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Review

Old, new and emerging functions of caspases

S Shalini[1], L Dorstyn[1], S Dawar[1] and S Kumar*[,1]

Caspases are proteases with a well-defined role in apoptosis. However, increasing evidence indicates multiple functions of caspases outside apoptosis. Caspase-1 and caspase-11 have roles in inflammation and mediating inflammatory cell death by pyroptosis. Similarly, caspase-8 has dual role in cell death, mediating both receptor-mediated apoptosis and in its absence, necroptosis. Caspase-8 also functions in maintenance and homeostasis of the adult T-cell population. Caspase-3 has important roles in tissue differentiation, regeneration and neural development in ways that are distinct and do not involve any apoptotic activity. Several other caspases have demonstrated anti-tumor roles. Notable among them are caspase-2, -8 and -14. However, increased caspase-2 and -8 expression in certain types of tumor has also been linked to promoting tumorigenesis. Increased levels of caspase-3 in tumor cells causes apoptosis and secretion of paracrine factors that promotes compensatory proliferation in surrounding normal tissues, tumor cell repopulation and presents a barrier for effective therapeutic strategies. Besides this caspase-2 has emerged as a unique caspase with potential roles in maintaining genomic stability, metabolism, autophagy and aging. The present review focuses on some of these less studied and emerging functions of mammalian caspases.
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Facts

- Caspases are involved in cell death mediated by apoptosis, pyroptosis, necroptosis and autophagy.
- Caspase function is not limited to cell death.
- Non-apoptotic roles of caspases include proliferation, tumor suppression, differentiation, neural development and axon guidance and aging.

Open Questions

- What are the mechanisms by which a caspase can mediate both cell death and non-cell death functions?
- *In vivo* validation of some of the proposed functions of caspases remains to be addressed.
- Are there other as yet undiscovered physiological roles for caspases?

Cell death is a fundamental process that maintains tissue homeostasis, removes unwanted or damaged cells and ensures recycling of cellular constituents promoting further growth and differentiation. Based on the morphology of dying cells two distinct modes of cell death commonly studied include apoptosis and necrosis, however, other types of cell death types have been recently described.[1–3]

Caspases (*c*ysteine-*asp*artic prote*ases)* are proteolytic enzymes largely known for their role in controlling cell death and inflammation. The function of caspases in cell death was identified more than two decades ago with the discovery of *ced-3* as an executioner of cell death during development of the nematode *Caenorhabditis elegans*.[4] The first mammalian *ced-3* homologs included interleukin-1-beta-converting enzyme (ICE), later renamed caspase-1,[5] and Nedd2, renamed caspase-2.[6,7] Owing to inconsistencies in naming caspases, 18 mammalian caspases are known.[8] However, newly identified caspases-15, -17 and -18 are absent in placental mammals with the exception of caspase-16 (Figure 1).[8] It is also important to note that caspase-5 is not present in mice and caspase-11 and -13 are the murine and bovine orthologues of caspase-4, respectively.[8,9] Caspase-12 exists in both truncated and full-length alleles in humans and as a full-length caspase in rodents.[10] Caspase-14 is expressed in the epidermis and has a primary role in cornification and protection of underlying layers of skin.[11]

Based on their function, mammalian caspase-2, -3, -7, -8, -9 and -10 are apoptotic caspases, where as caspase-1, -4, -5, -11 and -12 are involved in inflammation. The apoptotic caspases are subdivided into the initiators and the effectors based on the presence or absence of specific-protein

[1]Centre for Cancer Biology, University of South Australia, Adelaide, SA 5001, Australia
*Corresponding author: S Kumar, Centre for Cancer Biology, University of South Australia, Frome Road, Adelaide, SA 5001, Australia. Tel: +61 8 8222 3738; Fax: +61 8 8222 3162; E-mail: sharad.kumar@unisa.edu.au
Abbreviations: ROS, reactive oxygen species; Apaf-1, apoptotic protease-activating factor 1; CARD, caspase-recruitment domains; cFLIP, cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein; cIAP, cellular inhibitor of apoptosis protein; DED, death effector domains; DISC, death-inducing signaling complex; HCC, Hepatocellular carcinoma; NEMO, NF-κB essential modulator; FADD, FAS-associated death domain protein; MLKL, Mixed lineage kinase domain-like protein; MOMP, mitochondrial outer membrane permeabilization; PAMPs, pathogen-associated molecular patterns; PIDD, p53-inducible protein with a death domain; RAIDD, RIP-associated Ich-1/Ced-3 homologous protein with a death domain; RIP1, Receptor-interacting protein 1; RIP3, Receptor-interacting serine-threonine kinase 3; TLR3, Toll-like receptor 3; LTP, Long-term potentiation; TNFR, tumor necrosis factor receptor; TRADD, TNFR-associated death domain protein; TRAF, TNF receptor-associated factors; TRAIL, TNF-related apoptosis-inducing ligand
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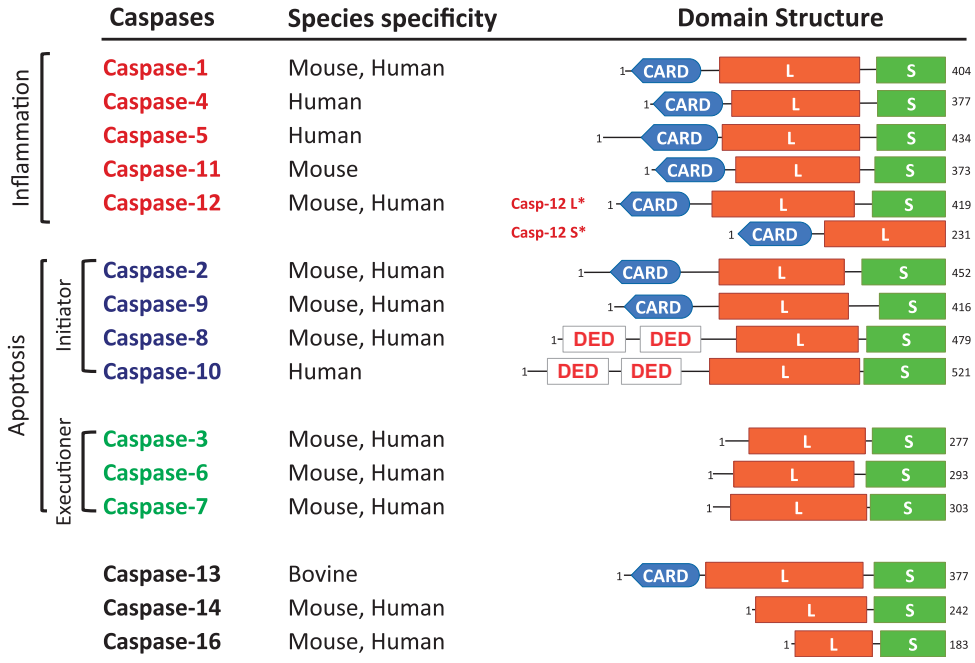

Figure 1 Domain structure and functional classification of placental mammalian caspases. Caspase-1, -4, -5, -11 and -12 are inflammatory caspases. Apoptotic caspase-2, -8, -9 and -10 are initiators, while caspase-3, -6 and -7 are key executioner caspases. CARD, caspase recruitment domain; DED, death effector domain; L, large subunit; S, small subunit; S*, short form; L*, long form

interaction domains toward the N-terminus (Figure 1). Initiator caspases comprise death effector domains (DED; caspase-8 and -10) or caspase-recruitment domains (CARD; caspase-2, -9, -1 and -11), which mediate their dimerization and/or recruitment into larger complexes to facilitate their activation.[12]

Initiator caspase activation during apoptosis is mediated by two main pathways; the mitochondrial or Bcl-2-regulated (intrinsic) pathway and the death receptor (extrinsic) pathway (Figure 2). The intrinsic pathway is activated in response to cellular stress (e.g., cytotoxic drugs, DNA damage) and is regulated by the Bcl-2 family of proteins. This pathway involves activation of the pro-apoptotic effectors BAX and BAK, which induce mitochondrial outer membrane permeabilization (MOMP) and cytochrome *c* release. Apaf-1 (apoptotic protease-activating factor 1) associates with cytochrome *c* into a large multimeric complex called the apoptosome to activate caspase-9.[13] Death receptor-mediated apoptosis is initiated following ligand-binding and activation of the death-domain-containing tumor necrosis receptor superfamily (e.g., TNFR, Fas, TRAIL).[14] This mediates recruitment and activation of caspase-8 or -10, through the death-inducing signaling complex (DISC) comprising FAS-associated death domain protein (FADD) and/or TNFR-associated death domain protein (TRADD) and other components. Caspase-8 also cleaves BID to a truncated form (tBID), which engages the mitochondrial pathway to amplify the apoptotic response.[15] Once initiator caspases are activated through the extrinsic or intrinsic apoptosis pathways, they mediate activation of effector caspases-3, -6 and -7.

