Review

Cell Death Signalling Pathways in the Pathogenesis and Therapy of Haematologic Malignancies: Overview of Apoptotic Pathways

(apoptosis / TNF-related apoptosis inducing ligand / TRAIL / granzyme B / ceramide / mitochondria / intrinsic apoptotic pathway / extrinsic apoptotic pathway)

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Abstract. Apoptosis, a Greek descriptive term for falling leaves or petals, plays an important role in the progression of many diseases. Apoptosis is essential for the development and survival of multi-cellular organisms. Malignant diseases, including haematologic malignancies, are associated with defects in the cell death mechanism. These defects are not only important for the growth advantage of malignant clones, but when understood can be used for specific therapeutic targeting of malignant cells while sparing normal cells. The cellular and molecular mechanisms of apoptosis have been extensively demonstrated and are reviewed in this article. In this part of the review we focus on basic details of the apoptosis pathways, key players of the receptor-mediated apoptosis, and molecules involved in the cross-talk between individual apoptosis pathways and apoptosis regulation.

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Abbreviations: Bcl-2 – B-cell lymphoma 2; CAD – caspase-activated DNAse; CTL – cytotoxic T lymphocyte(s); DISC – death-inducing signalling complex; ER – endoplasmic reticulum; FLICE - FADD-like interleukin-1-β-converting enzyme; IAP – inhibitor of apoptosis proteins; IL – interleukin; JNK – c-Jun N-terminal kinase; MAPK – mitogen-activated protein kinase; MOMP – mitochondrial outer membrane permeabilization; MPT – mitochondrial permeability transition; NK – natural killer; ROS – reactive oxygen species; Smac/DIABLO – second mitochondria-derived activator of caspase/direct IAP-binding protein with low pI; TNF – tumour necrosis factor; TRAIL – TNF-α-related apoptosis-inducing ligand.

Introduction

Apoptosis and necrosis represent two major mutually different mechanisms of cell death (Fink and Cookson, 2005). Necrosis is defined as uncontrolled process of cellular break-up executed as a consequence of massive and irreversible damage. Necrosis leads to the release of inflammatory cytokines such as TNF-α or IL-4. Apoptosis is a controlled, to a certain degree reversible process of programmed cell disintegration that usually doesn't induce inflammatory reaction. Although the understanding of detailed signalling pathways that execute apoptosis is incomplete, this process is controlled by numerous proteins and protein complexes that when activated by various triggers induce sequential activation of the effector mechanisms (Fadeel and Orrenius, 2005).

Apoptosis is triggered by two major apoptosis-initiating pathways, designated as the inner/intrinsic/mitochondria-mediated and outer/extrinsic/receptor-mediated pathway, respectively (Kroemer and Reed, 2000; Petak and Houghton, 2001; Malisan and Testi, 2003; Rossi and Gaidano, 2003; Green and Kroemer, 2004; Thorburn, 2004). Both pathways converge to a final apoptosis execution step resulting in the cleavage of cell regulatory and structural molecules. Both the extrinsic and the intrinsic pathways are interconnected at mitochondria, hence their distinction into two separate pathways of apoptosis is to a certain point simplistic. Apoptosis is an essential physiological process throughout the life of multi-cellular organisms important both in development and in the maintenance of tissue homeostasis. Apoptosis is involved in control of the cell number and proliferation during

embryogenesis, deletion of activated lymphocytes at the end of the immune response, elimination of self-reactive lymphocytes, in controlled destruction of damaged, aged, infected, transformed, and other harmful cells (Nagata 1997; Testa, 2004). The defect in apoptosis and in apoptotic regulatory mechanisms can result in various pathological states, including malignant transformation, tumour progression, or autoimmune and neurodegenerative diseases. In transformed cells, loss of sensitivity to apoptosis represents one of the key molecular mechanisms of resistance to chemo/immuno/radiotherapy. The detailed knowledge of apoptosis and apoptosis regulatory mechanisms is therefore essential for the understanding of pathogenesis of malignant disorders as well as for the development of more effective tumour therapies.

