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REVIEW ARTICLE

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Apoptosis: mechanisms and relevance in cancer

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Abstract Apoptosis or programmed cell death is a process with typical morphological characteristics including plasma membrane blebbing, cell shrinkage, chromatin condensation and fragmentation. A family of cystein-dependent aspartate-directed proteases, called caspases, is responsible for the proteolytic cleavage of cellular proteins leading to the characteristic apoptotic features, e.g. cleavage of caspase-activated DNase resulting in internucleosomal DNA fragmentation. Currently, two pathways for activating caspases have been studied in detail. One starts with ligation of a death ligand to its transmembrane death receptor, followed by recruitment and activation of caspases in the death-inducing signalling complex. The second pathway involves the participation of mitochondria, which release caspase-activating proteins into the cytosol, thereby forming the apoptosome where caspases will bind and become activated. In addition, two other apoptotic pathways are emerging: endoplasmic reticulum stress-induced apoptosis and caspase-independent apoptosis. Naturally occurring cell death plays a critical role in many normal processes like foetal development and tissue homeostasis. Dysregulation of apoptosis contributes to many diseases, including cancer. On the other hand, apoptosis-regulating proteins also provide targets for drug discovery and new approaches to the treatment of cancer.

Cell death by apoptosis

Different forms of cell death

Cell death is an essential strategy for the control of the dynamic balance in living systems, and two fundamentally different forms of cell death, apoptosis and necrosis, have been defined. Necrosis is an accidental passive process resulting in an early disruption of the cell membrane and in the progressive breakdown of ordered cell structures in response to violent environmental perturbations such as severe hypoxia/ischaemia, extremes of temperature and mechanical trauma. This type of cell death, associated with organelle swelling, will not be discussed further, but readers are referred to [1–4]. In contrast, apoptosis or programmed cell death involves the activation of an energy-requiring intracellular machinery, which is tightly regulated and conserved throughout evolution [5]. Apoptosis affects single cells asynchronously, typically in the absence of inflammatory changes [1]. It is involved in morphogenesis of embryonic tissues as well as in homeostasis of adult organs and tissues. For example, apoptosis contributes to normal structural maturation of the lung during foetal and postnatal development [6, 7]. Apoptosis will also eliminate cells exposing the organism to danger. For example, virally infected cells or cells with damaged DNA will be removed by apoptosis [8–10].

Apoptotic cells were first identified by a series of typical morphological changes, and morphology is still an important experimental proof of the underlying process [4]. Initially, cell membrane integrity is maintained while subtle changes, e.g. exposure of phosphatidylserine, occur. Other characteristics of apoptotic cells include cellular shrinkage, membrane blebbing, nuclear chromatin condensation and fragmentation. Eventually, the cell breaks into membrane-surrounded fragments (apoptotic bodies) which are engulfed in vivo by professional phagocytes (macrophages and dendritic cells). In cell cultures, apoptotic bodies will lose the integrity of the plasma membrane during the late stages of apoptosis, followed by complete cell disintegration, also called secondary necrosis [1].

Caspase activation: general features

All the typical signs of apoptosis are the final results of a complex biochemical cascade of events. Apoptotic signal-

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ling mainly converges in the activation of intracellular caspases, a family of cystein-dependent aspartate-directed proteases which propagate death signalling by cleaving key cellular proteins [11].

Caspases are synthesized in normal cells as inactive proenzymes; they can rapidly be activated by autoproteolytic cleavage or cleavage by other caspases at specific aspartic acid (Asp) residues [12]. Currently, 14 members of the caspase family have been identified, of which 7 mediate apoptosis (Table 1). During apoptosis, caspases with a long pro-domain function as upstream signal transducers ('initiator' caspases) and proteolytically activate downstream caspases ('effector' caspases) which contain a short pro-domain [13]. The initiator caspases-8 and -10 contain in their pro-domain a death effector domain (DED), which is involved in interactions with adaptor proteins. A caspase recruitment domain (CARD) is found in caspase-2 and caspase-9 and is also important for binding of adaptor molecules and activation of effector caspases (Table 1; Fig. 1) [14]. Caspases specifically recognize and cleave a tetrapeptide sequence on their substrate with an absolute requirement for an Asp residue (Table 1). Effector caspases act on a variety of substrates resulting in proteolysis of cellular proteins and death by apoptosis. The best characterized caspase substrate is poly-(ADP-ribose) polymerase (PARP), a nuclear protein implicated in DNA repair. PARP is one of the earliest proteins targeted for specific caspase cleavage [15]. Caspase cleavage and inactivation of ICAD (inhibitor of caspase-activated DNAse) allow CAD [also known as DNA fragmentation factor (DFF)] to translocate to the nucleus where it is responsible for internucleosomal DNA cleavage, generating oligonucleosomal DNA fragments [16, 17]. Caspase cleavage of lamins results in nuclear shrinkage; cleavage of cytoskeletal proteins like fodrin and actin leads to cytosolic reorganization [18–20]. Furthermore, caspase-dependent cleavage of DNA-protein kinase (DNA-PK), cell cycle regulators [e.g. retinoblastoma protein (pRb)], transcription factors (e.g. NF-κB) and cell signalling proteins [e.g. Raf, protein kinase B (PKB)] has been reported [21–25]. The cell survival factors Bcl-2 and Bcl-Xl are also cleaved during apoptosis, as are the pro-apoptotic proteins Bid and Bax [26–28]. Probably not all the key substrates are known yet, although caspase cleavage of different proteins is a critical event for apoptotic execution.