Caspase-2 is unique, in that it can be activated both upstream of MOMP and/or downstream of MOMP following various apoptotic stimuli. The activation of caspase-2 is primarily via CARD-mediated homo-dimerization and auto-proteolysis.[16,17] Caspase-2 is also recruited to a large multiprotein complex called the PIDDosome, comprising proteins RAIDD and PIDD,[18] and can also interact with RAIDD independent of PIDD. However, RAIDD and PIDD are not required for caspase-2 activation *in vivo*, which can occur normally in RAIDD- and PIDD-deficient mice.[19] Caspase-2 has been implicated in cell death induced by metabolic imbalance in oocytes, in mitotic catastrophe and in functions outside apoptosis.[20]

In recent years, it has become evident that the role of many caspases is not limited to apoptosis and that they function in other modes of cell death (necrosis, autophagy and pyroptosis). Furthermore, several other non-cell death functions have emerged. We begin by first addressing the role of caspases in cell death pathways other than apoptosis.

Caspases in Non-Apoptotic Cell Death

Necroptosis. Cell death by necroptosis is typically induced when caspase activity is blocked, and is morphologically characterized by an increase in cell volume, swelling of organelles and rupture of the plasma membrane.[21] Necroptosis can be initiated following stimulation of the Toll-like receptor TLR3, genotoxic stress or 'pathogen-associated molecular patterns (PAMPs).[22] However, the best-studied model is TNF-induced necroptosis.[23] Upon stimulation by TNF, TNFR1 recruits TRADD, RIP1, TRAFs and IAPs into a complex called TRADD-dependent complex I. Formation of this complex ensures activation of the canonical NFκB signaling pathway. Subsequently removal of polyubiquitin chains from RIP1, allows RIP1 to dissociate from the plasma membrane and interact with TRADD, FADD, procaspase-8 and cFLIP$_L$ as a TRADD-dependent complex IIa (also called

DISC; Figure 2). Caspase-8 then cleaves RIP1 and RIP3 and initiates apoptosis. Alternatively, by chemical or viral inhibition of caspase-8, or in the absence of cIAPs, another multi-protein complex called the ripoptosome is formed, which includes RIP1, FADD, caspase-8 and cFLIP$_L$. Ripoptosome formation is dependent on RIP1, which can then mediate either apoptosis or necroptosis depending on the presence and levels of cFLIP proteins and caspase-8 activity.[23] When caspase activity is inhibited, RIP1 and RIP3 are stabilized, undergo auto and trans-phosphorylation and recruit MLKL into a complex called the necrosome (complex IIb), that initiates necroptosis. Alternatively, caspase-8 activation leads to cleavage of RIP1 and RIP3, this prevents necroptosis but apoptosis can still occur (Figure 2).[23,24] RIP1 is thus a master regulator of cell fate that acts to regulate caspase-8-mediated apoptosis or promote RIPK3-MLKL-dependent necroptosis signaling pathway when apoptosis is blocked.[25,26] Blocking the activity of RIP1 completely inhibits necroptosis.[27] Interestingly, RIP1 can also inhibit necroptosis to mediate pro-survival and inflammatory signaling via TLR complexes and NFκB signaling.[25,26] Furthermore, recent studies have demonstrated that RIP1-caspase-8 signaling functions as the critical cell fate decision point that drives either cell survival or cell death. RIP1 functions to protect cells from excessive caspase-8-mediated apoptosis and TNF-driven inflammation to maintain cell homeostasis in barrier tissues such as the gut epithelium and skin.[28,29]

Pyroptosis. Cell death by pyroptosis was first observed in macrophages following infection with *Shigella flexneri* or *Salmonella typhimurium*.[30,31] This cell death was later characterized as a novel programmed cell death process, distinct from apoptosis. [32,33] Pyroptosis is a caspase-1- and -11-dependent cell death process and is considered an immune response to dispose of microbe-laden macrophages and clear intracellular pathogens in response to acute bacterial or viral infection or exposure to bacterial toxins. The process involves activation of these inflammatory