Intrinsic apoptotic pathway: mitochondria and endoplasmic reticulum

Factors that could initiate the intrinsic apoptotic pathway are physical and chemical injuries (e.g. UV radiation, heat shock, osmotic shock, hypoxia), changed expression of cellular oncogenes and tumour suppressor genes (e.g. c-*Myc*, c-*Fos*, *p53*, *PTEN*), disruption of cytoskeletal structures, DNA damage (e.g. mutagenic agents, cytostatics, antimetabolites), growth factor/cytokine deprivation, nucleotide/ATP deficiency, accumulation of misfolded proteins and other stress factors. While the initial triggers of diverse intrinsic apoptotic pathways may differ substantially, all of them inevitably converge to the pivotal events, which are the mitochondrial outer membrane permeablization (MOMP), increased production of reactive oxygen species (ROS), and mitochondrial fission (Fig. 1). Subsequently, cytochrome c and other pro-apoptotic molecules such as Smac/DIABLO (second mitochondria-derived activator of caspase/direct IAP-binding protein with low pl), AIF (apoptosis-inducing factor) and endonuclease G are released from the disrupted mitochondria to the cytoplasm (Liu et al. 1996; Susin et al. 1999b; Du et al., 2000; Li et al., 2001). The mechanisms regulating the release of pro-apoptotic molecules from the mitochondrial inter-membrane space are still incompletely understood. The pro-apoptotic Bcl-2 members Bax/Bak are believed to oligomerize in response to various stress signals at the mitochondria, where they form pores in the outer membrane, which directly elicits MOMP (Green and Kroemer, 2004). Distinct cell insults induce Ca^{2+} release from the endoplasmic reticulum (ER) stores, which is followed by rapid Ca^{2+} uptake by mitochondria (Breckenridge et al., 2003; Scorrano et al., 2003; Zong et al., 2003). Ca^{2+} accumulation stimulates production of mitochondrial ROS and leads to opening of the mitochondrial permeability transition (MPT) pores, which in turn results in dramatic mitochondrial inner transmembrane potential dissipation ($\Delta\Psi m$), osmotic swelling of the intermembrane matrix, MOMP, and the release of pro-apoptotic mediators (Brookes et al., 2004; Petrosillo et al., 2004). ER stress plays a central role in conveying certain pro-apoptotic stimuli and may be an important event in disorders such as myocardial infarction, stroke, and neurodegenerative disorders (Alzhemer's disease, amyotrophic lateral sclerosis (ALS), Parkinson's disease, prion disorders) (Rao et al., 2001; Oakes et al., 2003; Verkhratsky and Toescu, 2003; Bassik et al., 2004; Rao and Bredesen, 2004; Rao et al., 2004a, b; Sheikh and Huang, 2004).

In the cytoplasm the adaptor protein Apaf-1 binds (in the presence of ATP/dATP) cytochrome c and procaspase 9 to form a multiprotein complex, referred to as the apoptosome (Finucane et al. 1999; Susin et al. 1999a; Arnoult et al., 2003). The activation of procaspase 9 with subsequent activation of procaspase 3 results in the initiation of the common effector apoptotic pathway.