Not all caspases are directly involved in cell death; some carry out other physiological functions (Table 1). These caspases are involved in processing pro-inflammatory cytokines and in mediating inflammatory responses. Indeed, the first mammalian caspase, caspase-1, was originally identified as interleukin-1β converting enzyme (ICE) [29].

Intensive studies on how caspases are activated during apoptosis have revealed two important pathways of caspase activation: cross-linking of death receptors following external (extracellular) triggering and release of apoptogenic factors from mitochondria following internal (intracellular) signals (Fig. 1). In addition, two other apoptotic pathways are emerging: endoplasmic reticulum stress-induced apoptosis and caspase-independent apoptosis.

Table 1 Properties of the members of the caspase family

Name of caspase	Other names	Tetrapeptide preference	Function	Size of pro-domain	Pro-domain molecule	Adaptor protein
Caspases important for execution and signalling events of apoptosis						
Caspase-2	ICH-1/mNedd2	DEHD/ VDVAD	Initiator	Long	CARD	RAIDD
Caspase-8	MACH/FLICE/ Mch5	LETD/IETD	Initiator	Long	DED	FADD
Caspase-9	ICE-LAP6/ Mch6	LEHD	Initiator	Long	CARD	Apaf-1
Caspase-10	Mch4, FLICE2	IEAD	Initiator	Long	DED	FADD
Caspase-3	CPP32/ Apopain/Yama	DMQD/ DEVD	Effector	Short		None
Caspase-6	Mch2	VEID/VEHD	Effector	Short		None
Caspase-7	Mch3/ICE-LAP3/CMH-1	DEVD	Effector	Short		None
Caspases involved in control of inflammation						
Caspase-1	ICE	WEHD/ YEVD		Long	CARD	
Caspase-4	TX/ICH-2/ ICE_{REL}-II	LEVD/(W/L) EHD		Long	CARD	
Caspase-5	TY/ICE_{REL}-III	(W/L)EHD		Long		
mCaspase-11	ICH-3			Long		
mCaspase-12				Long		
Caspase-13	ERICE			Long		
mCaspase-14	MICE			Short		None

m, Murine
Amino acids used in the column "Tetrapeptide preference": *A* alanine, *D* aspartic acid, *E* glutamic acid, *H* histidine, *I* isoleucine, *L* leucine, *M* methionine, *Q* glutamine, *T* threonine, *V* valine, *W* tryptophan, *Y* tyrosine