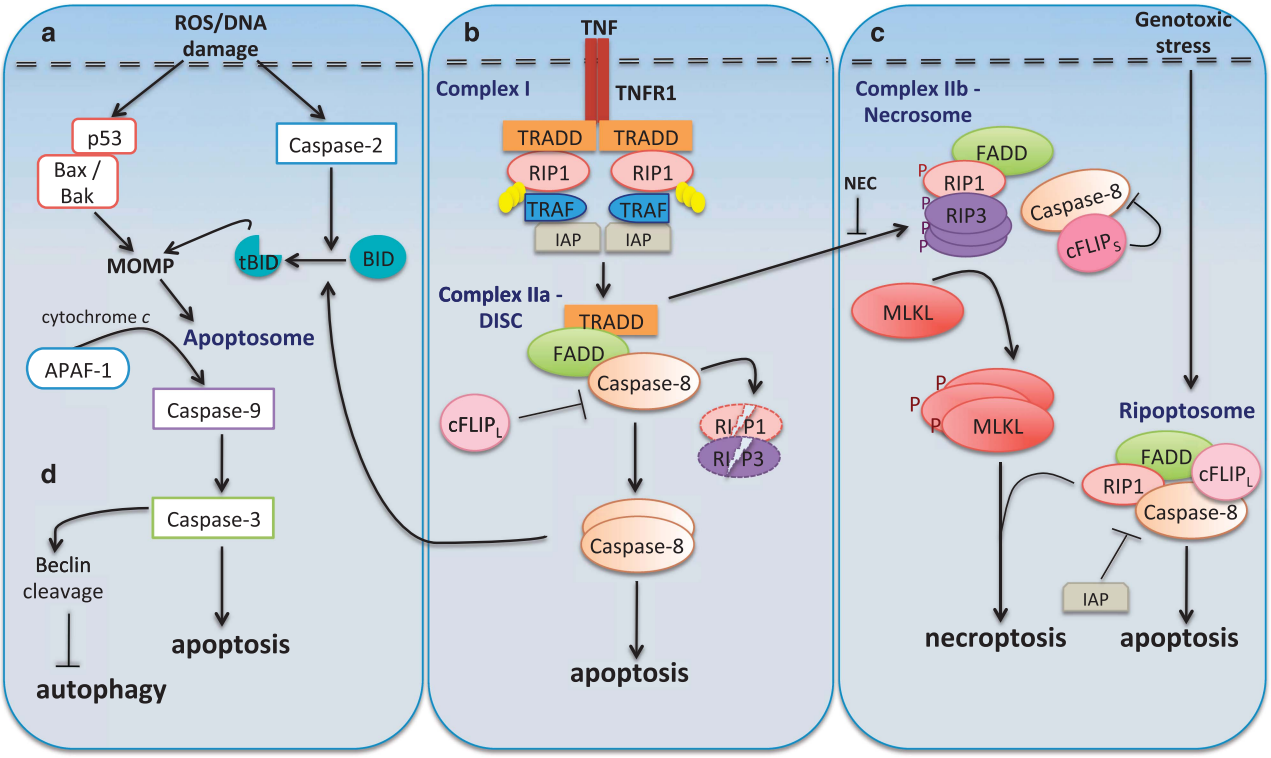

Figure 2 Caspases in cell death pathways. Caspases have been implicated in apoptosis, necroptosis and autophagy. Apoptosis can proceed in two ways: intrinsic or extrinsic. (**a**) Intrinsic apoptosis is initiated by a death-inducing stimulus, which causes activation of p53 and pro-apoptotic Bcl-2 proteins such as Bax and Bak. This leads to MOMP and activation of a death-inducing platform called the apoptosome. Following this caspase-9 is cleaved and activates other effector caspases such as caspase-3. Alternatively, caspase-2 gets activated following DNA damage and causes MOMP by cleaving Bid. (**b**) Extrinsic or receptor-mediated apoptosis involves complex formation by death-domain-containing proteins (called DISC), activation of caspase-8, which initiates apoptosis by directly activating effector caspases. Caspase-8 also cleaves Bid and this causes cytochrome *c* release, further amplifying apoptosis via the mitochondrial/intrinsic pathway. Depending on ligands (TNF and Fas) different complexes are formed. TNF stimulates TNFR1 that recruits TRADD, RIP1, TRAFs and IAPs into a complex called TRADD-dependent complex I. Subsequent removal of polyubiquitin on RIP1, dissociates this complex and allows it to interact with TRADD, procaspase-8, FADD and cFLIP as complex IIa/DISC. This results in caspase-8 activation, leading to apoptosis. (**c**) In certain conditions when caspase activity is blocked, RIP1, FADD and the FLIP$_L$–caspase-8 heterodimer, form a large multiprotein complex called the 'ripoptosome' following genotoxic stress. If caspase activity is lost, RIP1 and RIP3 are stabilized and associate in microfilament-like complexes called necrosomes (complex IIb). Recruitment of MLKL complex at the plasma membrane occurs, leading to pore formation and necroptotic cell death. Alternatively, RIPK1 and RIPK3 are inactivated by active caspase-8 and apoptosis occurs. Necroptosis can also occur following stimulation of TNF receptor or activation of Toll-like receptors (TLRs). (**d**) Some caspases such as caspase-3, mediate cleavage of crucial autophagic proteins and thus indirectly control autophagy. FADD, FAS-associated death domain; TNFR1, TNF receptor 1; TRADD, TNF receptor-associated death domain; cIAP1, cellular inhibitor of apoptosis protein 1; RIP1, receptor-interacting protein 1; MLKL, mixed lineage kinase domain-like; cFLIP, cellular FLICE-like inhibitory proteins; MOMP, mitochondrial outer membrane permeabilization

caspases, resulting in plasma membrane pore formation allowing water influx, cell swelling and osmotic lysis, followed by release of proinflammatory intracellular contents.[32,34] This demarcates it clearly from apoptosis where no loss of membrane integrity is observed. At the same time cytokines are secreted either through the newly formed plasma membrane pores or through microvesicles- or lysosome-mediated exocytosis.[35] The pyroptosis of macrophages also releases the bacteria that can be taken up by neutrophils as a secondary mechanism of phagocytic clearance and destroyed through the NADPH oxidase system.[36] Chromosomal DNA cleavage and nuclear condensation occurs in pyroptosis but in the absence of oligonucleosomal DNA fragmentation or cleavage of typical caspase substrates such as PARP and ICAD that are characteristic of apoptosis.[34,37]

Autophagy. Cross talk between autophagy and apoptosis is well established.[38] Initiation of apoptosis by inhibiting autophagy is beneficial for execution of cell death since autophagy is often considered a pro-survival mechanism. Several human ATG proteins can be cleaved by calpain or caspases. hATG3 has been shown to be cleaved by caspase-3, -6 and -8.[39] Beclin-1/ATG-6 is a caspase-3 target[40] and cleaved beclin-1 reduced autophagy and promoted apoptosis in HeLa cells.[41] Caspase-6 and -8 also cleave p62 that serves to inhibit autophagy.[42] Other studies have shown that hATG3 is a direct target of caspase-8, and cleavage of ATG3 blocked autophagy.[43] Recently, the *Drosophila* effector-caspase Dcp-1 was found to localize to mitochondria following starvation-induced stress and functions to positively regulate autophagy.[44]

A recent study suggests caspase-2 as a repressor of autophagy.[45] Cells/tissues lacking caspase-2 show upregulation of autophagy, which provides them with a survival advantage. Importantly, caspase-2-induced autophagy in a mTOR-AMPK dependent pathway with accompanying MAPK1/3 activation and MAPK14 repression.[45] Atg4 and AMBRA1 are examples of other autophagic proteins that are caspase targets.[46,47]

Mitotic catastrophe. Mitotic catastrophe is a cell death mechanism that occurs during mitosis in cells that have accumulated DNA damage due to dysregulated checkpoint mechanisms.[3] Such cells would normally arrest cell-cycle progression into mitosis and hence suppress catastrophic events until DNA repair has been achieved.[48] Although cell-cycle kinase such as cyclin B1-dependent kinase Cdk1, polo-like kinases and aurora kinases and other cell-cycle checkpoint proteins, are key players in regulating this process, cell death occurs via caspase activation, including caspase-2.[48]

Caspases in Inflammation and Immunity

The first indication of a role for caspases in inflammation came from the identification of caspase-1 function in the processing and maturation of interleukin (IL) cytokine family members IL-1β and IL-18, critical mediators of inflammation.[49,50] Mice deficient for caspase-1 exhibit defective maturation of pro-IL-1β and pro-IL-18, are resistant to LPS-induced endotoxic shock[50–52] and are protected from experimental mucosal inflammation.[53]

Additional studies in *Drosophila* unexpectedly identified the ortholog of caspase-8, Dredd, in a genetic screen as a regulator of the innate immune response.[54] In particular, Dredd interacts with a *Drosophila* NF-κB protein, Relish, in the immune deficiency (IMD) pathway, leading to Relish cleavage and activation, following exposure to Gram-negative bacteria.[54,55] Loss-of-function *dredd* mutants exhibit high susceptibility to Gram-negative bacteria, and defective production of antibacterial peptides, such as drosomycin[54] further substantiating an important role for this *Drosophila* caspase in immune regulation.

In mammals, there are now several caspases, which have been identified as important mediators of the innate immune response, termed the 'inflammatory caspases' and include human caspase-1, -4, -5, -12 and mouse caspase-1, -11 and -12.[34,56] Caspase-1 is the prototypical member of the inflammatory caspase family, while; the functions of caspase-4 and -5 are not well defined (caspase-5 is absent in mice and mouse caspase-11 is a paralogue of human caspase-4). Caspase-11 has been suggested to be an upstream activator of caspase-1 but unlike caspase-1, caspase-11 expression is induced by LPS at the transcriptional level.[57] Indeed, caspase-4, -5, and -11 act as cytoplasmic LPS receptors and have the ability to directly bind LPS via CARD domain, causing oligomerization and cell death. This represents a novel mechanism for inflammasome activation that is distinct from LPS sensing and activation of caspase-1.[58]

In contrast to the roles of other inflammatory cytokines, caspase-12 can abrogate the inflammatory response in mice by inhibiting caspase-1, in a protease-independent function.[59] As a result, mice deficient for *caspase-12* show increased bacterial clearance and resistance to sepsis.[60] Interestingly, full-length human caspase-12 protein is expressed only in ~20% of sub-Saharan African descendants, who as a consequence exhibit weakened inflammatory and innate immune responses and increased susceptibility to sepsis.[61]

The activation of inflammatory caspases is mediated through their recruitment to large multiprotein complexes known as 'inflammasomes'.[62,63] In response to specific internal or external cellular insults (viral or bacterial infection, toxin exposure, environmental irritants and metabolites), the specific activation of different pattern-recognition receptors (PRRs), leads to formation of distinct inflammasome complexes. There are three main classes of PRRs, activated in response to specific pathogenic stimuli, including (i) TLRs that can detect PAMPs derived from bacteria, viruses and fungi; (ii) retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), which detect virus infection; and (iii) the family of bacterial-sensing NOD-like receptor proteins (NLRs) or nucleotide-binding domain, leucine-rich repeat-containing proteins.[64]