Extrinsic apoptotic pathway: death ligands and receptors

Binding of a death ligand to the corresponding death receptor induces the extrinsic, receptor-mediated apoptotic pathway (Fig. 2). Death ligands are type II membrane glycoproteins that belong to the tumour necrosis factor (TNF) (super)family. TNF family of cytokines is involved in the control and manipulation of the immune system including cytotoxic effects (Gruss et al. 1996; MacEwan, 2002). Death ligands are physiologically either membrane-bound, or proteolytically cleaved (by specific metalloproteinases) into their respective soluble form. TNF (ligand) family comprises several protein members including TNF-α (TNFSF2, cachectin), Fas ligand (TNFSF6, FasL, CD95 ligand, Apo-1L), and TNF-α-related apoptosis-inducing ligand (TRAIL, TNFSF10). Receptors for the death ligands of the TNF family are type I membrane proteins that belong to a death receptor subgroup of the TNF receptor superfamily (TNF-R) (Ashkenazi and Dixit 1998). The TNF-R family proteins are characterized by the presence of cysteine-rich extracellular domains (CRDs) that mediate binding between ligands and these receptors. Based on the ability to transduce apoptotic signal these receptors can be divided into death receptors (DR) and decoy receptors (DcR). Highly conserved death domain (DD) motifs, responsible for conveying the death signal, are part of the intra-cytoplasmic portion of the death receptors. Death receptors comprise TNF-R1, Fas (TNFRSF6, Apo-1, CD95), DR4 (TNFRSF10A, TRAIL-R1, APO-2, MGC9365), DR5 (TNFRSF10B, TRAIL-R2, KILLER/DR5, TRICK2), DR3 and DR6 (Pan et al. 1997a, b; Sheridan et al. 1997; Walczak et al. 1997; Wu et al., 2000). TNF receptor family members that bind the death ligands but lack a functional DD and are unable to transduce the apoptotic signal are called decoy receptors: DcR1 (TNFRSF10C, TRAIL-R3, TRID), DcR2 (TNFRSF10D, TRAIL-R4, TRUNDD),

