of AiP have been developed, in which tissues are first subjected to a limited pro-apoptotic stimulus, and then they are allowed to regenerate lost tissue through compensatory AiP.^{17,18,64–67} These genuine/regenerative models have confirmed the requirement of JNK under physiological conditions, and have been useful for investigating the spatiotemporal dynamics of AiP.^{17,18,64–66}

The genuine/regenerative AiP models have also shown that effector caspases can promote proliferation through triggering the production of diffusible mitogens. For example, in hydra, caspase-3 activation in apoptotic cells promotes secretion of Wnt3, a key signal for proliferation and remodeling, at the site of head regeneration following mid-gastric bisection (Figure 2c).¹⁴ In apoptotic photoreceptor neurons in *Drosophila*, DrICE and Dcp-1 drive the upregulation and secretion of Hedgehog (Hh) which triggers activation of the Hippo growth

control pathway for AiP (Figure 2d).^{13,68} In mammalian tissues, caspase-3 and -7 can cleave and activate the calcium-insensitive phospholipase A₂ (iPLA₂), which in turn produces several phospholipid signals involved in phagocyte migration, apoptotic cell detection and clearance, and prostaglandin E₂ (PGE₂), a diffusible signal that promotes stem and progenitor cell proliferation (Figure 2e).^{69–73} Caspase-3-dependent production of PGE₂ is required for epithelial wound healing in the skin of mice, contributes to liver regeneration following partial hepatectomy, and has been implicated in tumor growth and tumor repopulation following targeted cytotoxic therapies.^{15,40} Caspase-7, while first linked to activation of iPLA₂, seems to be more important for activation of protein kinase C delta (PKCδ), which in turn mediates phosphorylation of Akt, p38 and JNK1/2 leading to mitogen production and tumor repopulation following radiation therapy (Figure 2e).⁴²

Multiple mechanisms of AiP: redundancy or context-specific? Controversies in the field of AiP are derived from the differences between undead and genuine/regenerative models. Depending on the model, the developmental stage, or the tissue (*eye versus wing imaginal discs*), different research groups conflict on the requirement and origins of specific mitogens such as Wg and Dpp.^{47,51,64,66} Even in our own recent work, we found that the role for hemocytes is not conserved among all forms of AiP. It is clear that Dronc triggers ROS production in response to pro-apoptotic stimuli in both undead AiP and genuine/regenerative AiP models in eye and wing discs.^{18,54} ROS is required for AiP in both contexts (Figure 2a and b); however, immune cell recruitment and TNF signaling does not appear to play a major role in the context of the transient regenerative period in genuine models, while it is clearly critical to the sustained proliferation in the undead model (Figure 2a and b).^{18,54}

These 'discrepancies' among the various models may in fact reflect flexibility—different mechanisms of caspase-driven AiP only activated under different contexts. There is already evidence for differences based on developmental context in flies—Dronc triggers AiP in undifferentiated eye tissue, but with a few more hours of development, the effector caspases DrICE and Dcp-1 become critical for driving AiP within the more mature differentiated tissues.¹³ Alternatively, 'discrepancies' could represent partial redundancy. Multiple AiP pathways may be activated sequentially or simultaneously depending on the extent and duration of the insult. Ongoing studies in *Drosophila* are taking advantage of the ever-increasing genetic toolkit to systematically test these possibilities. At the same time, there are opportunities in other model systems to examine the conservation of these mechanisms, probing for redundancy or variability. Although the function of caspases in initiating a ROS-dependent proliferation pathway is a new addition to the field of regeneration, the downstream functions of ROS in redox signaling, damage detection, and damage response have been the focus of many recent studies.⁷⁴ Notably, the mechanisms of ROS-dependent proliferation—in recruitment of immune cells and in activating MAPK cascades—have been linked to wound healing and regeneration in *Hydra*, *Drosophila*, *Xenopus* and zebrafish.^{75,76} Hydrogen peroxide (H₂O₂) is a diffusible ROS