Caspase-1 is activated by recruitment to multiple NLR-driven inflammasomes depending on the specific stimuli (Figure 3). There are currently four major, well-characterized inflammasomes: (i) NLRP1 inflammasome activated in response to the anthrax lethal toxin (LT)[62]; (ii) NLRP3/NALP3/cryopyrin inflammasome is activated by a wide range of PAMPs as well as whole pathogens, including fungi[56,62]; (iii) the NLRC4/IPAF inflammasome, which senses bacterial

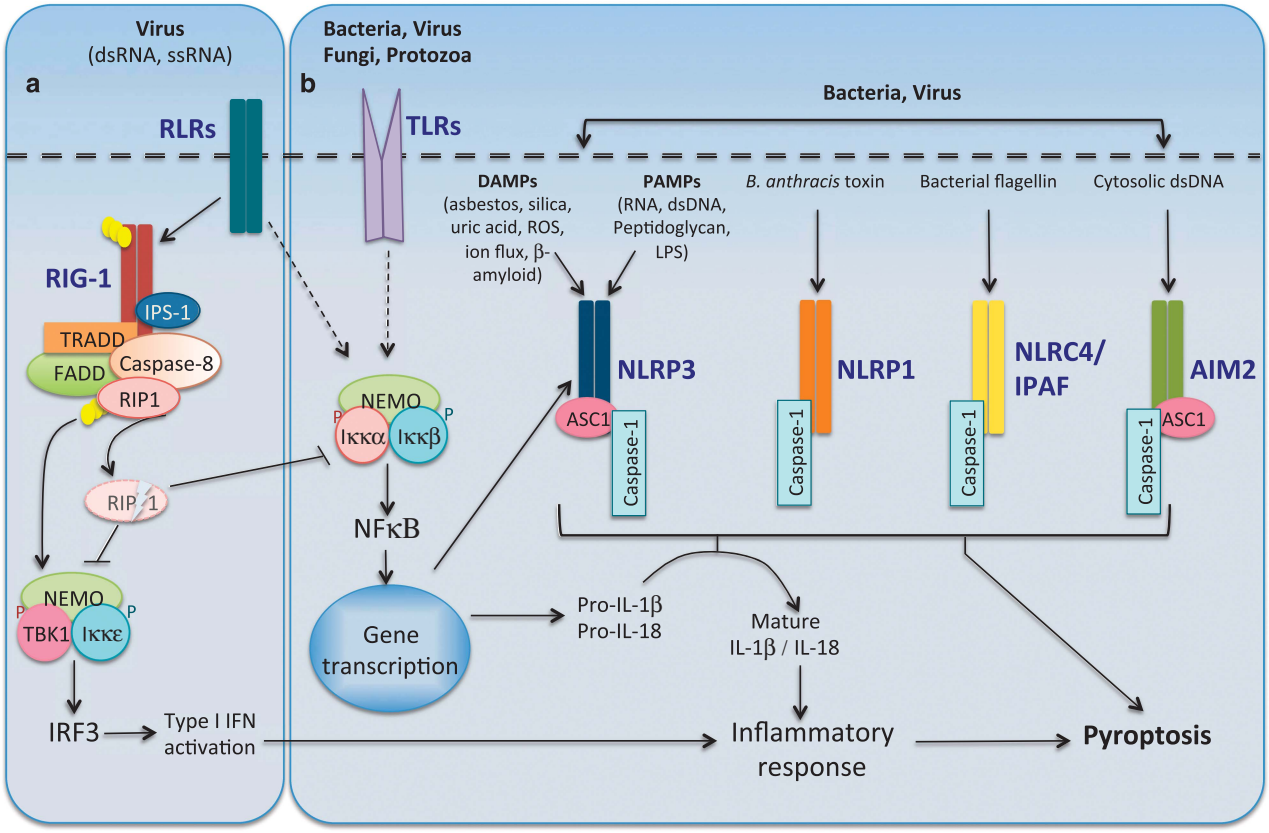

Figure 3 Caspase-mediated inflammatory responses. (**a**) Caspase-8 functions to restrict RIG-1 signaling. Activation of the retinoic-acid-like receptors (RLRs) are critical for anti-viral immunity. Following viral infection the caspase-8 DISC complex comprising FADD, TRADD and ubiquitin-conjugated RIP1 (Ub-RIP1) are recruited to the RIG-1 complex. Although Ub-RIP1 can enhance the phosphorylation of IRF3, it is also cleaved by activated caspase-8. Cleaved RIP1 inhibits IRF3 and NFκB thereby reducing the type I interferon (IFN) and IL-1β-/IL-18-mediated inflammatory responses. (**b**) Inflammasome complex formation is triggered by specific pathogen-associated molecular patterns (PAMPs) or by damage-associated molecular patterns (DAMPs). Activation of Toll-like receptors (TLRs) following pathogen exposure induces NFκB-mediated transcription of IL-1β, IL-18 and also NLRP3, and this is an important step in inflammasome priming and activation. Caspase-1 activation is mediated by its recruitment to different inflammasome complexes (NLRP3, NLRP1, NLRC4 and AIM2) in an ASC-dependent or independent manner. Activated caspase-1 mediates the cleavage and maturation of pro-inflammatory cytokines IL-1β and IL-18. Following acute infection and an acute inflammatory response, caspase-1 induces cell death by pyroptosis

flagellin and various secreted proteins from Gram-negative bacteria[65]; and (iv) AIM2 inflammasome, an atypical NLR, activates caspase-1 in response to cytosolic dsDNA.[66] NLRP1 and NLRC4 can bind and activate caspase-1 directly via a CARD-CARD-mediated interaction. In contrast, NALP3 and AIM2 do not comprise a CARD domain and require, the CARD/pyrin-containing inflammasome adapter, apoptosis-associated speck-like protein (ASC), to recruit caspase-1 to these inflammasomes. The NLRP1 inflammasome can also recruit caspase-5 via the CARD of the inflammasome adapter protein, CARDINAL,[62,67] but the requirement of CARDINAL in caspase-5 activation by NALP1 is unclear.

The first step toward an inflammasome-mediated immune response requires a process known as inflammasome priming, where stimulation of TLR or RIG leads to transcriptional activation of NALP3 and inflammatory cytokines (pro-IL-1β, IFN). Activation of the NALP3 inflammasome only occurs following detection of PAMPs as a second stimulus, and contrary to previous studies,[68] does not involve mitochondrial-mediated apoptosis signaling.[69] Recruitment and activation of caspase-1 by the inflammasome complex is a critical second step in the cleavage and activation of pro-inflammatory cytokines, IL-1β and IL-18, facilitating their secretion and promotion of innate immune responses. Although caspase-1 activation is required to activate inflammatory cytokines, it induces pyroptosis-mediated pro-inflammatory cell death following acute infection (Figure 3).

A novel role for caspase-8 in the regulation of inflammasome activation and maturation of pro-inflammatory cytokines has recently been revealed. Cleavage of pro-IL-1β can be mediated by both caspase-1 and caspase-8.[70,71] Interestingly, deletion of *caspase-8* in RIP3 knockout bone-marrow-derived macrophages (BMDMs) results in significant reduction of pro-IL-1β and TNF transcription levels, and reduced NALP3 activation following TLR stimulation. Importantly, this role for caspase-8 in the regulation of inflammasome priming is independent of apoptosis[69] and highlights an unexpected role for this caspase in immune homeostasis. Previous studies have also reported an inflammasome-independent role for caspase-8 in inhibiting extensive inflammation *in vivo*.[72,73] Conditional deletion of *caspase-8* in hepatocytes, augments Listeria infection in liver and chronic inflammation following hepatectomy.[73] *Caspase-8* deficiency also protects against inflammation-related hepatocarcinogenesis, while inducing

necrosis-mediated liver injury.[74] In addition, conditional deletion of caspase-8 in epidermal keratinocytes, is associated with chronic skin inflammatory disease associated with constitutive IRF3 signaling.[72] Interestingly, this role for caspase-8 in suppressing inflammation is independent of signaling mediated through TNF, IL-1β or TLR receptors and independent of NF$\kappa\beta$ signaling. Instead, caspase-8 regulates the activation of the IκK-related, TANK-binding kinase 1 (TBK1) and IRF3 to downregulate the inflammatory response.[72] Later reports demonstrated that caspase-8 suppresses inflammation following viral RNA-induced RIG-1 signaling[75] by interaction with the RIG-I signaling complex, RIP1 cleavage and inhibition of IRF3-mediated activation of interferons (Figure 3).[75]

Mutations in NALP3 and defective inflammasomes are associated with innate-immune-mediated diseases, and aberrant IL-1 processing has been reported in numerous auto-inflammatory diseases including gout, diabetes and juvenile-onset arthritis.[76] Mutations in *caspase-8* (and *caspase-10*) are also associated with autoimmune lymphoproliferative syndrome (ALPS).[9] Detailed understanding of the regulation of caspase activation during inflammation and secretion of pro-inflammatory cytokines will potentially provide new avenues for treatment of inflammatory and infectious diseases.