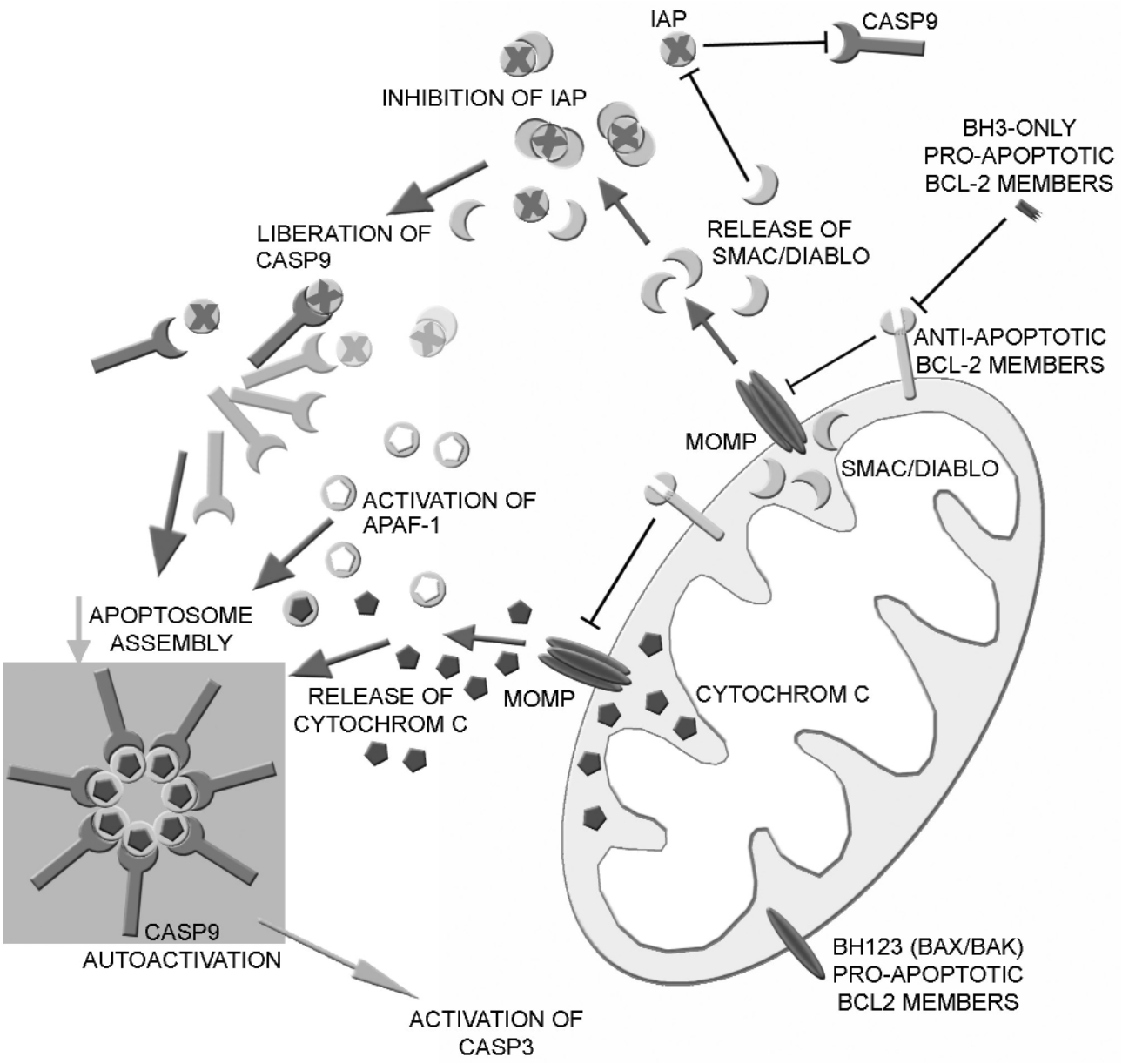

Fig. 1. Mitochondria-mediated/intrinsic apoptotic pathway overview. Various pro-apoptotic stimuli converge to MOMP with subsequent release of pro-apoptotic mediators. Some of the molecules (e.g. cytochrome c, Apaf-1) are directly involved in the assembly of the multiprotein apoptosis effector complex called „apoptosome" that activates caspase 9. Other molecules (e.g. SMAC/Diablo) consolidate the apoptotic signal propagation by blockage of apoptosis inhibitors (IAPs). See details in the text.

DcR3, osteoprotegerin (OPG) (Emery et al. 1998; Shipman and Croucher, 2003). Decoy receptors thus compete for specific death ligands with corresponding death receptors. The local concentration of death and decoy receptors might be critical for the sensitivity/resistance of cells to the death ligand-induced apoptosis (Ashkenazi and Dixit, 1998). TNF-R2 receptor represents an exception to the rule, as it does not contain a functional death domain, yet it remains a potent apoptosis inducer, via TRAF2 adaptor protein (MacEwan, 2002).

Members of the TNF ligand family (e.g. TNF-α, FasL, TRAIL) are arranged and function as stable homotrimers (Pitti et al., 1996; Hymowitz et al., 1999). The TNF ligand binding to the TNF receptor complex induces an activating conformational change or allows the formation of higher-order receptor complexes. The ligand-receptor binding leads to homophilic interaction of receptor-adaptor cytoplasmic death domains. The adaptor proteins Fas-associated death domain (FADD) or TNF-α-associated death domain (TRADD) allow recruitment of procaspase 8 (FADD-like interleukin-1-converting enzyme, FLICE) and 10 via homologous death effector domain (DED) motifs, which results in the formation of multiprotein death-inducing signalling complex (DISC) (Chinnaiyan et al., 1996; Kischkel et al., 2001; Seol et al., 2001; Kang et al., 2003). Procaspase

