OPINION

A fate worse than death: apoptosis as an oncogenic process

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Abstract | Apoptotic cell death is widely considered a positive process that both prevents and treats cancer. Although undoubtedly having a beneficial role, paradoxically, apoptosis can also cause unwanted effects that may even promote cancer. In this Opinion article we highlight some of the ways by which apoptosis can exert oncogenic functions. We argue that fully understanding this dark side will be required to optimally engage apoptosis, thereby maximizing tumour cell kill while minimizing unwanted pro-tumorigenic effects.

Apoptosis is a major type of regulated cell death in our bodies. This evolutionarily conserved process has crucial roles that range from tissue sculpting during embryonic development to execution of immune effector functions[1]. Moreover, too much or too little apoptosis has been implicated in diverse diseases, including neurodegeneration and autoimmunity[2,3]. Abundant evidence supports a role for inhibition of apoptosis in the promotion of cancer and blunting of therapeutic responses[4–6]. Accordingly, substantial excitement surrounds the therapeutic potential of engaging apoptosis in a tumour-specific manner; to this end, highly promising apoptosis-inducing therapies called BCL-2 homology domain 3 (BH3) mimetics were recently approved by the US Food and Drug Administration (FDA) for the treatment of 17p-deleted chronic lymphocytic leukaemia (CLL)[7,8]. Therefore, apoptosis is widely considered a positive process that can both inhibit and treat cancer.

In this Opinion article we present a more nuanced view of apoptosis, namely, that it can also have cancer promoting functions. We begin our discussion by providing a general overview of apoptotic signalling before briefly reviewing its role in restraining cancer. From this basis, we highlight the varied means by which apoptotic signalling can exert oncogenic effects; these include extrinsic effects of the dying cell and intrinsic effects in cells that survive apoptotic stress or owing to non-apoptotic functions of proteins typically considered apoptotic. We propose that improved understanding of these effects and their importance in cancer should optimize our ability to fully exploit apoptosis therapeutically.

Apoptotic signalling pathways

The protease activity of caspases is essential for the morphological and biochemical hallmarks of apoptosis[9]. In broad terms, caspases can be activated through one of two pathways — the extrinsic (also called death receptor) pathway and the intrinsic (also called mitochondrial) pathway (FIG. 1). As the name implies, the extrinsic pathway requires external stimulation; this occurs via a death receptor (DR) family member, such as tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptor 1 (TRAILR1, also known as DR4 and TNFRSF10A), TRAILR2 (also known as DR5 and TNFRSF10B), FAS (also known as CD95 and APO1) or TNF receptor 1 (TNFR1, also known as TNFRSF1A), located at the plasma membrane. After ligand binding, death receptors activate caspases, leading to widespread cleavage of caspase substrates and rapid cell death[9].

The intrinsic apoptotic pathway, which is often deregulated in cancer, is engaged by a wide array of stimuli that are sensed intracellularly, including cytokine deprivation, DNA damage and endoplasmic reticulum (ER) stress[10] (FIG. 1). These diverse apoptotic stresses converge to trigger one crucial event — mitochondrial outer membrane permeabilization (MOMP)[11]. The extrinsic and intrinsic pathways also cross-talk through caspase-8 cleavage of the BH3-only protein BH3-interacting domain death agonist (BID) — this generates the active, truncated form of BID (tBID) that triggers MOMP[12,13]. Following mitochondrial permeabilization, cytochrome *c* is released from the mitochondrial intermembrane space and induces caspase activation via a cytoplasmic complex termed the apoptosome[11]. Importantly, because MOMP often kills irrespective of caspase activity, it is considered a cellular death sentence[14]. Nevertheless, there are notable exceptions: for example, some types of neuron can circumvent the lethal effect of MOMP by inhibiting caspase activity through various means, including low expression of apoptotic protease activating factor 1 (APAF1) or degradation of cytochrome *c*[15,16]. Moreover, proliferating cells in which caspases are inhibited can also survive MOMP; this depends on glycolytic metabolism and autophagy[17].

Because of this pivotal role in dictating life and death, MOMP is highly regulated through interactions between BCL-2 family members[10]. There are three subfamilies of BCL-2 proteins: anti-apoptotic BCL-2 proteins (such as BCL-2 or BCL-X_L (also known as BCL2L1)), pro-apoptotic BH3-only proteins (including PUMA (also known as BBC3), BID and BIM (also known as BCL2L11)) and pro-apoptotic effector proteins (BAX, BAK and BOK). BH3-only proteins relay diverse apoptotic signals to activate BAX and BAK at the mitochondrial outer membrane, whereupon BAX and BAK trigger MOMP[10]. Anti-apoptotic BCL-2 proteins inhibit MOMP and cell death either by binding directly to BH3-only proteins or by binding to activated BAX and BAK[18]. This is therapeutically important, as competitive disruption of these interactions — thereby sensitizing to apoptosis — forms the mechanistic basis for the action of BH3 mimetics[19].