Caspases in Cell-Fate Determination and Differentiation

Early studies in *Drosophila* were instrumental in delineating a role for caspases in cell fate and differentiation. Loss-of-function mutation of the *Drosophila* Apaf-1 ortholog, *dark*, reduces activation of the initiator caspase, Dronc and abolishes developmental apoptosis.[77,78] Interestingly, *dark*-mutant flies expressing a dominant-negative form of Dronc, have defects in development with extra sensory organ precursor (SOP) cells.[79] These studies were the first to highlight an apoptosis-independent role for a caspase in regulating neural precursor cell formation in *Drosophila*. This process involves Dronc-mediated cleavage of Shaggy (Sgg46), which negatively regulates Wingless signaling required for SOP formation.[80] In turn, Dronc activity is positively regulated by Drosophila IκK, DmIKK, which degrades the caspase inhibitor protein DIAP1, to control SOP development.[81]

In mammals, caspase function in terminal differentiation has also been described in various cell types (Table 1), including keratinocytes, megakaryocytes, erythrocytes and lens cells.[82] A non-apoptotic role for the key effector caspase, caspase-3, was identified in lens fiber formation.[83] The process of differentiation of lens epithelial cell into a lens fiber involves enucleation (loss of nuclei), which was found to be caspase-3 dependent.[83] Other cells that demonstrate enucletion during differentiation include RBCs and keratinocytes. Several caspases, including caspase-3, -2, and -9 are transiently activated prior to enucleation during erythroid differentiation and involved cleavage of several proteins such as PARP, acinus and lamin B.[84] Expression of active caspase-3 was shown to be associated with cell proliferation and migration during neuronal differentiation.[85] Furthermore, in a mouse model of stroke, cleaved caspase-3 levels were increased in neuronal precursor cells (NPCs) during stroke recovery, without any associated increase in apoptosis.[86] Inhibition of caspase-3 activity significantly promoted the proliferation and migration of NPCs indicating that caspase-3 limits self-renewal capacity of regenerating neurons. Mechanistically, this was attributed to the ability of caspase-3 to reduce phosphorylation of Akt.[86]

Differentiation of muscle progenitor cells into myotubes and myofibres also requires caspase-3.[87] Later studies demonstrated the involvement of caspase-9 in this process. It was observed that depletion of caspase-9 or overexpression of Bcl-xL reduced caspase-3 activation and this had a limiting effect on the differentiation of myoblasts.[88]

Osteogenic differentiation of mesenchymal stem cells involves caspase-3 activity, and *caspase-3*-deficient mice exhibit attenuated differentiation of the bone marrow stromal stem cells and consequently decreased bone mineral density.[89] It is noteworthy that the process of normal osteoblast differentiation requires the transient and potent activation of other caspases, including caspase-8 and -2, without any increase in osteoblast apoptotic cell death.[90] Caspase-7 has an additional role in proper functioning of odontoblasts as *caspase-7* knockout mice show reduced dental mineralization.[91]

A role for caspase-3 in differentiation of embryonic stem cells (ESCs) and hematopoietic stem cells (HSCs) has also been reported,[92,93] with *caspase-3* deficiency-impeding differentiation

Table 1 Differentiation of many cell types involves caspases

Progenitor cells	Caspase activation	Mechanism	Reference (s)
Erythroid cells	Caspase-2, -3 and -9	Cleavage of lamin B and acinus	84
Keratinocytes	Caspase-3	Terminal differentiation of embryonic cells involving Casp3-Notch1 mechanism	102
Lens cells	Caspase-14	Caspase-14 induction and processing during differentiation of keratinocytes	98,99
	Caspase-3	Lens fiber formation	83
Muscle progenitor cell	Caspase-3 and -9	Muscle fiber formation	87,88
Bone marrow stromal stem cells	Caspase-3	Osteoblasts and bone formation involving TGFβ signaling	89
Osteoblasts	Caspase-2, -3 and -8	Activation of caspases in differentiating cells	90
Odontoblasts	Caspase-7	Activation of caspase-7 in bone-forming cells	91
Monocytes (bone marrow)	Caspase-3, -8 and -9	Macrophage formation	94
Neurons	Caspase-3	Neuronal differentiation, migration and plasticity	85
Embryonic stem cells	Caspase-3	Caspase-3-mediated cleavage of Nanog	92
Haematopoietic stem cell	Caspase-3	Maintenance of stem-cell quiescence and response to external stimuli	93

of these stem cell populations. Caspase activity is also required for macrophage colony-stimulating factor (M-CSF)-mediated differentiation of human peripheral blood monocytes.[94] Deletion of *caspase-8* in bone-marrow cells abrogates monocyte differentiation into macrophages.[95] In addition, caspase-8-mediated cleavage of RIP1, is required for downregulation of NF-$\kappa\beta$ during macrophage differentiation.[96]

Caspase-14, unlike other caspases, is present only in terrestrial mammals[97] and its expression is limited to epidermal tissue and epithelia, particularly the cornifying epithelia of the skin.[98,99] In the skin caspase-14 is expression is restricted to differentiating keratinocytes and absent in proliferating keratinocytes.[99,100] Caspase-14 was found to be involved in cornification, hydration and protection against UVB by correct processing and degradation of (pro)filaggrin.[101] In addition to caspase-14, caspase-3 functions in the commitment stage of embryonic keratinocytes to terminal differentiation.[102] During keratinocyte differentiation, caspase-3 activity is induced following Notch1 signaling and mediates cleavage and activation of PKC-δ, a positive regulator of keratinocyte differentiation.[102] In support of this role, mice deficient for *caspase-3* exhibit increased keratinocyte proliferation and deceased keratinocyte differentiation during embryogenesis.[102] Altered caspase-14 has also been observed in inflammatory skin disorders such as psoriasis.[103]

Caspases in Cell Proliferation and Tissue Regeneration

Apoptosis is closely linked to the process of regeneration that is evolutionary conserved. Invertebrates such as *Hydra*[104] and amphibians such as *Xenopus* show increased apoptosis during regeneration.[105] MAPK signaling in dying cells activates Wnt-ligand secretion that drives this compensatory proliferation in surrounding cells.[104,106]

The process of 'compensatory proliferation' was originally defined in *Drosophila*-larval-imaginal tissues, which carry extensive regenerative capacity and cell proliferation to compensate for cell loss through damage or injury, and thereby maintain tissue size and shape.[107] In the absence of effector-caspase activity these cells express high levels of activated Dronc but fail to die. Instead, Dronc activity is required for compensatory proliferation and functions to promote expression of the mitogens Wingless (Wg) and Decapentaplegic (Dpp) to promote proliferation of neighboring cells.[108–110] The effector caspases Drice and Dcp-1 can also induce compensatory proliferation in differentiating eye tissue, by activation of the Hedgehog signaling pathway.[106] These studies demonstrated critical roles for caspases in coordinating both cell death and proliferation during development and regeneration, and provided the first *in vivo* evidence for a non-apoptotic function for an initiator-apoptotic caspase.[109]