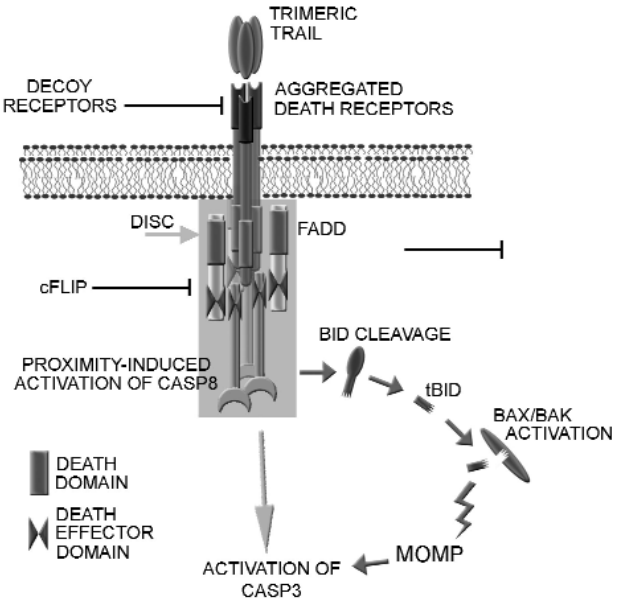

Fig. 2. Receptor-mediated/extrinsic apoptotic pathway overview. Ligation of death ligands to corresponding death receptors leads to aggregation of the receptors, which in turn provokes the assembly of multi-protein apoptosis effector called „death-inducing signalling complex" (DISC). DISC formation results in the proximity induced caspase 8/10 activation. Cross-talk between the extrinsic and intrinsic apoptotic pathways is effectuated by cleavage of Bcl-2 member Bid, which triggers mitochondrial damage. See details in the text.

8 (or 10) is autoactivated by a so-called proximity-induction mechanism. Substrates for fully assembled DISC are other procaspases such as procaspase 3 or Bid, the sentinel member of the Bcl-2 family (Chou et al., 1999; Metkar et al., 2003). Their cleavage represents a point of interconnection between the extrinsic and intrinsic apoptotic pathways.

Granzyme B/perforin apoptotic pathway

Apart from the two classical apoptotic pathways (intrinsic and extrinsic), there is at least one well-defined extrinsic accessory way of triggering apoptosis, the granzyme B pathway (Lord et al., 2003). Granzyme B is a lymphocyte granular enzyme (granzyme), serine protease, which specifically cleaves its substrates at Asp residues similarly to caspases. Granzymes are expressed by activated cytotoxic T lymphocytes (CTL) and natural killer (NK) cells (Trapani et al., 1994). Granzyme B binds its receptor, the mannose-6-phosphate/insulin-like growth factor II receptor, and is endocytosed but remains arrested in endocytic vesicles until released by perforin (Pinkoski et al., 1998; Trapani et al., 1998; Heibein et al., 1999; Barry et al., 2000; Motyka et al., 2000). Perforin-deficient mice thus are completely deficient in all granzyme-mediated cell-death pathways. In the cytoplasm, granzyme B directly cleaves and activates various procaspases, as well as other components of the apoptotic pathway (e.g. Bid), and downstream caspase targets (e.g. caspase-activated DNAse (CAD)) (Barry et al., 2000). Granzyme B-mediated cell death is still dependent on mitochondria disruption, since Bcl-2-overexpressing tumours are resistant to granzyme B-mediated apoptosis (Davis et al., 2000; Pinkoski et al., 2001; Metkar et al., 2003). Granzyme-mediated apoptosis is part of the immune surveillance mechanism where virus-infected and malignantly transformed cells are targeted by CTL and NK cells before widespread infection or cancer can ensue.

Ceramide/sphingomyelin pathways: essential counterpart to well-established apoptotic cascades