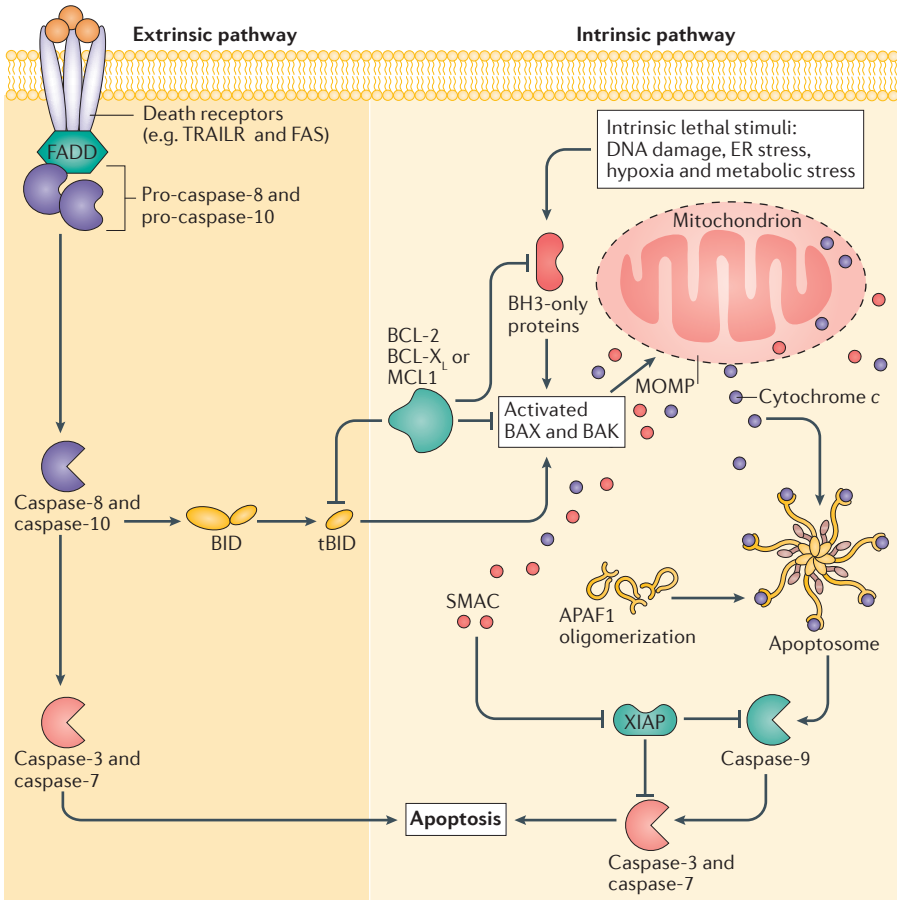

Figure 1 | **Extrinsic and intrinsic apoptotic signalling pathways.** In the extrinsic apoptotic pathway, upon binding to their cognate ligand, death receptors such as tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptor (TRAILR) and FAS can activate initiator caspases (caspase-8 and caspase-10) through dimerization mediated by adaptor proteins such as FAS-associated death domain protein (FADD). Active caspase-8 and caspase-10 then cleave and activate the effector caspase-3 and caspase-7, leading to apoptosis. The intrinsic (or mitochondrial) pathway of apoptosis requires mitochondrial outer membrane permeabilization (MOMP). Cell stresses engage BCL-2 homology domain 3 (BH3)-only protein activation, leading to BAX and BAK activity that triggers MOMP. Anti-apoptotic BCL-2 family proteins counteract this. Following MOMP, mitochondrial intermembrane space proteins such as second mitochondria-derived activator of caspases (SMAC) and cytochrome *c* are released into the cytosol. Cytochrome *c* interacts with apoptotic protease activating factor 1 (APAF1), triggering apoptosome assembly, which activates caspase-9. Active caspase-9, in turn, activates caspase-3 and caspase-7, leading to apoptosis. Mitochondrial release of SMAC facilitates apoptosis by blocking the caspase inhibitor X-linked inhibitor of apoptosis protein (XIAP). Caspase-8 cleavage of the BH3-only protein BH3-interacting death domain agonist (BID) enables crosstalk between the extrinsic and intrinsic apoptotic pathways. ER, endoplasmic reticulum; MCL1, myeloid cell leukaemia 1; tBID, truncated BID.

Cancer prevention and treatment

Apoptosis has long been considered a process that must be evaded to enable cancer to develop[4]. This view is supported by a wealth of data, including: the demonstration that inhibition of cell death, in combination with mitogenic oncogenes, can promote cancer in mouse models[20–22]; the discovery that many oncogenic pathways inhibit apoptosis, whereas tumour suppressors, such as p53, can engage apoptosis[23]; the positive correlation between apoptotic sensitivity and therapeutic efficacy in patient-derived cancer cells[24,25]; and the frequently observed upregulation of anti-apoptotic proteins in cancer[26]. Nevertheless, although inhibition of apoptosis may promote cancer, cancer cells are often not inherently apoptosis resistant. Indeed, as we will highlight, some human tumour types are actually more sensitive to apoptosis than their normal tissue counterparts[27,28]. Counter-intuitively, higher levels of apoptosis in the tumours of patients with cancer have also been shown to correlate with poorer prognosis in some cancer types[29–34]. Finally, and again paradoxically, high levels of anti-apoptotic proteins correlate with better prognosis in certain cancers. For example, as shown in TABLE 1, a high level of expression of anti-apoptotic BCL-2 family proteins is associated with favourable outcome in some human cancers, whereas high expression of pro-apoptotic BAX can correlate with a poor outcome[35–39]. Notably, increased anti-apoptotic BCL-2 protein expression does not necessarily translate into decreased apoptotic sensitivity; indeed the opposite can hold true. For example, some cancers, such as CLL, express high levels of anti-apoptotic BCL-2 proteins to buffer the intrinsic activity of pro-apoptotic BCL-2 family proteins[40]. Cancer cells in this state are referred to as 'primed for death' and are highly sensitive to apoptosis-inducing therapies[27]. As we now discuss, these and other findings challenge the view that apoptotic signalling serves solely to inhibit cancer, arguing instead that apoptosis also has a dark side that can actually promote cancer.

Cell-extrinsic effects of apoptosis

Although apoptotic cell death is a cell-autonomous event, its effects are not; dying cells affect their surrounding environment in various, yet not fully understood, ways. These can include stimulating the proliferation of neighbouring cells, affecting intra-tumoural cell competition as well as exerting paracrine effects on the tumour microenvironment. As these dying cells can promote cancer progression, they also represent potential nodes for therapeutic intervention.

Life after death — apoptosis-induced proliferation. Apoptotic cells can actively promote the proliferation of surrounding cells. As a physiological event, this may enable apoptotic cells within a tissue to control their replacement either during normal turnover or to induce a healing response following extensive tissue damage. This process has been best studied in *Drosophila melanogaster*, where cells undergoing apoptosis in the imaginal disc were shown to activate mitogen signalling, promoting the proliferation of neighbouring cells[41–43].