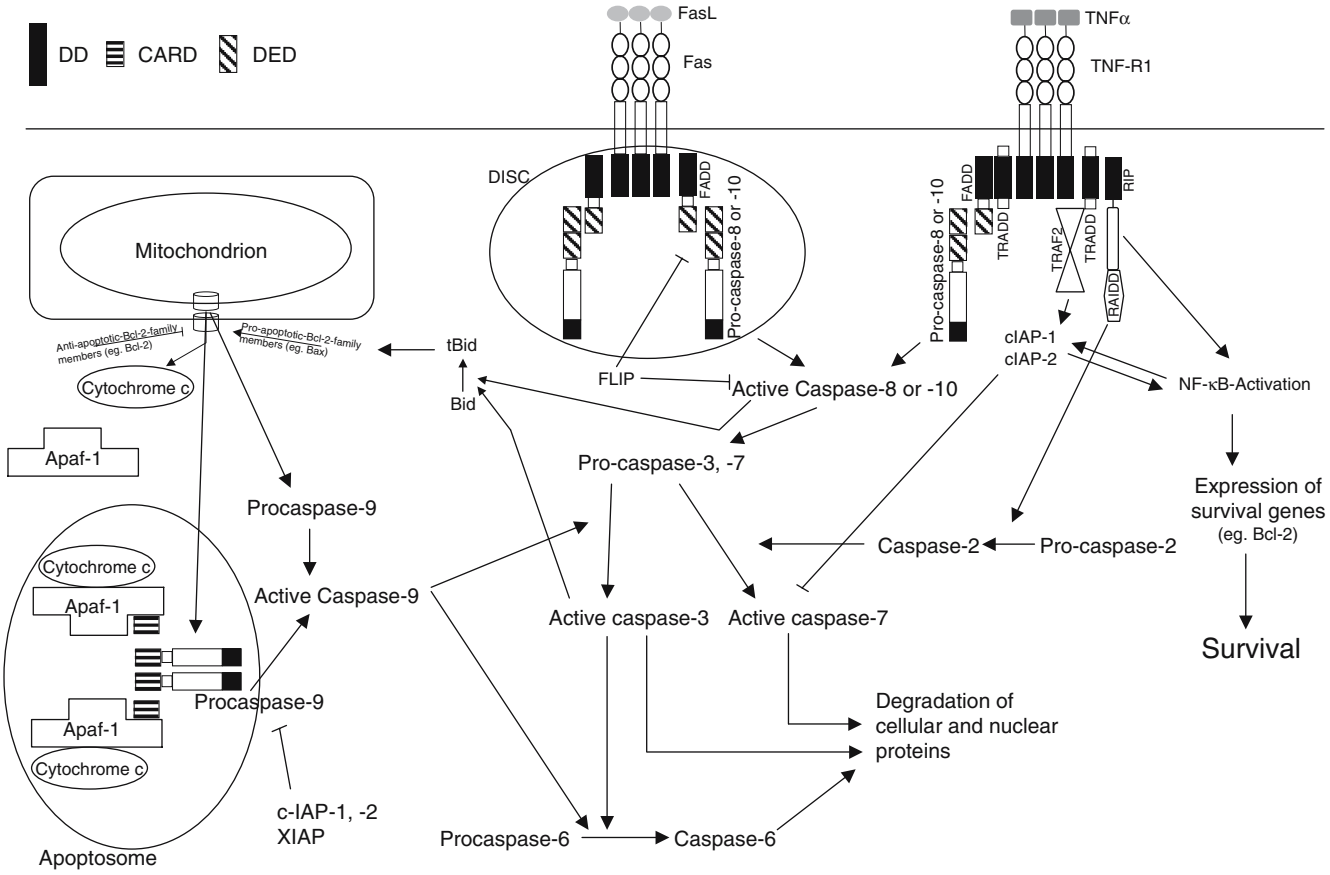

Fig. 1 Apoptosis signalling pathways. There are two important pathways of caspase activation, one involving death receptor and the other mitochondria; (→) activation; (⊣) inhibition

Death receptor-dependent pathway

Plasma membrane receptors triggering external apoptosis signalling belong to the tumour necrosis factor (TNF)-receptor superfamily. This family includes Fas (Apo-1 or CD95), TNF-receptor-1 (TNF-R1), death receptor-3 [DR3 or TNF-receptor-related apoptosis-mediating protein (TRAMP) or Apo-3], TNF-related apoptosis inducing ligand receptor-1 (TRAIL-R1 or DR4), TRAIL-R2 (DR5 or Apo-2) and DR6. The best studied death receptor is Fas; binding of Fas ligand (FasL) leads to receptor trimerisation and recruitment of specific adaptor proteins (Fig. 1) [30–32]. The Fas receptor contains a death domain (DD) in its cytoplasmic region which interacts with the adaptor protein, Fas-associated death domain protein (FADD), forming a death receptor-induced signalling complex (DISC) (Table 1, Fig. 1) [33–35]. Besides a DD, FADD contains a death effector domain (DED) and this recruits the DED-containing procaspase-8 into the DISC [36]. Procaspase-8 will be proteolytically activated to the enzymatically active caspase-8, which in turn will activate downstream effector caspases (Fig. 1) [12].

Other death receptors activate caspases in a similar manner. The binding of TNF-α to TNF-R1 leads to trimerisation of TNF-R1 and to binding of the TNF-R-associated death domain protein (TRADD). The death domain of TRADD interacts with the death domain of FADD, recruiting pro-caspase-8. Active caspase-8 in turn activates downstream effector caspases by cleaving pro-caspase-3 [37]. However, TNF-α binding to TNF-R alone rarely induces apoptosis; the binding of the adaptor molecule TNF receptor-associated factor-2 (TRAF2) to TRADD recruits cellular inhibitor of apoptosis (c-IAP)-1 and c-IAP-2, two anti-apoptotic proteins. The binding of the receptor interaction protein (RIP), the third protein able to interact with TRADD, leads to activation of the transcription factor NF-κB, resulting in transcription of anti-apoptotic genes and promoting cell survival (Fig. 1) [32, 35, 38, 39]. Alternatively, the RIP–TRADD interaction can also inititate apoptosis by recruiting caspase-2 through the adaptor molecule RIP-associated ICH-1/CED3 homologous protein with DD (RAIDD; Table 1) [40, 41]. Other death receptor (DR)-activating ligands include lymphotoxin, Apo3-ligand and Apo2-ligand; they bind with their respective receptors (TNF-R1, DR3, DR4 and DR5) and adaptor molecules.