Ceramides are sphingolipid signalling mediators involved in the regulation of differentiation, growth suppression, cell ageing, various stress responses, and apoptosis (Jayadev et al., 1995; Obeid and Hannun, 1995; Hannun, 1996; Mathias et al., 1998). Diverse stress stimuli converge toward activation of sphingomyelinases (SMases), enzymes that cause sphingomyelin hydrolysis with generation of phosphatidylcholine and ceramide. Besides the cytoplasmic membrane, lysosome, ER, and mitochondria are also supposed to represent sites of ceramide generation. Increase in the intracellular level of ceramide results in apoptotic cell death programme induction via only incompletely understood molecular pathways that involve, at least in part, direct mitochondrial disruption (Ghafourifar et al., 1999; Green, 2000; Birbes et al., 2001; Siskind et al., 2002). Apart from directly triggering apoptosis, ceramide seems to play a much more important role in apoptotic process amplification/consolidation. In healthy cells, enzymes called phospholipid translocases maintain plasma membrane lipid asymmetry, with sphingomyelin on the outer leaflet, and phosphatidylserine/phosphatidylethanolamine on the inner leaflet of the plasma membrane. During the early phases of apoptosis, activated caspases inhibit the translocases and activate specific scramblases, both of which results in the plasma membrane lipid scrambling (i.e. phosphatidylserine externalization and sphingomyelin internalization). In addition to that, caspases also cleave and activate SMases. Internalized sphingomyelin hydrolysis by caspase-activated SMases then increases the cytosolic ceramide levels, which amplifies death signals (that initially resulted in caspases activation) by triggering counterpart ceramide-mediated apoptotic cascades. Furthermore, the loss of sphingomyelin from the plasma membrane (by hydrolysis) results in concomitant loss of cholesterol molecules with subsequent swift deterioration of plasma membrane fluidity, all of which contributes to the ultimate demise of the cell. Besides caspases, death receptors activated by corresponding death ligands (i.e. TNF-α, FasL, and TRAIL)

belong to potent SMase inducers as well, which only emphasizes the interdependence of various „well-established" apoptotic pathways (Tepper et al., 1995; Gulbins, 2003; Gajate et al., 2004; Lee and Amoscato, 2004; Watanabe et al., 2004; Miyaji et al., 2005).

Common apoptotic pathway: disintegration step by step

The final stages of apoptosis are characterized by typical morphological changes, such as DNA fragmentation, condensation of chromatin, proteolysis of structural proteins (lamins, actins, cytokeratins), and cytoplasmic membrane alterations, including membrane blebbing with apoptotic bodies forming from the surface of the shrinking cell (Enari et al., 1998; Sakahira et al., 1998; Nagata, 2000; Robertson et al., 2000). In the course of apoptosis, phosphatidylserine is exposed from the inner to the outer leaflet of the cytoplasmic membrane, which is recognized by phagocytes and leads to the engulfment of the apoptotic cell. The execution phase of apoptosis is carried out by cysteine proteases called caspases (cysteine aspartic acid–specific proteases) (Nunez et al., 1998; Earnshaw et al., 1999; Chen and Wang, 2002;). Caspases are synthesized as inactive procaspases (zymogens). Procaspases are activated by proteolytic cleavage. Caspases create a cascade (like the haemostatic one), in which the upstream caspases cleave and activate downstream caspases. Caspases can be divided into two groups: enzymes that participate in apoptosis (caspases 2, 3, 6, 7, 8, 9, and 10), and enzymes that play a role in cytokine processing and inflammation (caspases 1, 4, 5, 13, and 14). Apoptotic caspases can be further subdivided into initiator caspases (2, 8, 9, and 10) and effector caspases (3, 6, and 7). The initiator caspases are auto-activated during apoptosome and/or DISC formation at the initial phases of apoptosis by a proximity-induction mechanism and are responsible for the activation of effector caspases (Boatright et al., 2003). Effector caspases, in coordination with the initiator caspases, proteolytically cleave the cell structural proteins, mediators and regulators of apoptosis, cellular DNA repair proteins, and cell cycle-related proteins to execute the terminal phases of apoptosis. Various enzymes that are involved in cellular disintegration are activated by caspases, such as CAD, which is responsible for DNA fragmentation (DNA ladder), or gelsolin (actin-depolymerizing enzyme) and p21-activated kinase-2, both of which are responsible for cell membrane blebbing (Kass et al., 1996; Rao et al., 1996; Sahara et al., 1999; Daugas et al., 2000; Deschesnes et al., 2001; Li et al., 2001b; Wu et al., 2002; Kivinen et al., 2005). Caspases 3 and 7 can cleave and inactivate poly(ADP-ribose) polymerase (PARP), a DNA repair enzyme that is activated by DNA breaks, and catalyse the attachment of ADP-ribose polymers to acceptor proteins. The PARP repair function is associated with cell ATP store depletion, which may result in the switch from the programmed cell death, apoptosis, to energy-independent cell death, necrosis (Heibein et al., 1999; Motyka et al., 2000).