molecule, which when initially produced from the site of injury can form a chemoattractant gradient for leukocytes within the first few hours following the trauma.^{61,62} This transient ROS burst and the responding immune cells contribute to the temporary healing of the wound site. In contrast, sustained ROS production is required over the following 12–72 h for the regeneration of complete structures (e.g., amputated tails of *Xenopus* tadpoles).^{77,78} While the initial transient burst of ROS may simply be explained as nonspecific oxidative stress released from the traumatized cells, the sustained ROS are more likely actively produced by oxidases over this extended time period. The sustained ROS signal in *Xenopus* activates JNK and generates many of the known AiP-associated mitogens (Wnt, TGF β , BMP, etc), similar to the undead fly model. These findings, taken together with the previously reported requirement for Caspase-3 in *Xenopus* tail regeneration,¹² highlight one of many excellent opportunities to look for conserved mechanisms of caspase-driven AiP, connecting the dots between trauma, caspases, and eROS signaling.

Intriguingly, Dronc is the only initiator caspase to date conclusively shown to drive AiP independently of effector caspases, while examples of effector caspase-driven AiP are seen from hydra to humans. It is possible that *Drosophila* has a uniquely evolved mechanism of Dronc-dependent AiP; however, the parallels described above would suggest further studies of caspase-2,-8 or -9-specific contributions to AiP in other systems are warranted. All future studies should be open to the possibility of multiple paths from caspases to proliferation.

The regulation of caspases in AiP: a matter of life or death

While the mechanisms downstream of caspases for some forms of AiP are better understood, essential questions about the regulation of caspases in AiP remain. AiP does not occur after every cell death. It rather appears to be confined to stress-induced apoptosis. How is this distinction regulated? In general, what distinguishes apoptotic from non-apoptotic functions of caspases? Currently, there are no definitive answers to these and related questions. However, in

the following we review some recent findings on the regulation of Dronc in AiP that give a glimpse at the potential answers.

Regulating the enzyme: non-degradative mono-ubiquitylation regulates Dronc in apoptosis and AiP. It has long been known that the RING domain of DIAP1 inhibits the activity of Dronc through ubiquitylation.^{33–35} However, the outcome of this ubiquitylation (degradation *versus* non-degradative inhibition) was less clear.^{34,35,79} Recently, we have identified a novel regulatory mechanism by which