It is of direct relevance to cancer that apoptosis-induced proliferation also occurs in mammals. Apoptotic cells can stimulate the proliferation of stem cells in a caspase-dependent manner[44]. Moreover, genetic deletion of caspase-3 or caspase-7 inhibits regeneration in two model systems — skin wound healing and liver regeneration after partial hepatectomy[44]. How can apoptotic

cells stimulate proliferation? Various studies have implicated prostaglandin E_2 (PGE_2) as a key mediator of apoptosis-induced proliferation in mammalian systems. During apoptosis, caspases cleave and activate calcium-independent phospholipase A_2 ($iPLA_2$, also known as PLA2G6), increasing the production of arachidonic acid, which is converted via cyclooxygenase 1 (COX1) and COX2 (also known as PTGS1 and PTGS2) to PGE_2 (REF. 45). This may provide a mechanistic explanation for intriguing observations made in the 1950s (REF. 46) that mixing viable tumour cells with cells destined to die actually accelerated tumour growth, a finding subsequently observed by others[47] (FIG. 2a). Indeed, recent data argue that signalling by apoptotic tumour cells has an important role in tumour regrowth after radiotherapy; tumour repopulation in a mouse xenograft model of breast cancer is promoted in a caspase-3- and $iPLA_2$-dependent manner, probably through the production of PGE_2 (REF. 48). In bladder cancer, production of PGE_2 by apoptotic tumour cells promotes chemoresistance by stimulating cancer stem cell proliferation[49]. The therapeutic relevance of targeting apoptosis-induced proliferation is supported by the finding that neutralization of PGE_2 signalling reduced the emergence of chemoresistance in this model[49]. Importantly, PGE_2 has pleiotropic functions; for example, not only does it promote proliferation, but it can also skew immune responses towards a tumour promoting, anti-inflammatory phenotype[50]. In doing so, generation of PGE_2 by apoptotic tumour cells could have dual tumour promoting functions — simultaneously driving proliferation and inhibiting antitumour immunity.

Apoptosis, cell competition and cancer. Many influences, including the availability of nutrients and growth factors, combine to affect the ability of the cell to grow. Proliferating cells constantly compete with one another; 'winner' cells out-compete 'loser' cells either by, for example, taking up nutrients more effectively or through elimination of the competition. Apoptosis can contribute to the latter, generating a vacant niche into which cancerous cells can expand. The key tumour suppressor protein, p53, might contribute to this effect[51]. In a transplantable mouse model of lymphoma, deletion of p53 from haematopoietic progenitors per se does not confer a competitive advantage over wild-type cells unless the system is stressed (in this case by irradiation)[52,53]. p53-deficient cells survive irradiation whereas wild-type cells are eliminated by p53 activity (through either apoptosis, cell cycle arrest or senescence). This provides a vacant niche into which p53-deficient cells can proliferate, facilitating the accumulation of genetic lesions that lead to cancer[52,53].

Similarly, a direct role for apoptosis in promoting cancer by killing healthy cells has been shown in *Puma*-deficient mice. PUMA is essential for p53-mediated apoptosis in thymocytes, and its loss enhances *Myc*-induced lymphomagenesis[54–57]. Counter-intuitively, loss of PUMA prevents rather than promotes thymic lymphoma following irradiation[58,59]. Similarly, in a carcinogen-induced liver cancer model, deletion of *Puma* or overexpression of anti-apoptotic BCL-2 also delays tumour development[60,61]. Finally, deletion of pro-apoptotic BID inhibits rather than promotes hepatocarcinogenesis following various chronic liver injury insults[62,63]. Why would inhibition of apoptosis prevent rather than promote cancer? The answer probably relates to our earlier discussion of p53 and cell competition. As the authors propose, PUMA-mediated apoptosis of healthy cells could remove the competitive pressure that normally acts to restrain proliferation of aberrant cells, which could lead to cancer. In support of this, glucocorticoid treatment eliminates cells in a PUMA-independent manner and leads to thymic lymphoma in *Puma*-deficient mice[58]. Relating to our discussion above, particularly in liver cancer, apoptosis may also promote cancer by actively engaging compensatory proliferation of neighbouring cells[60].

At least in mice, these studies strongly support a cancer-promoting role for apoptosis through an ability to generate a vacant niche. However, to what extent might the role of apoptosis in cell competition affect human malignancies? One possibility is in the development of myelodysplastic syndrome (MDS), which can progress to MDS-related acute myeloid leukaemia (MDS-AML)[64]. MDS leads to sudden blood cell cytopenia. In many cases its aetiology is unknown but its increasing incidence with age strongly suggests that cumulative damage plays a major part[65]. MDS is also a relatively common, secondary consequence of DNA-damaging cancer therapy[66]. MDS arises as a clonal disease of haematopoietic

Table 1 | **Prognostic value of BAX and BCL-2 expression in various human cancers**

Cancer type	Comments	Refs
Correlation of increased anti-apoptotic BCL-2 expression and good cancer prognosis		
Breast cancer	Increased BCL-2 expression (IHC) is significantly associated with better DFS and OS	147
	Following chemotherapy, higher BCL-2 expression (IHC) correlates with better survival rate	148
	Increased BCL-2 expression (IHC) correlates with improved OS	149
	High BCL-2 (IHC) predicts better survival for early-stage cancers	35
Colorectal cancer	BCL-2 expression (IHC) correlates with improved survival in Dukes B colon carcinoma	150
	Increased BCL-2 expression (IHC) in the context of p53 deficiency correlates with improved survival	151
NSCLC	High BCL-2 expression (IHC) correlates with better survival in patients with lymph node infiltration	152
	High BCL-2 (IHC) expressers have higher median survival	36
	BCL-2 expression (IHC) is associated with increased survival	37
Mesothelioma	High BCL-2 expression (IHC) confers a survival advantage	153
Correlation of increased pro-apoptotic BAX expression and poor cancer prognosis		
AML	High *BAX* and *BAD* mRNA levels correlate with decreased survival	154
ALL	Increased *BAX*/*BCL2* ratio (mRNA) was found in patients with a high risk of relapse such as those with chromosomal abnormalities	39
	High BAX protein expression correlates with increased risk of relapse	38
Non-Hodgkin lymphoma	BAX expression is associated with short survival	155

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; DFS, disease-free survival; IHC, immunohistochemistry; NSCLC, non-small cell lung cancer; OS, overall survival.