In addition to death receptors, decoy receptors (DcR1, DcR2, DcR3, osteoprotegerin) have been identified. These receptors compete with the death receptors for ligand binding, but they do not transduce apoptotic signals [42].

Mitochondrial-dependent pathway

Participation of mitochondria to apoptosis induction mainly involves the release of caspase-activating proteins into the cytosol. The release of cytochrome *c* from the mitochondria results in the activation of the apoptotic protease activating factor-1 (Apaf-1). In the presence of cytochrome *c* and ATP, the CARD domain of Apaf-1 binds with the CARD domain of procaspase-9 (Table 1), and this forms the mitochondrial DISC, also designated as 'apoptosome' [43]. Following activation, the apoptosome-associated caspase-9 will in turn activate downstream caspases like caspase-3, caspase-6 and caspase-7 (Fig. 1) [44].

The mechanism of cytochrome *c* release has not yet been completely unraveled. It has been suggested that a decisive role is played by the mitochondrial permeability transition pore (PTP) formed at the contact sites between the inner and the outer mitochondrial membranes. The main components of the PTP include the adenine translocator (ANT) at the inner membrane and the voltage-dependent anion channel (VDAC), also called porin, at the outer membrane. The PTP also includes several other proteins: hexokinase II (HKII), mitochondrial creatine kinase (mtCK), cyclophilin D (Cyp-D) and peripheral benzodiazepine receptor (PBR), which all have a role in modulating the activity of the PTP [45, 46]. The pore opening is influenced by numerous endogenous effectors, e.g. ions (mainly Ca^{2+} and Mg^{2+}), protons, local ADP/ATP concentrations, mitochondrial membrane potential and changes in composition or function of the Bcl-2 complex [47]. There is emerging evidence that the pro-apoptotic members of the Bcl-2 protein family are important regulators of PTP opening. The Bcl-2 family of proteins is located or translocated to the outer mitochondrial membrane and includes anti-apoptotic members (e.g. Bcl-2, Bcl-Xl) and pro-apoptotic members (e.g. Bax, Bak). Both groups of proteins share homology in one to four Bcl-2-homology (BH) domains (BH1, BH2, BH3, BH4). A subset of the pro-apoptotic proteins only has the central short BH3 domain (e.g. Bid, Bak) (Table 2) [48–59]. The different family members can homo- or hetero-dimerize, and the relative ratios of anti- and pro-apoptotic proteins will determine the susceptibility of cells to apoptotic stimuli [60–62]. Bcl-2 family proteins modulate permeabilization of the inner and/or outer mitochondrial membranes and regulate in this way the release of cytochrome *c* [63]. However, a single mechanism by which the Bcl-2 family members regulate apoptosis has not been completely determined. Phosphorylation and dephosphorylation of Bcl-2 family proteins may be a crucial event in the regulation of its function [64, 65]. Besides cytochrome *c* release, the Bcl-2 family proteins have also been reported to control the release of other proteins from mitochondria including certain caspases (caspase-2, caspase-3 and caspase-9), apoptosis inducing factor (AIF), second mitochondria-derived activator of caspase/direct IAP-binding protein with low PI (Smac/Diablo), Omi/HtrA2 and endonuclease G [66–69]. Smac/Diablo can interact with inhibitors of inhibitors of apoptosis (IAPs) like X-linked inhibitor of apoptosis (XIAP) and prevent their activity [70, 71]. Omi/HtrA2 is a serine protease that contributes to caspase-dependent and caspase-independent cell death and that interacts with IAPs [70, 72]. Endonuclease G translocates to the nucleus during apoptosis and induces caspase-independent DNA fragmentation [73].

Table 2 Bcl-2 family members

Anti-apoptotic members				
Bcl-2	Bcl-W	Boo/Diva	Mcl-1	Bfl-1/A1
Bcl-Xl	Bcl-B			
Pro-apoptotic members				
Pro-apoptotic members with two or three distinct BH domains				
Bax	Bcl-Xs	Bfk	Bcl-Xs	Bok/Mtd
Bak	Bcl-G_L			
Pro-apoptotic members with a BH3 domain only				
Bid	PUMA	Spike	Hrk/DP5	Bik/Nbk/Blk
Bak	Bad	Nix	Bim/Bod	BNIP3/nix
Bmf	Nip3	Noxa	Harakiri	

A molecular link between the death receptor and the mitochondrial apoptosis pathways can be found at the level of caspase-8 cleavage of cytosolic Bid, a member of the BH3 domain-only subgroup of Bcl-2 family [28, 74]. Cleaved Bid [truncated Bid (tBid)] translocates from the cytosol to mitochondria and activates the mitochondrial-dependent apoptotic pathway (Fig. 1) [75, 76]. Caspase-3 can also cleave Bid, thereby inducing cytochrome *c* release and apoptosis [74].