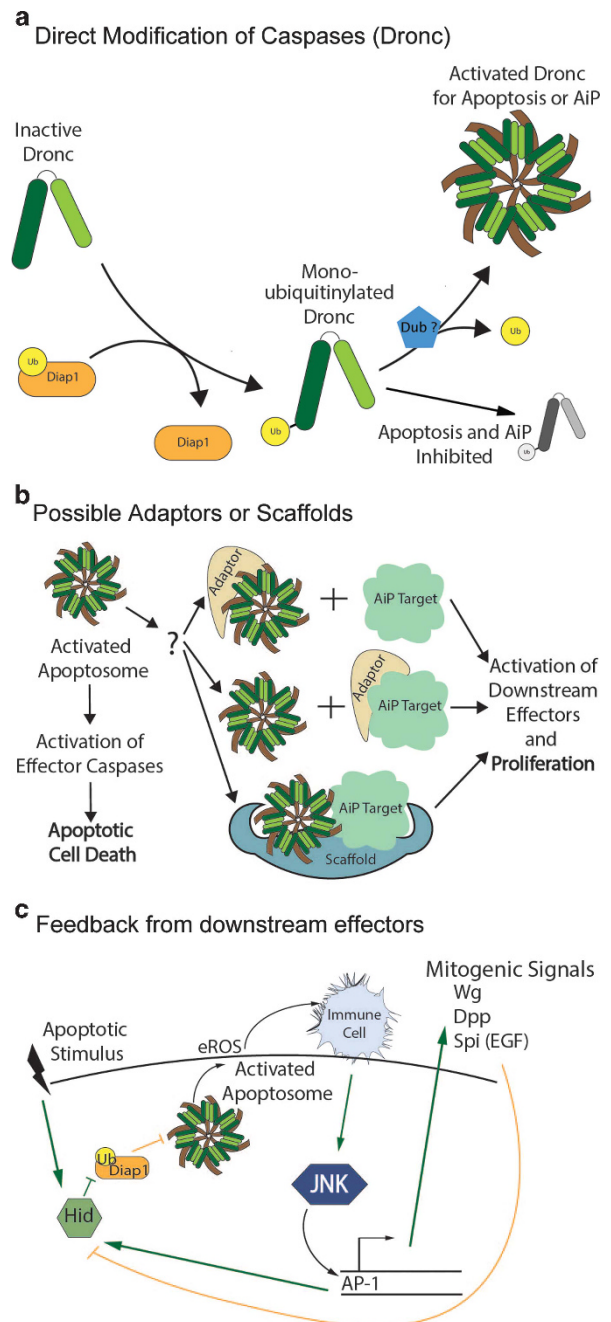


Figure 3 Regulation of caspases in AiP. Caspase activation may lead to apoptotic cell death or AiP. The exact mechanisms that direct caspase activity toward death or proliferative functions are not yet clear. When considering what is known regarding one of the better characterized pathways, the Dronc-dependent AiP in *Drosophila* epithelium, there are several possible regulatory mechanisms which could direct Dronc toward AiP. (a) Direct biochemical modification of caspases: Non-degradative mono-ubiquitylation of Dronc inhibits both apoptotic and AiP activity. (b) Direction of caspases by adaptors or scaffolds: other non-apoptotic functions of Dronc rely on adaptors to localize the caspase to the non-apoptotic target. It is possible that Dronc-mediated AiP may be facilitated by an as of yet unidentified adaptor or scaffolding protein. (c) Feedback loops allow for amplification (green arrows) or inhibition (orange lines): positive feedback from JNK upregulating the pro-apoptotic factor *hid* increases Dronc activity and yields a robust AiP response. Negative feedback from EGFR signaling can inhibit Hid activity, potentially dampening the AiP response. As new downstream targets are uncovered for both initiator and effector caspase-dependent AiP, we will likely develop a better understanding of how a balance among these varying positive and inhibitory signals drive caspases towards a proliferative function

ubiquitylation controls the activity of Dronc. Dronc from embryonic, larval, pupal and adult stages is ubiquitylated only on one Lysine residue, K78, and this ubiquitylation is a mono-ubiquitylation which is not a mark for proteasomal degradation (Figure 3a).³⁶ A ubiquitylation-deficient mutant of Dronc (Dronc^{K78R}) interacts more efficiently with the scaffolding protein Dark and thus induces more apoptosis, suggesting that K78 mono-ubiquitylation of Dronc inhibits the interaction with Dark which explains at least partially the inhibitory role of ubiquitylation.³⁶ Removal of K78 mono-ubiquitin by a deubiquitylase (DUB) may facilitate recruitment of Dronc into the Dark apoptosome (Figure 3a). While a specific DUB for this activity is not known, the recently described DUBA is a good candidate.⁸⁰

Very surprisingly, a ubiquitylation-deficient and catalytically dead (due to the change of the catalytic Cys318 to Ala) double mutant of Dronc (Dronc^{K78RC318A}) rescues the strong lethality of homozygous *dronc* null mutants which the catalytic-only mutant Dronc^{C318A} was unable to do.³⁶ Because expression of *dronc*^{K78RC318A} does not rescue the inability of *dronc* mutants to induce apoptosis,^{36,55} these observations suggest that the non-apoptotic functions of Dronc are more important for the survival of the fly than its apoptotic one. Moreover, in contrast to *dronc*^{C318A}, co-expression of *dronc*^{K78RC318A} with *p35* does induce AiP,³⁶ suggesting that mono-ubiquitylation of Dronc serves also an inhibitory role for the ability of Dronc to induce AiP (Figure 3a).

This observation also implies that the catalytic activity of Dronc is not strictly required for AiP and may offer an answer to another question that centers on the proteolytic target of Dronc for AiP. Although Dronc cleaves DrICE and Dcp-1 for induction of apoptosis, a specific cleavage target of Dronc for AiP has not been identified although there is evidence that Dronc has additional cleavage targets.⁸¹ The observation that Dronc^{K78RC318A} can induce AiP may suggest that Dronc does not have a specific cleavage target for AiP. All Dronc needs for AiP is incorporation into the Dark apoptosome¹⁷ which is facilitated in the absence of K78 ubiquitylation.³⁶ Therefore, the Dronc^{K78RC318A} mutant opens up exciting opportunities for future investigations not only regarding AiP but also other non-apoptotic functions of Dronc that are independent of effector caspase activation.