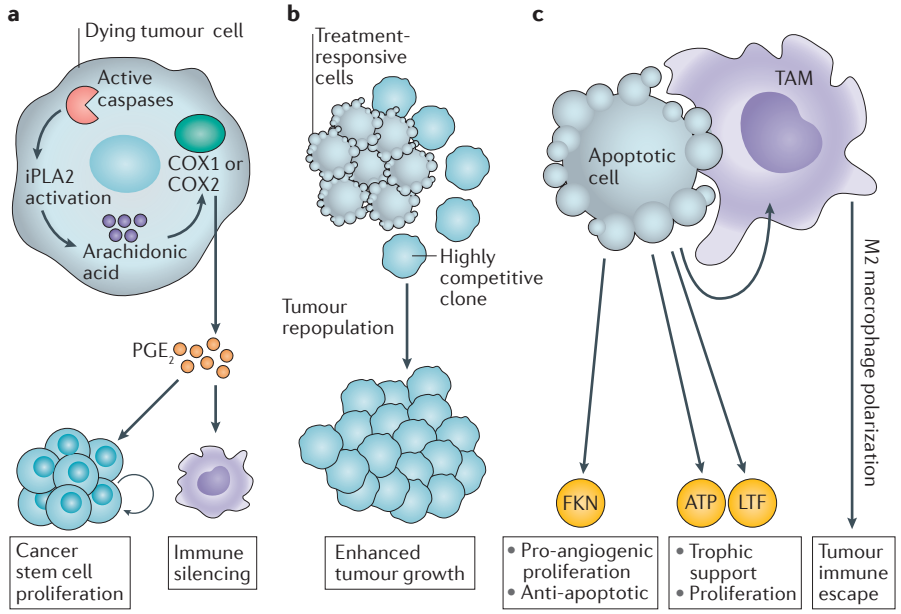

Figure 2 | **Cell-extrinsic pro-oncogenic effects of apoptotic cell death. a** | During apoptosis, caspases cleave and activate calcium-independent phospholipase A_2 (iPLA2), leading to the generation of prostaglandin E_2 (PGE_2). PGE_2 can have pleiotropic effects on surrounding cells, including pro-proliferative and immune-silencing effects. **b** | Apoptosis of tumour cells that are responsive to treatment can provide a vacant niche into which more competitive tumour cells can proliferate, ultimately leading to tumour regrowth and therapeutic resistance. **c** | Apoptotic cells release various 'eat-me' and 'find-me' molecules (such as fractalkine (FKN), ATP and lactotransferrin (LTF)) to signal their removal by phagocytes. These signals can have various pro-tumorigenic effects, including turning tumour-associated macrophages (TAMs) towards a pro-oncogenic state. More precisely, TAMs can stimulate angiogenesis, tumour cell motility and dissemination, prepare the premetastatic niche and silence immune surveillance by preventing natural killer (NK) cells and T cells from attacking cancer cells. COX, cyclooxygenase.

stem cells (HSCs)[64]. Through successive rounds of DNA damage, proliferation and apoptosis, clones are selected that ultimately give rise to AML[64]. The role of apoptosis in this disease may be multifaceted, but one possibility is that, similar to the PUMA models described above, a proportion of highly apoptosis-sensitive HSCs die, leaving a vacant niche that is filled by mutated HSCs. In an accelerated process of Darwinian selection, rounds of these events may lead to the development of aggressive and apoptosis-resistant AML. Indeed, in support of this idea, inhibiting apoptosis — through either ectopic BCL-2 expression or loss of PUMA expression — delays leukaemia progression in mouse models of MDS[67,68]. As the authors of these studies suggest, if these findings hold true in humans, inhibition of apoptosis may actually delay MDS progression. By similar means, engagement of apoptosis by cancer therapies may also inadvertently promote more aggressive disease. In this scenario, killing of sensitive cancer cells ('losers') may remove competitive pressure enabling aggressive clones to dominate and 'win' (FIG. 2b).

Effects of apoptotic cells on the microenvironment. An estimated one million cells in our bodies undergo apoptosis every second[1]. Nevertheless, analysis at any given time point fails to reveal this wholesale carnage, largely because apoptotic cells are efficiently engulfed and destroyed by phagocytic cells. Caspase-dependent events actively orchestrate recruitment of phagocytes towards the apoptotic cell by releasing 'find-me' signals, and promote the engulfment of the dying cell by exposing 'eat-me' signals[69]. Several find-me signals released by apoptotic cells have been identified, including the lipid lysophosphatidylcholine (LPC), nucleotides such as ATP, the proteins fractalkine (FKN; also known as C-X3-C motif chemokine ligand 1 (CX_3CL1)) and lactotransferrin (LTF) (FIG. 2c). These signals may exert oncogenic functions through pleiotropic effects that include but are not limited to their role as find-me signals. For example, *in vitro*, FKN can stimulate angiogenesis and hypoxia-induced proliferation of prostate cancer cells and can enhance oncogenic ERBB2 receptor signalling[70,71]. Besides being a powerful chemokine released by apoptotic cells[72], ATP can also have a major influence on cancer. Although numerous studies support an antitumorigenic effect of ATP, there is increasing realization that adenosine (a degradation product of extracellular ATP), can be oncogenic, supporting tumour growth, angiogenesis and immune escape[73].

Various data also support the idea that apoptosis can promote tumorigenesis through the recruitment and activation of phagocytic macrophages at the tumour site. For example, several studies have found that high levels of apoptosis or macrophage infiltration correlate with poor prognosis for cancer patients[29–32,74]. Is there a relationship between tumour-associated macrophages (TAMs), apoptosis and cancer progression? Similarly to macrophages involved in the wound healing process, TAMs promote tissue remodelling and angiogenesis while exerting anti-inflammatory effects. In doing so, TAMs promote cancer progression[75]. Because apoptotic cells are in close proximity to TAMs in the tumour microenvironment, a functional interconnection is possible. Direct support for a causal effect has come from recent research demonstrating that inhibition of mitochondrial apoptosis in a mouse model of B cell lymphoma impairs angiogenesis and thereby tumour growth[76]. In this study, the authors found that apoptotic tumour cells promoted TAM recruitment to the tumour, leading to TAM activation and proliferation. Importantly, TAM infiltration within tumours directly correlated with levels of tumour cell apoptosis — in apoptosis-proficient tumours TAM infiltration was much higher than in apoptosis-deficient settings. These results, together with transcriptomic and *in vitro* co-culture experiments, led the authors to propose a model whereby tumour cell apoptosis reprogrammes TAMs towards a wound-healing type response that fuels tumorigenesis. The authors also found that the pro-oncogenic effect of apoptotic cells extended to a mouse model of melanoma[76]. This study provides strong experimental evidence that apoptotic cells can promote tumorigenesis in a non-cell-autonomous manner (FIG. 2c). Clearly, many questions remain, not least in understanding how apoptotic tumour cells promote TAM activation.