Endoplasmic reticulum and apoptosis

In the endoplasmic reticulum (ER), proteins obtain their mature conformation after proper post-translational modification, folding and oligomerization. Accumulation of misfolded proteins in the ER activates the unfolded protein response (UPR), a conserved signalling pathway leading to repair of ER folding or, in case of severe damage, to initiation of apoptosis. In the lumen of the ER, Ca^{2+} is stored, and disturbance of the Ca^{2+} homeostasis initiates apoptosis [77, 78]. Mitochondrial involvement in ER stress-induced cell death has been shown by the release of cytochrome *c* from mitochondria after induction of ER stress. In addition, Bcl-2 has been shown to inhibit ER stress-induced apoptosis [79, 80]. Members of all Bcl-2 classes are also localized on the intracellular membrane of ER and regulate ER Ca^{2+} homeostasis, probably by influencing membrane permeability. Ca^{2+} release may induce apoptosis by influencing the mitochondrial PTP or by direct activation of caspase-12 [79]. This caspase is localized on the ER membrane and is specifically activated during ER stress-induced apoptosis [81, 82]. The mechanism of caspase-12 activation is not completely clear; it probably involves both a calpain-dependent removal of the pro-domain and self-cleavage [79]. Calpains are Ca^{2+}-dependent cytosolic cysteine proteases which can also mediate caspase-independent apoptosis [83]. Besides caspase-12, other ER-associated pro-apoptotic molecules have been reported, e.g. Bap31, which

is both a regulator of procaspase-8 and a substrate of caspase-8 itself [84].

Inhibition of apoptosis

Since most cells can undergo apoptosis, it is clear that there is a need for apoptosis inhibitory mechanisms. One group of endogenous inhibitors of DR-induced apoptosis belongs to the FADD-like ICE inhibitory proteins (FLIP)-family. Their DED regions compete for binding to DED-containing initiator caspases-8 and -10 or adaptor proteins, thereby inhibiting the recruitment to the DISC and inhibiting apoptosis [85–88]. Both the mitochondrial pathway and the death receptor pathway are under suppression of a family of inhibitors of apoptosis (IAP), originally discovered in baculoviruses and containing one to three baculoviral IAP repeats (BIR) [89]. Several IAPs have been discovered in humans, including c-IAP-1, c-IAP-2, XIAP, BIR repeat containing ubiquitin-conjugating enzyme (BRUCE), neuronal apoptosis inhibitory protein (NAIP), survivin and livin [90–93]. IAPs inhibit directly or indirectly the members of the caspase family, e.g. XIAP, c-IAP-1 and c-IAP-2 directly bind to and inhibit caspase-3, caspase-7 and caspase-9 [94–96]. c-IAP-1 and c-IAP-2 have also been shown to be induced and activated by the transcription factor NF-κB (nuclear factor-κB), providing a positive feedback loop [96]. Survivin, an IAP family member, also inhibits caspases directly; recently, a role for survivin in mitotic spindle formation was shown [92]. IAPs themselves are negatively regulated by IAP-binding proteins such as Smac/Diablo, a mitochondrial protein that can be released in the cytosol during apoptosis induction [69]. Omi/HtrA2 has also been described as an IAP-antagonist [70]. The recently identified tumour-up-regulated CARD-containing antagonist of caspase-9 (TUCAN) suppresses caspase activation induced by the Apaf-1 activator cytochrome *c* [97].

The highly conserved heat shock proteins (Hsp), synthesized in response to stress, facilitate cell survival by inhibiting apoptosis. Different Hsp families can rescue cells at different phases of the apoptosis signalling cascade. Hsp27 can protect mitochondria during apoptosis by inhibiting the activity of pro-apoptotic Bcl-2 family proteins, or they can, more downstream, disrupt the apoptosome function [98]. Hsp10, Hsp60 and Hsp90 also have pro-apoptotic capacities and can chaperone the cell to death. The role of Hsp proteins in regulation of apoptosis is extremely complex and beyond the scope of this review. Their role in apoptosis is discussed in different interesting reviews [99, 100].