Mammals have greatly reduced regenerative ability but some tissues, such as liver and skin undergo extensive regeneration. Both *caspase-3*- and *-7*-deficient mice have impaired liver regeneration and wound healing.[111] Mechanistically, this was attributed to calcium-independent phospholipase A2 activity (iPLA$_2$). Both caspase-3 and -7 cleave iPLA$_2$ which increases secretion of arachidonic acid and lysophosphocholine that further induce the secretion of prostaglandin E2 (PGE$_2$), which then promotes stem cell proliferation, tissue regeneration and repair (Figure 4).[111] PGE$_2$ also has been shown to interact with the Wnt pathway at the level of β-catenin destruction to control HSC formation *in vivo*.[112] This highlights that caspases are capable of cell repair by modulating the

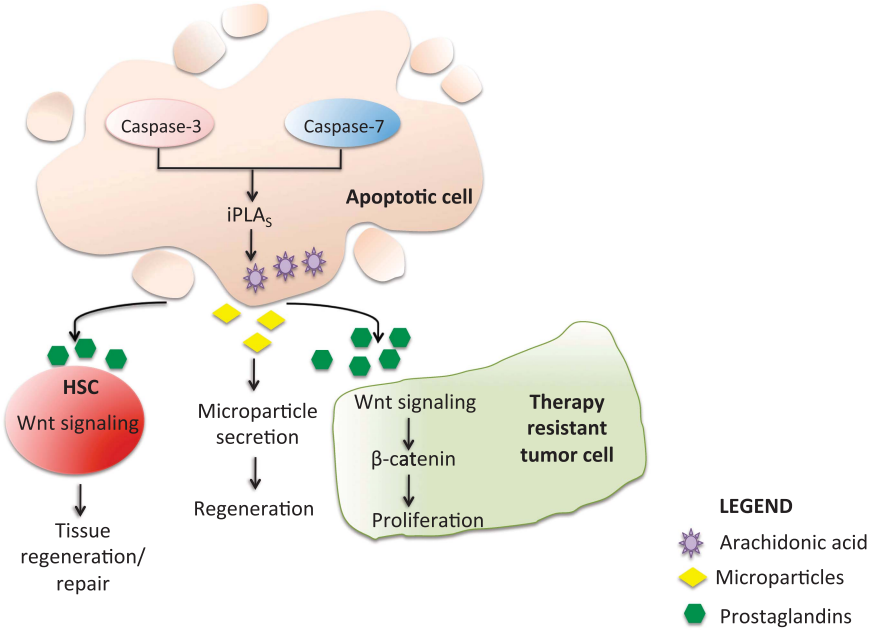

Figure 4 Caspase function in tissue regeneration and tumor cell proliferation. Activation of caspase-3 and -7 during radiotherapy or during tissue injury causes cleavage and activation of iPLA$_2$. This results in AA synthesis and subsequent PGE$_2$ activation. The secreted PGE$_2$ promotes proliferation in surrounding tumor cells by activation of Wnt/β-catenin signaling. This facilitates tumor cell repopulation and acts as a limiting factor of effective therapy. Shedding of microparticles by apoptosing cells (pancreatic beta cells) stimulates proliferation/differentiation of neighboring cells through activation of regenerating (*reg*) genes. PGE$_2$ also interacts with Wnt signaling pathway to regulate stem cell/progenitor formation and function. iPLA$_2$, calcium-independent phospholipase A2; AA, arachidonic acid; PGE$_2$, prostaglandin E2; HSC, hematopoietic stem cells

secretion of paracrine factors independent of their cell death function. Further evidence for indirect control of cell growth and repair comes from another study involving conditional *caspase-8* knockout mice. Mice with hepatocyte-specific deletion of *caspase-8* (Casp8$^{\Delta hepa}$) show greatly improved liver regeneration due to increased NFκB signaling[113] and wound healing. Caspase-7 has another important role in osteogenesis. Adult *caspase-7* knockout mice display reduced bone mineral density in endochondronal (long bones of limbs) and reduced bone volume (intramembraneous bones such as mandibular and alveolar bone). This is attributed to reduced gene expression of key bone-forming genes, *Smad1* and *Msx1*.[114]

There is increasing evidence that mammalian caspases influence cell proliferation in a cell autonomous manner. Studies using caspase inhibitors, implicate a role for caspase-8 in regulating lymphocyte proliferation.[115–117] *Caspase-8* deletion in T cells causes normal thymocyte development, but causes a substantial reduction in peripheral T cells and impaired T-cell response to stimuli.[118] Further with advancing age, these mice develop a lymphoproliferative phenotype characterized by lymphoadenopathy, splenomegaly and T-cell infiltration in several organs.[119] These findings implicate an essential role for caspase-8 in T-cell homeostasis and immunity. In support of this role, patients with *caspase-8* homozygous mutations have defects in lymphocytes and natural killer cell activation.[120] FADD, is also essential for cytokine-induced proliferation of hematopoietic progenitor cells.[121] These results, at least in part, could suggest that that loss of caspase-8 or FADD makes cells undergo necroptosis, rather than caspase-8 or FADD only having a direct effect on cell proliferation. However, the DISC complex comprising Fas/FADD/c-FLIP$_L$/caspase-8 also controls cell-cycle progression of hepatocytes following EGF stimulation.[122] Like caspase-8, caspase-1 and -6 have also been shown to regulate B-cell proliferation and maintain lymphocyte homeostasis.[123,124]

In a contrasting role, *caspase-3*-deficient B cells show hyperproliferation that is dependent on p21, a cyclin-dependent kinase inhibitor.[125] Many caspase-3 substrates are cell-cycle regulators, including cyclin-dependent kinase (CDK) inhibitor p27, a mediator of lymphoid cell proliferation.[126] Caspase-3 also cleaves p21 and this blocks p21 interaction with proliferating-cell nuclear antigen (PCNA), and consequently prevents cell proliferation.

Caspases in Tumorigenesis

The phenomena of cell growth and cell death are closely linked. Basically, tumors arise due to unregulated increase in proliferative activity although the cause for this is multifactorial. The most likely reason for increased cell number would be loss of cell death control. Several genes with well-established roles in cell death have tumor suppressor function. Activation of Bcl-2 due to chromosomal translocation and gene amplification, blocks caspase activation and is frequently detected in non-Hodgkin's lymphoma (NHL) and small cell lung cancers.[127,128] Several clinical trials targeting Bcl-2 proteins as cancer therapeutics are ongoing. The cell death-inhibitor proteins, IAPs, are also promising targets for cancer research, as they suppress apoptosis either by inhibiting caspase activity or by modulating growth by targeting NFκB signaling pathways.[129] Caspases promote cell death and it would be expected therefore that loss of caspases promotes tumor development. They, however, are also involved in tumorigenesis through non-apoptotic pathways by affecting proliferation, invasion and migration.[130]

Caspase-1. Caspase-1 tumor-suppressor function has been widely demonstrated. Overexpression of caspase-1 enhances the sensitivity of androgen-independent prostate cancer cell lines, to irradiation-induced cell death.[131] It is also known that *caspase-1* is frequently downregulated in human cancers, particularly prostate cancer.[132,133] When caspase-1-overexpressing renal cancer cells were injected into flanks of syngenic mice, not all mice developed solid tumors, while all control mice did.[134] This was attributed to increased cell death following caspase-1 overexpression. It was also shown that while caspase-1 expression in renal cancer cell lines varies, tumor cells frequently have low caspase-1 levels, and inducing gene expression by demethylation increases apoptosis and could prevent tumor growth. Further evidence for its role as a tumor suppressor comes from a mouse model of colitis-associated colorectal cancer.[135] *Caspase-1*-deficient mice showed enhanced tumor formation, characterized by early stage increase in epithelial cell proliferation in the colon and reduced apoptosis during advanced disease stage.[135] Recent studies have shown that originally generated *caspase-1*-deficient mice also harbor a mutation in the *caspase-11* locus, which mediates LPS-induced lethality in these mice.[136]