Besides promoting tumour growth, apoptotic cells may also facilitate metastatic tumour progression[77]. It is well known that pregnancy reduces the overall risk of developing breast cancer; however, when

breast cancer is diagnosed in recently postpartum women (0–5 years after pregnancy) it is often more aggressive and has a poorer prognosis than disease diagnosed in nulliparous women and women diagnosed more than 5 years after pregnancy[78,79]. Potentially contributing to this, postpartum involution is characterized by massive cell death, leading to extensive efferocytosis (clearance of dead cells). A recent study in the mouse mammary tumour virus (MMTV)–polyomavirus middle T antigen (PyMT) mouse model of breast cancer found that a high level of efferocytosis in parous animals promoted TAM infiltration, stimulation of a wound-healing cytokine response and increased metastasis, implicating the clearance of apoptotic cells in cancer progression[77].

Cell-intrinsic oncogenic effects

The only way in which apoptosis can be oncogenic in a cell-intrinsic manner is if the cell in question can survive apoptosis signalling. In this section, we discuss this point, as well as giving an overview of emerging evidence that some apoptotic proteins can exert oncogenic functions through non-apoptotic mechanisms.

Failed apoptosis and cancer. Recent data show that cells that initiate apoptosis but do not die — we term this failed apoptosis — sustain potentially oncogenic damage, such as genomic instability and gene amplification[80–82] (FIG. 3). The failure to commit suicide is puzzling given what we know about apoptosis. First, activation of caspases can unleash various feed-forward mechanisms to fully engage apoptosis[12,13]. Secondly, live-cell imaging studies have shown that during apoptosis, MOMP itself is an explosive event that occurs in all mitochondria over a 10 minute window[83,84]. Finally, the level of caspase activity that is required to kill a cell does not seem to be very high[85]. These points notwithstanding, various studies have found that cells can engage caspase activity and survive, sustaining damage in the process[80–82].

Intense interest has surrounded the potential of TRAIL (also known as TNFSF10) ligands as anticancer therapies because many tumour types are selectively sensitive to TRAIL-mediated apoptosis[86]. One of the first studies to demonstrate engagement of caspase activity in the absence of cell death focused on TRAIL-induced apoptosis[87]. X-linked inhibitor of apoptosis protein (XIAP) inhibits apoptosis by inhibiting the activity of caspase-9, caspase-3 and caspase-7 (FIG. 1). The authors found that under conditions in which MOMP was inhibited and XIAP function was compromised (thereby facilitating caspase activity) TRAIL-treated cells could engage limited caspase activation yet survive — an example of failed apoptosis. In support of an oncogenic role for failed apoptosis following TRAIL treatment, another study found that sub-lethal doses of TRAIL (and FAS ligand) led to caspase-dependent mutations and genomic instability in surviving cells[81]. Why might limited caspase activity prove oncogenic? The answer relates to specific caspase substrates; one caspase-dependent hallmark of apoptosis is extensive DNA fragmentation mediated by a protein called caspase-activated DNase (CAD, also known as DFFB)[88]. Under conditions of failed apoptosis, low levels of caspase activity might trigger limited CAD activity, leading to DNA damage. Indeed, glioma cells and mouse fibroblasts that survive TRAIL treatment engage DNA damage in a caspase- and CAD-dependent manner[81] (FIG. 3).