Caspase-independent apoptosis

Some forms of cell death cannot be easily classified as apoptosis or necrosis, e.g. when cell death occurs in the presence of the caspase inhibitor Z-VAD.fmk without DNA fragmentation, DNA condensation or caspase activation [101, 102]. Z-VAD.fmk binds irreversibly to the catalytic site of all caspases through an aspartic acid residue mimicking the cleavage site and a fluoromethyl ketone (fmk) group forming a covalent inhibitor–enzyme complex [103]. If caspases alone are responsible for the execution of apoptosis, cells should survive apoptotic treatment in the presence of Z-VAD.fmk. The opposite observation led to the idea of caspase-independent apoptosis. Because not all caspases are inhibited to the same extent by Z-VAD.fmk, experiments using this artificial inhibitor do not provide indisputable evidence for caspase-independent apoptosis. Bax-triggered cell death and different mitochondrial changes (loss of mitochondrial membrane potential and release of cytochrome *c* and AIF) were reported not to be inhibited by Z-VAD.fmk [102, 104, 105]. There are also reports of programmed cell death with originally necrotic or non-apoptotic morphology. For example, TNF induces, depending on the cell type, apoptotic cell death with caspase activation or cell death with necrotic morphology and without caspase activation [106–108]. It should also be envisaged that, if cells escape from caspase activation, it would be very dangerous for the organism to depend on a single family of proteases for removing unwanted cells [109]. Observation of non-caspase proteases including cathepsins, calpains and serine proteases like granzyme A/B and Omi/HtrA2 suggests that the role of caspases in apoptosis is substitutable [72, 110, 111]. These proteases can cooperate with caspases, but they can also trigger caspase-independent apoptosis [112].

Apoptosis and disease

Considering that apoptosis is an integral part of life, it is obvious that it could play a role in the pathogenesis of many diseases. Normally, apoptosis will remove unwanted, injured and virus-infected cells, but disease can occur when this process is disturbed. There are diseases linked with suppression of apoptosis like cancer, atherosclerosis and autoimmune disorders. Other diseases are linked with increased apoptosis such as viral infections (e.g. AIDS), bacterial infections (e.g. *Neisseria meningitidis*), neurodegenerative disorders (e.g. Alzheimer's disease), autoimmune disorders (e.g. multiple sclerosis), haematological disorders (e.g. myelodysplastic syndromes), ischaemic injury (e.g. myocardial infarction) and toxin-induced diseases (e.g. alcohol-induced hepatitis) [113]. The process of aging seems to be associated with dysregulated apoptosis. Different studies showed age-related changes in apoptosis-regulating proteins, compatible with the higher prevalence of malignancies, autoimmune diseases and neurodegenerative disorders in older people [114]. Below, some issues on the role of apoptosis in cancer development and the possibilities of apoptosis-based therapy will be highlighted. For more information on the apoptosis-linked diseases mentioned above, readers are referred to [113–116].

Apoptosis and cancer

The accumulation of too many cells in cancer is a result of excessive cell proliferation and/or insufficient apoptosis. Both inactivating mutations in pro-apoptotic genes and increased expression or activity of anti-apoptotic proteins result in insufficient apoptosis and growth advantage of malignant cells [117]. In this regard, mutations in the pro-apoptotic *p53* tumour suppressor gene and alterations in the expression of proteins of the Bcl-2 family have received the most attention.

Alterations in the amount of Bcl-2 protein family members have been associated with a variety of pathological conditions. The anti-apoptotic *Bcl-2* gene, identified in the t(14;18) chromosomal translocation in follicle centre cell B-cell lymphomas, is overexpressed in lymphomas, acute leukaemias and in many solid tumours; its overexpression has been correlated with poor prognosis [118–120]. Overexpression of other Bcl-2 family proteins has also been identified; increased Mcl-1 expression has been reported in acute leukaemias after relapse from chemotherapy, and elevation in Bcl-Xl level has been found in chronic myeloid leukaemia (CML) and multiple myeloma [121–124]. Insufficient expression of the pro-apoptotic members *Bax* and *Bak* has been reported in several human cancers, e.g. colon cancer and hematological malignancies [125, 126]. A high ratio of Bcl-2 to Bax protein is correlated with poor prognosis and decreased rates of complete remission [127]. In addition, the genome of several pathogenic viruses such as Epstein–Barr virus (EBV) encodes Bcl-2 homologues [128].