Molecular cross-talk between „individual" apoptotic pathways

It has become evident that there is an intensive cross-talk between the extrinsic (death receptors) and intrinsic (mitochondrial) pathways. The best characterized connection from the extrinsic to the intrinsic pathway is effectuated by the Bcl-2 family member Bid (BH3-interacting DD agonist). Bid is first cleaved by activated caspase-8 to yield truncated Bid (tBid). tBid then translocates to the mitochondrion where it binds and activates pro-apoptotic BH3-123 Bcl-2 members Bax/Bak. Hence, tBid cross-talk creates a pro-apoptotic mitochondrial amplification loop of the receptor-mediated apoptotic pathway (Kuwana et al., 1998; Li et al., 1998; Luo et al., 1998; Chou et al., 1999; Gross et al., 1999; Ruffolo et al., 2000) (Fig. 2). Cells that die after the activation of death receptors without the mitochondrial amplification are called type I cells. In other words, in type I cells activation of caspase 3 by caspase 8 appears sufficient for further propagation of the death signal. In type II cells, however, apoptosis transduction from death receptors fully relies on the tBid-mediated intrinsic pathway death signal amplification. Tumour type II cells, with a somatic knock out of Bax, held resistant to TRAIL-mediated apoptosis but could be sensitized to it by reintroducing Bax (Li et al., 1998; Chou et al., 1999; Ruffolo et al., 2000).

Another recently described cross-talk mechanism between the extrinsic and intrinsic apoptotic pathways represents the ceramide generation by death receptor-activated sphingomyelinases. Apart from triggering many diverse signalling pathways, ceramide directly interferes with the mitochondrion, where it induces MPT pore activation, which results in the MOMP, release of mitochondrial inter-membrane pro-apoptotic messengers and apoptotic cascade induction (see also above).

Besides tBid and ceramide, apoptotic pathways can influence each other through a plethora of molecular connections. Death receptors, for example, can trigger parallel signalling cascades, apart from apoptosis. Such pathways are initiated by distinct adaptor molecules, like receptor interacting protein (RIP), or members of the TNF receptor-associated factor family (TRAF). These adaptors represent upstream components of signal conduction pathways that lead to activation of many diverse protein kinases and/or transcription factors. Protein kinases, in turn, activate/block a variety of proteins by phosphorylation, while transcription factors induce/repress expression of a broad spectrum of genes. TRAF adaptors, for example, lead to activation of

nuclear factor kappa B (NFκB), a pivotal pro-survival transcription factor. TRAF can also represent upstream components of mitogen-activated protein kinase (MAPK) pathways, which comprise both the pro-survival extracellular signal-regulated kinase (Erk), and the stress-related c-Jun N-terminal kinase (JNK) and p38 MAPK pathways, respectively. The JNK and NFκB belong, in addition to the induction of apoptosis, to the most important signalling cascades triggered by ligand-activated death receptors. In general, the JNK pathway seems to exert primarily pro-apoptotic, while the NFκB pathway anti-apoptotic functions. Evidence suggests that the JNK cascade promotes Bax translocation to mitochondria through phosphorylation of the 14-3-3 protein, a cytoplasmic anchor of Bax (Tournier et al., 2000; Tsuruta et al., 2004). Moreover, activated JNK participates in posttranslational modification of Bid and Bim (Lin et al., 2000; Gabai et al., 2002; Deng et al., 2003). Translocation of NFκB to the nucleus induces transcription of NFκB target genes, including IAP (Jeremias et al., 1998). Interestingly, NFκB seems to exert its anti-apoptotic functions at least in part via inhibiting the JNK pathway (Tang et al., 2001; Deng et al., 2003). Thus, JNK and NFκB pathways represent another important extrinsic-to-intrinsic apoptotic pathway cross-talk.