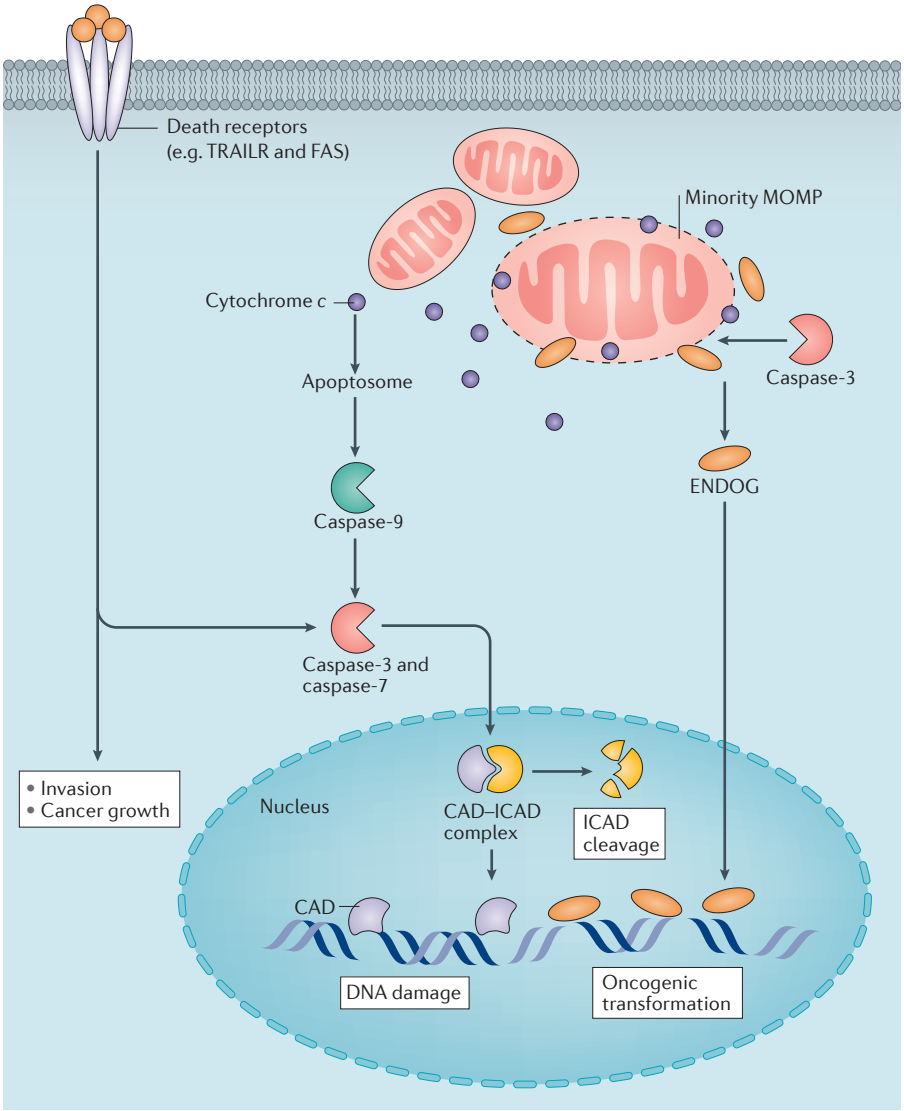

Figure 3 | **Oncogenic effects of engaging sub-lethal apoptotic signalling.** Death receptors such as FAS and tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptor (TRAILR) can have non-apoptotic signalling functions that include promotion of growth, invasion and survival — all of which support cancer development and progression. Death receptors can also engage caspase-activated DNase (CAD) activation, leading to DNA damage and mutagenesis. Intrinsic apoptotic stimuli can engage limited mitochondrial outer membrane permeabilization (minority MOMP), which activates effector caspases to sub-lethal levels. The endonucleases CAD and endonuclease G (ENDOG) (released following MOMP) cleave double-stranded DNA, causing oncogenic mutations. ICAD, inhibitor of CAD.

Most stimuli, including established anticancer therapies such as etoposide, paclitaxel or more recently developed BH3 mimetics, initiate apoptosis through the intrinsic pathway by engaging or sensitizing cells to MOMP[89]. On the basis of live-cell imaging studies, the binary all-or-nothing nature of MOMP makes it difficult to envisage how intrinsic stimuli could act in any other way but to kill a cell. However, several years ago we demonstrated that some mitochondria could resist MOMP, owing to higher expression of BCL-2, a situation we termed incomplete MOMP (iMOMP)[90]. These findings revealed intracellular heterogeneity in the sensitivity of mitochondria to MOMP. Recently, we have found that different stresses applied at sub-lethal doses can engage MOMP in limited numbers of mitochondria without killing the cell — we call this minority MOMP[80]. Importantly, minority MOMP triggers sub-lethal caspase activity (similar to TRAIL), causing DNA damage and genome instability in a CAD-dependent manner (FIG. 3). Furthermore, repeated engagement of sub-lethal stress was shown to promote transformation and tumorigenesis in a MOMP- and caspase-dependent manner[80].

Other studies support the oncogenic effects of failed apoptosis. For instance, mitotic arrest has been shown to trigger DNA damage in a MOMP- and caspase-dependent manner[91,92]. Moreover, transgenic expression of pro-apoptotic BAX has been found to promote lymphomagenesis — characterized by genomic instability — in a manner that is suppressed by co-expression of BCL-2 (REF. 93). Besides CAD, caspase-3-dependent release of endonuclease G (ENDOG) from the mitochondria has also been found to promote radiation-induced DNA damage and transformation[82]. Deletion of caspase-3 inhibited transformation following DNA damage and reduced tumorigenesis in a chemically induced model of skin cancer, implying a direct oncogenic role for caspase-3[82].

Is there any evidence that DNA damage induced by failed apoptosis actually promotes cancer? One possibility is the following. The mixed lineage leukaemia gene *MLL* (also known as *KMT2A*) encodes a histone-methylating enzyme that functions as an epigenetic regulator. The *MLL* locus is highly susceptible to breakage and rearrangement, which can generate oncogenic MLL fusion proteins that lack methyltransferase activity[94]. Rearrangements in *MLL* are recurrent oncogenic drivers in various leukaemias, including AML and acute lymphoblastic leukaemia (ALL)[95]. Importantly from the perspective of this discussion, *MLL* is the most commonly rearranged gene in cancer therapy-related neoplasms, including AML and MDS[95]. Although *MLL* rearrangements are associated with topoisomerase II inhibitor treatment, several lines of evidence argue that they do not occur solely as a result of inhibition of topoisomerase II function; in these settings apoptosis may have a role in generating *MLL* rearrangements. Indeed, various studies have shown that inhibition of caspase or CAD function reduces the incidence of *MLL* rearrangements after topoisomerase II inhibitor treatment[96–98]. This suggests a model whereby failed apoptosis causes caspase- and CAD-dependent break points in the *MLL* gene, thereby promoting oncogenic rearrangements in surviving cells.

How else might failed apoptosis-induced DNA damage or genome instability have an impact on cancer? In inflammation-associated cancers, although DNA damage is required for tumorigenesis, the cause of DNA damage often remains unclear[99]. Potentially, engagement of failed apoptosis could serve to induce DNA damage under inflammatory conditions. Another possibility may be that failed apoptosis promotes acquired resistance to apoptosis-inducing anticancer therapies. In support of this idea, repeated culturing of cells in the BCL-2-targeting BH3 mimetic venetoclax (also known as ABT-199 and Venclexta), led to the development of acquired resistance[100]. Resistance was found to be due to a mutation in BCL-2 that surprisingly enabled it to maintain anti-apoptotic function but prevented its inhibition by venetoclax[100]. DNA damage engaged by failed apoptosis may potentially have given rise to the acquisition of resistance in this case, although it is also possible that a pre-existing clone harbouring these BCL-2 mutations may have been selected for by drug treatment. Finally, it is also important to note that DNA damage acquired during failed apoptosis may also have protective, anticancer functions. For example, BH3-mimetic treatment can induce senescence in a caspase-dependent manner[101]. This study, as well as others[91,92], supports a role for DNA damage induced by failed apoptosis in feeding forward to activate p53 transcriptional responses. By doing so, p53 can exert tumour suppressor functions. Importantly, non-lethal functions for caspases have also been described in various physiological processes, including differentiation, memory and neurite pruning[102]. However, it is unknown whether caspase activity also engages DNA damage during these processes and, if not, how cells would suppress these effects.