Regulating the targets: a role for adaptors and scaffolds directing AiP versus apoptotic death? It is clear that there is highly specific signaling that leads to AiP, but another ongoing question is how are caspases directed to their non-apoptotic targets to promote AiP instead of apoptosis. As described above, Dronc interacts with the scaffolding protein Dark/Apaf-1 during the initiation of apoptosis. The apoptosome provides an environment that facilitates zymogen activation, and aggregation of caspase activity to cross the threshold to trigger apoptosis.^{82,83} When considering other non-apoptotic functions of Dronc, there are examples of accessory adaptors interacting with this scaffold to regulate and direct activity (Figure 3b). For example, in spermatid differentiation in *Drosophila*, active Dronc within the apoptosome is required for cellular remodeling that resolves the individual spermatids from a larger syncytium.⁸⁴ Interestingly, during this non-apoptotic process the active apoptosome

does not induce cell death. The apoptosome is activated and directed to the proper sub-cellular localization by an adaptor molecule, Tango7.^{85,86} Tango7 is required for Dronc to cleave its targets during this remodeling, with mutant alleles of Tango7 resulting in male sterility due to a failure of sperm individualization.⁸⁵

In contrast, the unconventional myosin Crinkled serves as an adaptor for caspase substrates during Dronc-dependent patterning, specifically guiding kinases such as GSK3 β to active Dronc for cleavage and regulation of its activity.⁸⁷ Both of these interactions were also found in mammals, with the Tango7 homolog PCID1 interacting with caspase-9, and the unconventional myosin MYO7A binding to and regulating a different initiator, caspase-8.^{86,87} Taken together these findings in *Drosophila* and mammals raise the possibility that some as of yet unidentified co-factors could physically interact with caspases to guide them to AiP targets preferentially over the cell death effectors, or bring AiP targets to active caspases while cell execution proceeds (Figure 3b).

Regulating the response: feedback loops modulate AiP activity. As detailed above, Dronc triggers AiP through JNK. While JNK is activated downstream of Dronc, JNK activation also leads to upregulation of pro-apoptotic *hid*, amplifying the initial apoptotic stimulus, caspase activity, and caspase-driven pathways like AiP (Figure 3c).^{8,10,17,53,88} In the undead AiP model, the ROS-recruited hemocytes contribute to this JNK/Dronc feedback amplification (Figures 2b and 3c).¹⁸ Blocking hemocytes in this model not only blocks JNK activity and AiP but also leads to reduced caspase activity in the undead cells, a feature not seen when blocking AiP at other downstream points.¹⁸ Adding yet another layer, there is some evidence that in certain contexts there is JNK-dependent non-autonomous positive amplification of the apoptotic pathway in surrounding cells (apoptosis-induced apoptosis), leading to increased caspase activity, and potentially further activating a wider AiP response.^{89,90} Conversely, EGFR signaling, another player activated downstream of JNK in AiP,¹⁷ negatively regulates Hid activity and reduces caspase-driven apoptosis in certain cell populations, reducing their susceptibility to cell death.^{91–93} This type of feedback could provide a mechanism to limit caspase-driven AiP preventing unregulated proliferation.

The AP-1 transcription factors c-Jun and c-Fos are important for many developmental control programs and, when specifically phosphorylated by JNK, can activate genes important for death, survival, cellular remodeling, or proliferation, depending on the context.^{57,94,95} In the context of regenerative proliferation, it was recently demonstrated that AP-1 can directly bind to a stress-responsive promoter element of the *wg* gene which is only activated during regenerative responses.⁹⁶ How JNK-activated AP-1 is directed to this element only under regenerative conditions is unknown, but other downstream pathways in AiP might contribute to regulation. For example Atg1, a serine/threonine kinase known for its role in autophagy, was recently identified as functioning downstream of JNK to promote AiP independently of autophagy.⁹⁷ Therefore, Atg1 might act as a modulation factor directing the correct JNK-dependent cellular

response to stress situations as in AiP. As new downstream factors like Atg1 are identified and characterized, we should expect to find more regulatory feedback opportunities fine-tuning AiP.