Caspase-2. A tumor suppressor function for caspase-2 was first proposed based on an evidence that the human *CASP2* gene region on chromosome 7q is commonly deleted in leukemia.[137] Although somatic mutations of *CASP2* are not common in acute leukemia or solid cancers,[138] the reduced expression of caspase-2 is correlated with poor prognosis in some cancers,[139,140] and high caspase-2 levels are associated with remission and survival in adults with ALL and AML.[141] Furthermore, reduced expression and somatic missense mutations in *CASP2* have been identified in some gastric and colorectal cancers.[142]

Studies using *caspase-2* knockout mice and cells generated from these mice provided the first direct evidence that loss of *caspase-2* enhances oncogene-induced cellular transformation and tumorigenic potential.[143] Loss of *caspase-2* augments tumorigenesis in several different mouse tumor models, including *EμMyc*-driven lymphomagenesis,[143] *MMTV/c-neu* mammary carcinogenesis[144] and *ATM$^{-/-}$*-mediated thymic lymphomagenesis.[145] Although the mechanism by which caspase-2 functions as a tumor suppressor is still unclear, it does not appear to be a PIDDosome dependent.[146] Loss of caspase-2 has been shown to impede undefined radiation (IR)-induced DNA damage response in primary murine embryonic fibroblasts by significantly reducing p53 activation and function.[147] Recently, a tumor suppressor role for caspase-2 was demonstrated in a mouse model of *KRas*-driven lung cancer. Tumors lacking *caspase-2* responded better to chemotherapy, however, were more prone to relapse due to increased proliferation. It was observed that following DNA

damage-mediated PIDD activation, caspase-2 cleaves mdm2, thus stabilizing p53 and preventing tumorigenesis.[148] These studies suggest that caspase-2 cooperates with established tumor suppressors such as p53. However, *caspase-2* deficiency does not influence IR-induced thymoma development in mice or affect spontaneous or IR-mediated thymoma in *p53*-null mice.[146] In addition, loss of *caspase-2* does not influence MCA-driven fibrosarcomas in mice.[149] Interestingly, while caspase-2 acts as a tumor suppressor in cells with high *c-myc* expression, loss of *Pidd* had only slightly delayed tumor onset and had no additive effect. [149] In contrast to a tumor suppressor function, *caspase-2*-deficient mice showed significantly delayed onset and development of *MYCN*-mediated neuroblastoma, and high caspase-2 levels are associated with poor survival outcome in human neuroblastoma patients.[150] Together these studies indicate that caspase-2 role as a tumor suppressor is tissue and context specific.

Caspase-3. Recently, Human Genome Epidemiology review and meta-analysis polymorphisms of *CASP3* and *CASP7* genes were found to be linked to cancer risk.[151] Although somatic *CASP3* mutations are rare in most tumors,[152] *CASP3*-variant alleles have been detected in squamous cell carcinomas of the head and neck (SCCHN) and associated with increased risk of SCCHN.[153] In addition, *CASP3*-variant alleles are associated with risk of endometrial cancer [154] and NHL [155] and multiple myeloma.[156] *CASP3* polymorphisms in some lung cancer patients also show significantly decreased risk of disease.[157] Although it was reported that caspase-3 expression is significantly reduced in breast cancer cells, cancer tissue and even in the normal parenchyma surrounding tumors,[158] several groups have demonstrated the converse. Higher levels of caspase-3 protein were found in malignant, compared to nonmalignant breast tissue, and coincided with increased apoptosis.[159] Indeed, it was observed that caspase-3 is activated in dying tumor cells and causes increased secretion of PGE_2 that results in tumor cell repopulation and resistance to radiotherapy.[160] Further support to this study comes from human trials where two different cohorts of head and neck cancer patients and breast cancer patients, had high expression of active caspase-3 (cleaved caspase-3) and associated increased recurrence of the disease. This suggests that apoptosis is an essential aspect of tumor growth, and paracrine signals released by dying cells act to promote repopulation of these tumor cells and aids further growth.

Caspase-6 and -7. Mutations in *CASP6* and *CASP7* are rare in most human cancers.[161] *CASP6* mutations associated with reduced caspase-6 expression have been detected in colonic and gastric cancers.[142] Somatic mutations in *CASP7* are associated with reduced apoptosis[161] and have been detected in colon carcinoma, esophageal carcinomas and head/neck carcinomas, but not stomach, urinary bladder or lung cancers.[161] *CASP7* polymorphisms can promote susceptibility to lung cancer and endometrial cancer.[162]

Caspase-8. The connection between caspase-8 and carcinogenesis has been extensively studied. A comprehensive review on caspase-8 expression and different types of tumors is available in the Human Protein Atlas.[163] The studies demonstrate that in most epithelial-derived cancers, such as colon carcinoma, gastric cancer and breast cancer that progress through multiple stages, the loss of *caspase-8* is a rare event and loss of expression is not detected. On the contrary, *CASP8* mutations have been detected in 5% of invasive colorectal carcinomas (but not adenomas), and are associated with dominant-negative caspase-8 function and decreased apoptosis.[164] Somatic mutation of *CASP8* has also been detected in hepatocellular carcinomas (HCCs)[165] and advanced gastric cancers,[166] and are associated with decreased cell death.[165] Interestingly, a *CASP8* polymorphism (D302H) is associated with significantly reduced overall risk breast cancer.[167] In addition, malignant neuroendocrine tumors frequently show loss of *caspase-8*, such as neuroblastoma,[168] primitive neuroectoderm tumors,[169] medulloblastoma,[170] relapsing glioblastoma[171] and small cell lung carcinoma.[172] Loss of *caspase-8* renders neuroblastoma cell lines resistant to apoptosis[173] and enhances metastatic potential in chick embryos.[174] In a mouse *MYCN* neuroblastoma model, deletion of *caspase-8* promotes bone marrow metastasis.[175] Increased caspase-8 expression has been observed in many advanced-stage tumors suggesting that caspase-8 might promote metastasis.[176] Further evidence in support of its metastatic role comes from the fact that caspase-8 has been shown to interact with the focal adhesion complex,[177] thereby promote cell migration. The complex observations on caspase-8 can be attributed to the fact that this caspase is involved in apoptosis and also protected against necroptosis. Hepatocyte-specific deletion of *caspase-8* ($Casp8^{\Delta hepa}$) was found to be protective against hepatocarcinogenesis in $NEMO^{\Delta hepa}$ mice that develop spontaneous HCC.[74] Thus caspase-8-induced apoptosis and compensatory proliferation is important in HCC progression, but the increased necrosis and liver damage observed in the absence of caspase-8, is insufficient for tumor progression.

Caspase-10. The absence of caspase-10 in mice has perhaps resulted in delay in understanding potential roles of this caspase. Nonetheless, inactivating *CASP10* mutations have been detected in NHL.[178] *CASP10* mutations have also been detected in T-cell acute lymphoblastic leukemia and multiple myeloma,[179] as well as colon, breast, lung, HCCs[180] and gastric cancers.[181] In a very interesting study, it has been shown that myeloma cells require caspase-10 for survival. Caspase-10 dimerizes with cFLIP to inhibit autophagy by preventing cleavage of the Bcl-2-interacting protein BCLAF1.[182]

Caspase-14. Expression of caspase-14 is reduced in the colon, cervical and ovarian tumor tissue and associated with more advanced-stage cancers.[183] However, ductal carcinomas showed increased caspase-14 levels compared to surrounding mammary tissue.[183] Caspase-14 overexpression in a human salivary gland adenocarcinoma has been shown to promote growth inhibition and prevent tumorigenicity in athymic mice.[184]

Caspase Activation During Aging

Aging is often described as a process of progressive decline in function of organs/organism largely due to accumulation of damaged cells. Ever since caspases were discovered and apoptosis established as an intricate cell death mechanism, it was causally linked to organismal aging. Apoptosis was implicated in the aging process in two ways: by clearing multicellular organism of dysfunctional or unwanted cells, it maintains normal homeostasis; or by removing functionally important post-mitotic cells such as neurons or cardiac myocytes, it may hasten aging-associated pathologies.