The tumour suppressor gene *p53* can be activated by a variety of conditions including DNA damage, hypoxia and heat shock. *p53* regulates the transcription of several genes involved in cell cycle arrest (*p21*, *Gadd45*) or induction of apoptosis (eg. *Bax*, *Apaf-1*, *caspase-9*, *Fas*, *DR5*, the *p53*-inducible gene (*PIG)* and *Noxa*) [52, 129–132]. Recently, *p53* has been shown to directly activate the pro-apoptotic protein Bax, contributing to apoptosis [133]. Mutated or deleted *p53* is the most frequent genetic abnormality in cancer; more than 50% of human tumours contain mutations in *p53* that inactivate its function [134–136]. Alteration of *p53* abrogates its tumour suppressor activity and contributes to tumourigenesis. Cells expressing mutant *p53* are also sensitive to drug-induced apoptosis [137, 138]. Individuals with Li-Fraumeni syndrome have germline mutations in *p53* and are predisposed to an increased risk of malignancy, in particular, breast cancer and sarcoma [139]. In addition, the presence of wild type *p53* does not necessarily indicate that the p53 pathway is intact: mutations or altered expression of upstream p53 regulators (ATM, Chk2, Mdm2 and p19 (ARF)) and human papillomavirus (HPV)-E6 oncoprotein-triggered p53 proteolysis have also been described in human tumours [138, 140, 141].

Alterations of other apoptotic regulators have also been implicated in the pathogenesis of various malignancies. Germline mutations in the human *Fas* gene are associated with autoimmune lymphoproliferative syndrome (ALPS) [142]. Somatic mutations in the *Fas* gene have been described in myeloma, T-cell lymphoblastic leukaemia and human T-cell leukaemia/lymphoma virus type I (HTLV-I)-related adult T-cell leukaemia [143–147].

Mutations in caspase-encoding genes are less described. Germline mutations in the *pro-caspase-10* gene have been identified in some patients with ALPS [148]. Mutations in the genes encoding pro-caspase-8 or caspase-9 have been described in neuroblastoma, and caspase-8 mutants inactivate cell death in gastric carcinomas [149, 150].

The IAPs normally protect cells from apoptosis, but they are also correlated with malignancies. The IAP survivin is commonly overexpressed in cancer and counteracts apoptosis at the G_2/M checkpoint, contributing to aberrant mitosis in many cancers [92, 151, 152]. A longer median survival was seen in acute myeloid leukaemia (AML) patients with low levels of another IAP, XIAP. Chromosomal translocations involving the c-IAP-2 gene have been observed in mucosa-associated lymphomas of extranodal tissues (MALT lymphomas) [153–156].

Besides tumourigenesis, defects in apoptosis can also underlie drug resistance [157]. Tumour cells can resist death receptor-mediated apoptosis following reduction or loss of Fas from the cell surface (eg. in leukaemia) [144, 145, 158]. Overexpression of Bcl-2 and Bcl-Xl renders cells resistant to chemotherapy; increased Bcl-2 levels have been shown to inhibit apoptosis in response to dexamethasone and to chemotherapeutic agents like etoposide, camptothecin, doxorubicine, vincristine and actinomycine D [159–162].

Apoptosis and cancer therapy

Most drugs currently used in anti-cancer therapy kill target cells by induction of apoptosis, both by receptor-mediated and mitochondrial-mediated pathways. Disruption of the mitochondrial membrane potential, cytochrome *c* release and activation of different caspases have been described following treatment of cells with diverse chemotherapeutic agents [102, 163]. For example, chemotherapy-induced increase in the transcription of the p53 response gene *Bax* leads to cytochrome *c* release and caspase activity. Activation of the Fas system has been observed in different systems, e.g. induction of FasL and upregulation of Fas following treatment of different tumour cell lines with doxorubicin, cisplatin, methotrexate, cytarabine and etoposide [137, 164–167]. In addition, treatment of CML with the death receptor ligand interferon α brings about the upregulation of Fas on CML progenitors [168]. Patients with Fas-positive AML were shown to have a better therapeutic response in comparison with Fas-negative AML patients [169].

The improved understanding of the mechanisms of apoptosis and resistance to apoptosis have provided new insights for the development of new anti-cancer agents. TRAIL, a member of the TNF family of ligands, binds to the cell surface death receptors DR4 and DR5 and is expressed by most cells; most normal cells appear to be re-

sistant to TRAIL-induced apoptosis due to expression of decoy receptors (DcR1 and DcR2), while transformed cells are sensitive [32, 145, 170]. Indeed, TRAIL induced apoptosis in leukaemic and solid tumour cell lines, while normal prostate cells, fibroblasts and smooth muscle cells were unaffected. Furthermore, no observable TRAIL-induced toxicity was observed in nude mice and non-human primates [171]. Phase II clinical trials are currently ongoing to evaluate the potential of HGS-ETR1, an agonistic monoclonal antibody to TRAIL-R1, in the treatment of non-small-cell lung cancer, colorectal cancer and non-Hodgkin's lymphoma (Human Genome Science, Rockville, MD, USA; http://www.hgsi.com) [172].