The emerging clinical significance of caspase-driven AiP in cancer

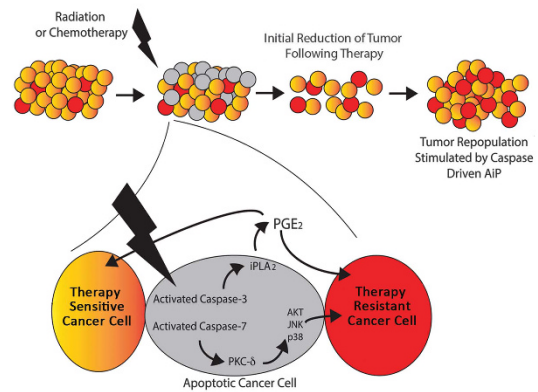
Given that the undead AiP model exploits a sustained induction of proliferation and results in abnormal, hyperplastic overgrowth, it has been proposed that this compensatory mechanism contributes to the aberrant proliferation seen in cancer.⁹⁸ Indeed, a number of recent studies have been exploring the ways in which these physiological regenerative pathways may be deregulated in human disease and even hijacked in malignant transformation and growth.

Caspase-dependent tumor repopulation following cytotoxic therapies. Increased cell proliferation and rapid repopulation of tumors following cytotoxic therapy (either ionizing radiation or chemotherapies) has been a long known factor in poor treatment response in human cancers, but the exact mechanism behind this phenomenon had remained elusive.^{99,100} Following the description of caspase-3-driven PGE₂-mediated proliferation in the context of tissue regeneration (Figure 2e),¹⁵ examples of caspase-dependent PGE₂ production directly supporting tumor growth and repopulation following radiation therapy were found in mouse models and human cancer cell lines (Figure 4a).^{40,41} Since then, possible roles in repopulation for other AiP-associated factors including caspase-7, PKCδ, Akt, p38, JNK1/2, SHH and HMGB1 have been identified (Figure 4a).^{42,43,101,102} A similar caspase/PGE₂ mechanisms of repopulation has also been found following cytotoxic chemotherapy, confirming this is not a radiation-specific response.¹⁰³

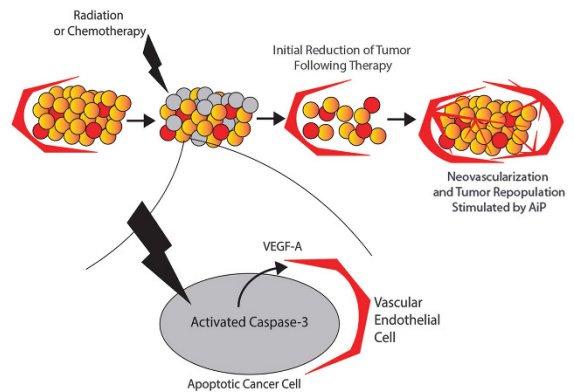
AiP, chronic inflammation, and cancer risk. Separate from their direct role in tumor survival, apoptotic caspases may also contribute to tumor initiation, caspase-dependent inflammation, and sustained AiP in tumor initiation. Tumors have been described as analogous to wounds that do not heal,¹⁹ and tumor-promoting inflammation is an emerging hallmark of

cancer.¹⁰⁴ Chronic inflammatory states, often characterized by repeated cycles of cell death and incomplete wound healing, are known to predispose to the development of certain cancers (Figure 1c).²⁰ Inflammatory bowel diseases such as ulcerative colitis increase the risk of colorectal carcinomas;¹⁰⁵ Marjolin's ulcers, a type of epidermoid carcinoma, can be found arising from chronically irritated skin or non-healing scars;¹⁰⁶ inflammation of the lungs in severe types of chronic obstructive pulmonary disease increases the risk of squamous cell lung cancer, beyond the risk from smoking exposure alone.¹⁰⁷