Non-canonical oncogenic roles for apoptotic proteins. Besides their role in engaging apoptosis, various non-apoptotic roles have been ascribed to almost all proteins classically viewed as apoptotic[103–105]. Some of these non-apoptotic functions may be oncogenic. For brevity, we have restricted our discussion of non-apoptotic protein functions and oncogenesis to death receptor signalling. BCL-2 family proteins have been implicated in various non-apoptotic functions that may also impinge on tumorigenesis. These include regulation of calcium homeostasis, metastasis and autophagy — although how they regulate the latter remains controversial[106–109].

Focusing on the death receptors the TRAILRs and FAS, our discussion highlights that any consideration of pro-oncogenic effects of apoptosis signalling must also take into account these non-canonical functions (FIG. 3). As discussed, many human tumour types are selectively sensitive to TRAIL-induced apoptosis owing to increased expression (relative to many normal tissues) of TRAILRs[110]. Although this provides therapeutic opportunities, it suggests that cancer cells must gain some advantage from expressing TRAILRs. Addressing this, a recent study has shown that TRAILR signalling can promote cancer independently of its role in canonical apoptosis signalling[111]. Here the authors found that TRAILR2 signalling could promote various effects, including invasion, proliferation and migration, independently of its apoptotic function but dependent on PI3K signalling. Unlike humans, mice express only one TRAILR. In line with its functional importance, prevention of TRAILR signalling (through deletion of mouse *Trailr*) reduces metastasis while increasing survival in a mouse model of mutant-KRAS pancreatic adenocarcinoma[111]. In clinical support, high TRAILR2 expression correlates with reduced metastasis-free survival in human KRAS-mutant colorectal cancer[111]. In other *in vitro* settings, TRAILR signalling has also been found to stimulate cell migration in a caspase-dependent manner[112]. In a process dependent on failed apoptosis, TRAILR signalling can lead to caspase-dependent cleavage of RHO-associated

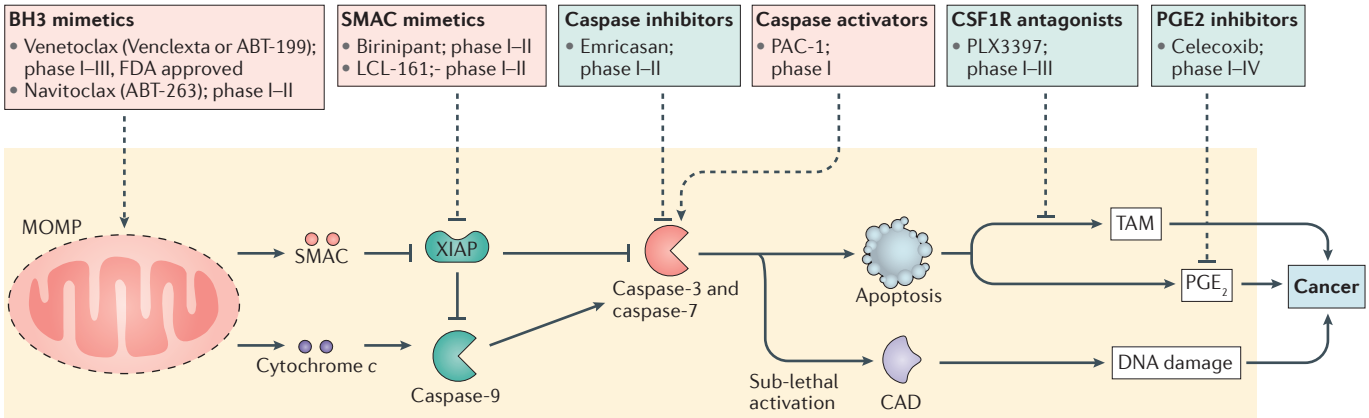

Figure 4 | **Enhancing apoptosis while minimizing damage.** Possible enhancers of apoptosis are in pink boxes, and inhibitors of unwanted effects are in blue boxes; examples of therapeutic agents and their clinical trial status (source: clinicaltrials.gov) are also shown. Given that they do not prevent death following widespread apoptotic mitochondrial outer membrane permeabilization (MOMP), caspase inhibitors could prevent unwanted effects of caspase activity such as DNA damage and prostaglandin E_2 (PGE_2) production. Inhibitors of PGE_2 activity such as celecoxib could be applied in combination with apoptosis-inducing cancer therapies. Similarly, inhibiting colony stimulating factor 1 (CSF1) signalling may either deplete tumour-associated macrophages (TAMs) or switch them to an antitumorigenic phenotype. Following failed apoptosis, enhancement of caspase activity using second mitochondria-derived activator of caspases (SMAC) mimetics (to antagonize X-linked inhibitor of apoptosis protein (XIAP)) or other means may promote apoptosis. Likewise, direct, caspase-activating molecules may preferentially promote death in tumour cells that have undergone failed apoptosis and that already have a low level of caspase activity. CAD, caspase-activated DNase; CSF1R, CSF1 receptor; FDA, US Food and Drug Administration.

protein kinase 1 (ROCK1), activating RHO GTPase and causing membrane blebbing and cell migration[112,113]. In summary, these findings suggest that inhibition of TRAILR signalling may provide therapeutic benefit in some settings whereas TRAILR agonists could engage unwanted side effects in specific tumour types.

Engagement of FAS triggers extrinsic apoptosis in a manner similar to TRAILR. Like TRAIL, various non-apoptotic, pro-oncogenic functions have also been described for FAS signalling, including stimulation of proliferation and migration[114]. Increased levels of FAS ligand are observed in several cancers, and inhibition of FAS signalling (through deletion of *Fas*) inhibits tumorigenesis in various mouse tumour models[115]. Besides having pro-proliferative effects, FAS signalling can also exert pro-survival functions. This paradoxical role has been revealed in various cancer cell types, including breast, colon and ovarian cancer stem cells, in which elimination of FAS signalling actually causes cell death[116,117]. The mechanism or mechanisms underlying this type of cell death, termed death induced by CD95R/L elimination (DICE), is unclear but does not seem to involve any known regulated pathway of cell death[115,116].