The dysregulation of different members of the Bcl-2 family in many cancer types led to the search for small inhibitors of this protein family. Small molecules with high affinity for the BH3-domain on the surface of Bcl-2 induce apoptosis [173–176]. Gossypol, a natural product found in cottonseeds, interacts with the BH3-binding pocket of four anti-apoptotic proteins [177, 178]. Several other Bcl-2 family inhibitors are in pre-clinical investigation; HA14-1, BH3I-1, antimycine analogues, certain theaflavins and epigallechatechins [179–181]. Recently, seliciclib (CYC202 or R-roscovitine), a cyclin dependent kinase inhibitor, has shown activity in multiple myeloma via downregulation of Mcl-1 [182]. In addition, peptides mimicking BH3 and antisense oligonucleotides targeting Bcl-2 can enhance apoptosis in tumour cells. The *Bcl-2* antisense drug Genasense (Genta, Inc., Berkeley Heights, NJ, USA) is currently in phase III clinical trials for malignant melanoma, multiple myeloma, chronic lymphocytic leukaemia and non-small-cell lung cancer (http://www.genta.com) [183–185]. *Bcl-2* antisense oligonucleotide therapy trials are ongoing in patients with non-Hodgkin's lymphoma, acute leukaemia and small-cell lung cancer [186–190]. Tumour-selective expression of pro-apoptotic Bax by adenoviral gene transfer resulted in selective toxicity on tumour cells [191].

Some compounds (e.g. PK11195, a peripheral bezodiazepine receptor ligand; methoxyoestradiol, an oestrogen derivative; lonidamine, derived from indazole-3-carboxylic acid and arsenite) have been shown to directly impact on mitochondrial function [192, 193]. Lonidamine has been shown in combination chemotherapy phase II and phase III trials to improve the overall response rate of breast cancer and non-small-cell lung cancer [194–196].

Different strategies for targeting tumours with p53 mutation or dysregulation are also being exploited, including development of small molecule inhibitors and inhibition of expression and/or function of p53 regulators (e.g. Mdm-2) [197].

The suppression of NF-κB is another strategy for developing new cancer therapies. This transcription factor induces expression of several anti-apoptotic genes (Bcl-2, Bcl-Xl, c-IAP-2), and NF-κB-overexpression has been found in different cancers [198, 199]. Small molecule inhibitors of the IκB protein, the regulator of NF-κB, have been identified [200]. Furthermore, chemical inhibitors of IKK, the protein responsible for the phosphorylation of IκB, with pro-apoptotic properties have been described including BMS-345541, Bay 11-7082, SC-514 and beta carbolines [201–204]. In addition, the proteasome inhibitor bortezomib (PS-341) (Velcade) inhibits the degradation of ubiquitinated IκB and is in clinical development for cancer treatment (Millenium Pharmaceuticals Inc., Cambridge, MA, USA) [205]. It has demonstrable clinical activity in relapsed, refractory multiple myeloma [206]. Bortezomib sensitizes its effects on NF-κB suppression-induced apoptosis by downregulating Bcl-2 [207, 208]. On the other hand, the level of the BH3-only proteins Bik and Bim is increased in several cell lines by bortezomib, indicating that these proteins are important mediators of the anti-tumour activity [209].

Recently, the Smac/Diablo protein was discovered. This protein binds to and inhibits the IAP-family proteins and promotes apoptosis [68]. Peptides from the N terminus of Smac can reverse the caspase inhibition by IAP. This has opened up a new field for drug development; more small molecules and oligonucleotides with effect on IAP are being developed [210, 211]. The recent finding that the caspase inhibitor survivin is abundantly expressed in transformed cell lines but not in normal adult tissue generated interest for selective targeting of cancer cells [92]. Indeed, an antisense oligonucleotide targeting survivin sensitizes cancer cells to etoposide treatment [212] (ISIS Pharmaceuticals/Lilly Inc.). Furthermore, downregulation of XIAP induced apoptosis in ovarian cancer [213]. Heat shock proteins are also pharmacological targets. Geldanamycin, the first Hsp90 inhibitor, showed clear anti-tumour effects, but high toxicity in clinical trials led to the synthesis of geldanamycin hybrids with improved selectivity and efficiency [100, 214].

For all these therapeutic options, the basic idea of selective activation of apoptosis in transformed cells remains the key issue and may result in the development of new therapeutic agents, more active and/or less toxic than the ones used currently. In the future, patient-specific profiles of apoptosis-related genetic alterations and genetic comparisons between chemotherapy-sensitive and chemotherapy-resistant cells will open the way for patient-specific apoptosis-based therapy with hopefully fewer adverse effects [146, 215].

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