a AiP Drives Tumor Repopulation Following Therapy



b AiP Promotes Angiogenesis Following Therapy



c Apoptotic Vessels Can Stimulate AiP in Tumors

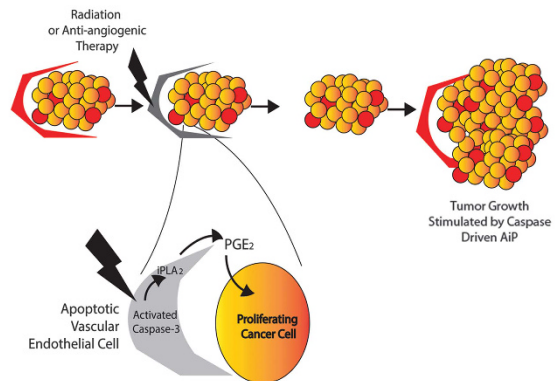


Figure 4 AiP in cancer and treatment failure. Emerging clinical studies have found that high levels of activated caspase-3 in tumors is associated with poor prognosis. Possible mechanisms by which caspase-mediated AiP could be contributing to tumorigenesis are modeled here. (a) Tumor repopulation following radiation or chemotherapy: cancer cells sensitive to the cytotoxic treatment initiate apoptosis and some cells trigger an AiP response through the production of PGE₂ and other proliferative cytokines. Overall tumor bulk is initially reduced due to the apoptotic death, but the remaining cancer cells—including therapy-resistant cells—are stimulated via AiP to proliferate, repopulating the tumor. Over repeated cycles this could lead to extremely resistant tumors and eventual treatment failure. (b) Growth signals from dying tumor cells stimulate angiogenesis: caspase activation in dying tumor cells has been directly linked to VEGF-A production, a growth factor that promotes angiogenesis and neovascularization of the tumor. (c) Dying vascular endothelial cells also trigger AiP: caspase activation in the VE cells results in PGE₂ production, which may stimulate further proliferation of the tumor cells

Just as there are opportunities to connect the dots between caspases and mechanisms of regeneration, recent studies have raised intriguing opportunities to connect AiP with inflammation-associated tumorigenesis. The initiator caspase-dependent model of AiP in *Drosophila* as described above has demonstrated direct connections between ROS, macrophage-like immune cells, JNK stress responses, EGF-mediated signaling, hyperplasia and dysplasia.^{6–9,16–18,63} In models of human inflammatory pathologies, the connections are not characterized as rigorously, but conservation of key players is striking. For example, in a mouse model of ulcerative colitis in which colonic epithelial cells are exposed to oxidative stress, JNK signaling and non-apoptotic caspase activity were linked to each other through mutual feedback, to cell survival despite DNA damage, to increased proliferation, and to morphological changes in the epithelium consistent with early transformation.¹⁰⁸ In another example, a chronic-injury model of liver carcinogenesis in mice showed compensatory proliferation and subsequent tumor formation is mediated by an EGFR-dependent activation of liver macrophages following hepatocyte cell death.¹⁰⁹ Downregulation of apoptotic caspases in the parenchyma is sufficient to prevent the development of these tumors.¹¹⁰

AiP and vasculogenesis in the tumor microenvironment.

Thus far, we have focused on the effects of AiP triggered by caspase-activated cells of one type acting on neighboring cells of the same type, with or without an immune intermediate. However, caspase-dependent AiP can extend to other cell types in the microenvironment. While cytotoxic radiotherapy targeting tumor cells can paradoxically drive AiP-mediated tumor repopulation as described above (Figure 4a), it can also trigger neovascularization and angiogenesis via mitogens acting on vascular endothelial (VE) cells. This post-irradiation angiogenesis (PIA) is thought to contribute to radiotherapy failure in certain cancers, and new evidence suggests this could be partly due to a form of caspase-3-dependent AiP acting on VE cells (Figure 4b).^{44,111} Blocking caspase-3 in irradiated glioma cells attenuates new vessel formation, which in turn suppresses overall tumorigenicity.¹¹¹ Caspase-3-dependent production of VEGF-A by these tumor cells appears to be one of the major factors contributing to PIA, but several caspase-3-activated signaling pathways may contribute to a pro-angiogenic microenvironment for VE cell proliferation.⁴⁴