Mutations in NALP3 gene, which increase caspase-1 activity and consequently IL-1β and IL-18, lead to systemic inflammatory diseases that increases during aging.[185] Increase in oxidative stress and low-grade inflammation are the hallmarks of the aging process, which have been linked to caspase-1 activity.[186] In another study, improvement in memory was observed in rats injected intracerebroventricularly with a caspase-1 inhibitor (Ac-YVAD-CMK),[187] indicating that inhibition of caspase-1 can delay aging-associated pathologies.

Alzheimer's disease is an age-associated pathology and active caspase-6, which plays an important role in the etiology of this disease.[188] Sarcopenia, age-related muscle loss, is associated with decreased differentiation of a population of stem cells, called satellite cells (SCs), into myofibrils. In a study, analyzing caspase activity in young and aged individuals, an increased rate of apoptosis and increased *CASP2*, *CASP6*, *CASP7* and *CASP9* levels in aged SCs, was observed, indicating an enhanced susceptibility of human SCs to undergo apoptosis with age.[189] This would diminish the stem cell population and trigger increased muscle loss or damage.

Age-associated hearing loss occurs with accompanying cochlear degeneration and high levels of apoptosis. Calorie restriction, CR, which is known to delay aging in mice and other laboratory animals prevented apoptosis and hearing loss.[190] The underlying mechanism is reduced ROS and mitochondrial damage during CR.[191] Another quality control process that declines with aging is the unfolded protein response (UPR). Aged mice fail to employ UPR, especially when sleep deprived and simultaneously showed increased caspase-12 activity and apoptosis. This was not observed in young mice and suggests increased caspase activity to compensate a poor ER-stress-induced adaptive response.[192]

Zhang *et al.*[193] first demonstrated a role for caspase-2 in delaying aging. Latter it was shown that *caspase-2*-deficient mice have reduced antioxidant ability and experience increased oxidative stress that accounts for their shorter lifespan.[193] Aged *caspase-2* knockout mice tissues also exhibit increased oxidative DNA damage and double-strand DNA breaks (γH2AX).[194] Paraquat-induced pulmonary lesions in aged caspase-2 knockout mice were more severe and associated with hepatocyte karyomegaly.[195] It was recently shown that the increase in mitochondrial ROS in aged *caspase-2* knockout mice is associated with mitochondrial complex-III activity.[196] The early-aging phenotype observed in *caspase-2* knockout is distinct and involves non-apoptotic properties of this protease including its role in oxidative stress. A proposed model depicting how presence or absence of caspases can lead to aging is shown in Figure 5.

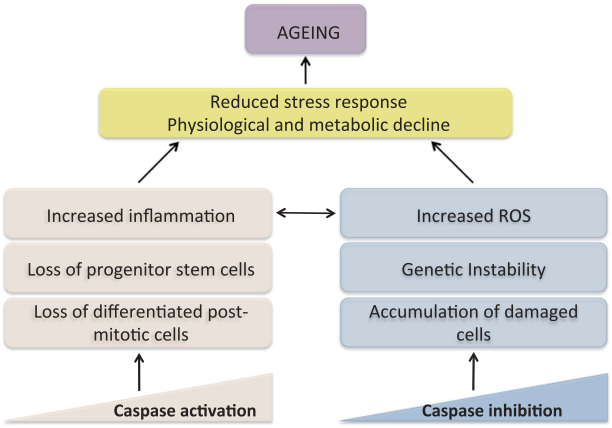

Figure 5 Caspase activation occurs during aging, which is normally accompanied by a general decline in stress tolerance and functional strength. On one hand, this increased caspase activity, accelerates aging by increasing inflammation (e.g., caspase-1). Increased activity of caspases also results in removal of stem cells (e.g., loss of satellite cells by caspase-6), while in conditions such as stroke/ischemia, it causes loss of highly differentiated cells such as neurons and cardiomyocytes (e.g., caspase-9), causing functional decline and reduced regenerative ability. In another alternate scenario, loss of apoptosis may also hasten the aging process by preventing removal of damaged cells, increasing genetic instability and oxidative stress (e.g., caspase-2)

Caspases in Neural Development

Apoptosis plays a crucial role during neural development both in embryos and during adult life.[197] Caspase-3, -9 and Apaf-1 are involved in forebrain development as mice lacking these proteins show exencephaly and neural overgrowth, attributed to increased cell death both in NPCs and developing neurons.[197] However, caspases also affect neural development in non-apoptotic manner (Table 2).

One such process is neuronal sculpting by dendrite pruning that results in removal of aberrant dendrites/axonal connections. Pioneering work in *Drosophila* revealed that Dronc is essential for dendrite pruning during larval development and the consequent formation of the adult nervous system.[198] This process is dependent on ubiquitin-mediated degradation of DIAP1, to permit Dronc activation, and altered Dronc localization in axon and dendrites.[198] Similar to apoptosis, the effector phase of pruning of dendrites involves cytoskeletal disruption, cell fragmentation and phagocytic clearance, indicating that Dronc activation may induce these morphological changes in the absence of cell death.

Recently, caspase-3 was found to control spine density and dendrite morphology without any observed increase in cell death.[199] In support to these findings supernumerary spines have been reported in *caspase-3* knockout mice.[199] In a related study, *caspase-2*-deficient J20 APP transgenic mice did not show age-related reduction in cognitive function. This was attributed to an important role for caspse-2 in controlling dendrite spine density.[200] Caspase-2 was found to activate the RhoA/ROCK-II signaling pathway, leading to collapse of dendritic spines.

Caspases are also involved in axonal guidance and synaptogenesis. Mice lacking *caspase-9* and *Apaf1* display misrouted axons, impaired synapse formation and defects in sensory neurons responsible for olfaction. However, no

Table 2 Non-apoptotic roles of caspases in neuronal function

Process	Caspase	Mechanism	Reference
Dendritic pruning	Caspase-2	Activation of Rho/ROCK-II	200
	Caspase-3	Activation of caspase-3	199
Axon guidance and synaptogenesis	Caspase-3 and -6 are involved in axonal guidance and synapse formation	Mice lacking caspase-3 and -6 have developmental delay in pruning retinocollicular axons	202
	Caspase-9 and Apaf regulate synapse formation	*Casp9*$^{-/-}$ and *Apaf*$^{-/-}$ mice have misrouted axons, impaired synapse and defects in olfaction. Cleavage of semaphorin 7A is vital for proper projection of axons	201
Normal synaptic plasticity and function	Caspase-3 is important in LTD	Caspase- 3-mediated internalization of AMPA	203

defects in neuronal cell death were observed.[201] The underlying mechanism involves caspase-9-mediated cleavage of an essential protein, semaphorin 7A that is crucial for proper projection of axons.[201] Caspase-3 and -6 have also been implicated in the process of axonal guidance.[202]

Normal brain functions depend on proper synaptic activity. Long-term potentiation (LTP) and long-term depression (LTD) are long-lasting modifications of synapses, in the hippocampus region. Caspase-3 is essential for LTD.[203] During LTD, AMPA receptor in the post-synaptic membrane is internalized and the process is facilitated by caspase-3 and blocked by peptide inhibitors of caspase-3 and -9. It is again worth noting that during LTD, transient caspase-3 activation occurs without causing cell death.[202]

Conclusions

Ever since their discovery almost two decades ago, caspases have been shown to modulate cellular functions in more than one way. Although the mechanism and their activation platforms during cell death and apoptosis are well characterized, many of their non-apoptotic functions are poorly defined. Caspases mediate most of their activity by cleaving their target proteins and this substrate specificity guides cellular behavior. Several caspases are involved in tissue differentiation, cell proliferation, tumor formation and metastasis, determining immune response, synaptic plasticity, DNA damage and stress response, metabolism and aging. Although activation of caspases in some processes involves apoptosis/cell death, some others are independent of their apoptotic activity. Thus a better understanding of these functions may have much broader implications than previously thought. A better knowledge about caspase function may provide greatly improved strategies to design effective therapeutics in the area of inflammation, autoimmune disease and cancer.

Conflict of Interest

The authors declare no conflict of interest.

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