Modelling apoptotic oncogenicity

Mouse models have proved invaluable in helping to define the role of apoptosis in tumour suppression, and as a bone fide cancer therapeutic target[20,118–121]. To investigate the pro-oncogenic potential of apoptosis, we propose that mouse models should be extended to address the following interrelated questions: Can apoptosis exert a pro-oncogenic function during tumorigenesis? and Do we observe correlations between sub-lethal caspase activation, tumorigenesis and treatment responses?

To ask whether apoptosis can exert tumour promoting functions, given that apoptosis has clear tumour suppressor functions, a key challenge will be to develop models that permit temporal inhibition or induction of apoptosis during cancer progression. Nevertheless, inducible systems based around tamoxifen–oestrogen receptor type control methods should enable this[122]. In a complementary approach, experimentally engaging different levels of tumour cell apoptosis *in vivo* would directly enable the effects of tumour cell apoptosis on tumour progression to be tested. To this end, we have developed a method called mito-priming, which should prove useful[123].

In parallel, it will be necessary to develop ways to report processes such as failed apoptosis or sub-lethal caspase activation *in vivo* to ascertain whether these correlate with tumour progression. Along these lines, fluorescent reporters have recently been developed that accurately detect caspase activity even at sub-lethal levels[80,124]. Although apoptosis has been extensively imaged *in vitro*, very few studies have extended these analyses to an *in vivo* setting[125–127]. Particularly with respect to a possible role for apoptotic cell–immune cell interactions in promoting cancer, we speculate that real-time intravital imaging of tumour cell death may provide compelling new insights into this process.

Targeting apoptosis

How can we better target apoptosis by improving tumour cell killing while preventing unwanted oncogenic effects? One possibility might be to induce intrinsic apoptosis while inhibiting caspase activity (FIG. 4). Although this may seem counter-intuitive, as previously discussed, inhibition of caspase function does not ultimately protect against cell death following MOMP[14]. Triggering apoptosis in the presence of caspase inhibitors may prevent detrimental caspase-dependent effects. In support of this idea, some studies have shown a positive effect of caspase inhibition in combination with chemotherapy or radiotherapy[128,129]. These potentiating effects may be due to various mechanisms, including inhibition of tumour vascularization and enhanced antitumour immunity[128,129]. Nevertheless, inhibition of caspase function may have untoward and potentially unwanted effects. For example, MOMP in the absence of caspase activity can engage interferon responses dependent on activation of the stimulator of interferon genes (STING, also known as TMEM173) pathway[130,131]. The role of STING-dependent interferon signalling in cancer is complex as it can exert

both pro- and antitumorigenic effects[132,133]. Moreover, caspase inhibitors can completely block extrinsic apoptosis and are thereby unsuitable when this is expected to exert a therapeutic benefit[134]. Another strategy might be to target specific, caspase-dependent effects, for example, by blocking the activity of PGE_2 generated by apoptotic cells using COX inhibitors such as celecoxib[49].

The overall balance between cell proliferation and cell death dictates whether a tumour grows or regresses. Besides tumour-intrinsic mechanisms (for example, apoptotic sensitivity) that affect this, cell death triggered by antitumour immunity also has an important role in regulating this balance[135]. It is now widely accepted that immune cells can be both anti- and pro-tumorigenic[135]. First, they can prevent cancer by eliminating precancerous lesions (immune surveillance), and increased tumour infiltration by T cells and natural killer (NK) cells correlates with improved prognosis for several malignancies[136]. Secondly, the same immune response can also promote cancer growth[136]; for example, tumour cell death can switch macrophages to become tumour promoting. Understanding how this occurs should reveal possible ways to block it. Additional approaches might be combining apoptosis-inducing anticancer therapies with modulators of macrophage (specifically TAM) signalling. For example, inhibition of colony stimulating factor 1 (CSF1) signalling (the main trophic support factor for macrophages) has been shown to have multiple beneficial effects by either depleting TAMs or switching them towards a more tumour suppressive state[137,138].

In cancer cells that have engaged caspase activity but survived, how do we push them over the edge and kill them? One avenue may be by antagonizing endogenous caspase inhibitors such as XIAP using inhibitors called second mitochondria-derived activator of caspases (SMAC, also known as DIABLO) mimetics[139] (FIG. 4). However, there are also various other XIAP-independent mechanisms whereby caspase activity can be restrained[11,140]. Another possibility could be to enhance caspase cleavage of specific substrates, thereby triggering death. A prime candidate might be BID, of which minimal cleavage is required to kill a cell[141]. One possibility is to use inhibitors for the kinases that phosphorylate BID and inhibit its caspase-mediated activation[142,143]. Finally, cancer cells with sub-lethal caspase activity may be more sensitive than healthy tissue to additional caspase activity, providing a therapeutic window for direct small-molecule caspase activators[144].

Outlook

Clearly, apoptosis has abundant beneficial effects in restraining and treating cancer — the approval of BH3 mimetics for clinical use attests to this. Nevertheless, as we have discussed, apoptosis is responsible for various effects that may be tumour promoting. As such, tumour progression may be governed not only by the balance between proliferation and cell death, but also by the balance between the tumour suppressor and oncogenic functions of apoptotic signalling. The relative importance of these oncogenic versus tumour suppressor effects needs to be defined *in vivo*. The importance of oncogenic apoptotic effects may also be tumour-stage or tumour-type specific; in this respect, studies investigating apoptotic levels and/or BCL-2 protein expression with prognostic outcome (TABLE 1) should help to guide investigation to specific tumour types. Finally, the end goal is to investigate the clinical relevance of these effects in primary patient samples. Beyond defining this dark side of apoptosis, some of these oncogenic effects also offer promising potential to improve cancer cell killing; for example, understanding how cancer cells tolerate failed apoptosis and survive could provide new strategies to subvert this process to kill these cells.

Besides apoptosis, it is likely that other forms of cell death similarly affect cancer in a multifaceted manner. For example, inflammation associated with non-regulated, necrotic cell death can have both tumour promoting and tumour inhibitory effects[145,146]. Taking all these considerations into account, we envision that an improved understanding of the role of apoptosis in cancer will enable us to fully harness its potential as a therapeutic target.

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Competing interests statement
The authors declare no competing interests.

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