However, simply blocking angiogenesis is not a panacea.¹¹² VE cells activate caspase-3 following cytotoxic treatments triggering their own production of the diffusible AiP factor PGE₂, enhancing tumor growth which can lead to therapy resistance (Figure 4c).¹¹³ In addition, independent of formal angiogenesis, some especially aggressive tumor types can generate a blood and nutrient supply via a process known as vasculogenic mimicry (VM).¹¹⁴ VM appears to also involve a non-apoptotic function of caspases, as caspase-3 activity contributes to the proliferation of vessel-like channels formed from highly aggressive cancer cells in certain human melanomas.³⁸ Very little is known about the precise mechanisms of VM, but this may represent more of a caspase-dependent invasion and metastasis phenotype than true AiP.

Ongoing work in model organisms, is currently investigating the relationship between caspases, extracellular matrix remodeling, and metastatic potential.¹¹⁵

Clinical implications for prognosis and improved treatments.

Several independent patient cohort studies are now confirming the clinical relevance of caspase-dependent repopulation and other non-apoptotic functions of caspases in these tumors (Figure 4a), finding significant associations between poor prognosis and elevated caspase-3 expression and activity. In one study, increased active caspase-3 in biopsy samples before initiating treatment correlated with significantly shorter overall survival time in gastric, cervical, colorectal, and ovarian tumors.¹¹⁶ In another study, elevated circulating serum levels of caspase activity correlated with shorter time to progression despite chemotherapy in head and neck cancers.¹¹⁷ In a third study, activated caspase-3 and increased HMGB1 expression were found in colorectal cancers and were correlated with poor prognosis, independent of their function in metastasis.⁴³

As the details of caspase-dependent AiP and their vital significance in humans are clarified, the logical next questions center on employing this knowledge to refine cancer therapy or even prevent it before it starts. A few experiments have started to address these questions and have shown promise in leveraging our understanding of AiP to improve cancer treatment strategies. For example, knowing that there is redundancy in AiP signaling, targeting one AiP pathway is likely not enough, and subsequent cycles of AiP might expedite and amplify drug-resistance. One study found modest success in improving colorectal cancer cell killing *in vitro* by combining a pro-apoptotic chemotherapeutic with a cocktail of inhibitors targeting AKT, STAT3 and ERK1/2, three likely AiP-associated survival and proliferation pathways.¹¹⁸ Two other studies have successfully employed *in vivo* administration of celecoxib, an inhibitor of the enzyme cyclooxygenase-2 (COX2) that synthesizes PGE₂ downstream of iPLA₂, as adjuvant therapy following standard cytotoxic treatments to prevent tumor progression and suppress tumor repopulation in a mouse model of melanoma and a xenograft model of bladder cancer, respectively.^{103,119}

Yet, other questions remain wide open. Are there key regulatory points in these caspase-dependent pathways that can be targeted, to distinguish between the various pleiotropic effects, enhancing the desired anti-tumor functions while suppressing the undesired pro-tumor functions? What upstream factors or environmental conditions are driving cancer cells to have such high caspase levels, even before the stress of treatment, priming them for a robust AiP response? Can this knowledge of AiP be harnessed for the field of regenerative medicine in a way that does not increase future risk of malignancy?

Conclusion

Caspases are critical not only for apoptotic cell death but can also stimulate proliferation in response to either death or non-lethal stresses. Caspase-dependent AiP is not a single pathway, but an evolutionarily conserved process which provides mechanisms for healing and adaptation in the face

of an injurious world. Despite the incredible progress made over the past decade, there are likely more mechanisms of AiP that have yet to be uncovered, more upstream regulators influencing life versus death functions of caspases and more downstream caspase targets to effect those functions. Continued work probing AiP in genetically tractable model organisms will provide clues for which players and pathways may be at work in human diseases, while clinical investigations will guide the search for non-apoptotic caspase involvement in new contexts, potentially informing novel therapies and improving patient outcomes.

Conflict of Interest

The authors declare no conflict of interest.

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