On the other hand, many factors that trigger the inner apoptotic cascade modulate, to a certain degree, the receptor-mediated pathway. p53 tumour suppressor protein is a typical example of such intrinsic-to-extrinsic cross-talk (Haupt et al., 2003). Apart from binding directly to the mitochondrial membrane, p53 enhances transcription of genes coding for death receptors, which increases susceptibility of the cells to receptor-mediated programmed death (Schuler et al., 2000; Karawajew et al., 2005). A whole spectrum of other important transcription factors (c-Myc, c-Fos, c-Jun) appear to cross-talk in a similar way. Several cytotoxic drugs (anthracyclins, cis-platin, etoposid, 5-FU, etc.) then lead to upregulation of death receptors indirectly, by means of p53 activated by genotoxic damage (Marchenko et al., 2000; Haupt et al., 2003).

Regulation of apoptosis: interplay of proteins

The Bcl-2 (B-cell lymphoma 2) family of proteins probably represents the most important and best characterized group of apoptotic regulators (Kluck et al., 1997; Yang et al., 1997). The family can be divided into the anti-apoptotic and the pro-apoptotic group. The pro-apoptotic Bcl-2 proteins have developed during evolution as molecular signals corresponding to a plethora of stress impulses from the environment or from within the cell, and their major role lies in conveying the stress stimuli into the apoptotic pathway. The pro-apoptotic group can further be divided into a subgroup of proteins that contain in their structure only one Bcl-2 homology domain (Bcl-2 homology domain 3, BH3), called BH3-only proteins. BH3-only proteins comprise pro-apoptotic modulators Bad, Bid, Bik, Bim, Bmf, Puma, Noxa, etc. They act either to activate multidomain Bax and Bak members or to interfere with the anti-apoptotic Bcl-2 family (see later) (Letai et al., 2002; Kandasamy et al., 2003). Multidomain (BH-123) proteins Bax and Bak represent the latter, effector subgroup of Bcl-2 pro-apoptotic molecules (Wei et al., 2001). Activated Bax/Bak proteins translocate from the cytoplasm to the mitochondrial outer membrane, oligomerize, and by forming pores they precipitate MOMP, which leads to mitochondrial disruption and subsequent apoptosome assembly.

Anti-apoptotic members of the Bcl-2 family contain in their structure four Bcl-2 homology domains (BH1-BH4) and inactivate pro-apoptotic Bcl-2 proteins mainly via forming dimers with one another (Cheng et al., 2001). The anti-apoptotic Bcl-2 family consists of Bcl-2, Mcl-1, Bcl-xL, Bcl-w, Boo/Diva, A1, etc. The Bcl-2 protein is a key anti-apoptotic regulator (Kluck et al., 1997; Yang et al., 1997). It constitutively associates with both the mitochondrial and ER membranes.

The extrinsic pathway of apoptosis has a wide range of regulators as well. Decoy receptors together with cFLIP protein (FLICE-inhibitory protein) belong among the most potent ones (Muzio et al., 1996). cFLIP, a structural homologue of caspase 8 with enzymatically inactive pseudocaspase domain, acts as a dominant-negative competitive inhibitor of caspase 8. Decoy receptors compete for death ligands with death receptors, but lack death domains in their intracytoplasmic portion, thereby blocking the propagation of the apoptotic signal (Riccioni et al., 2005).

Heat-shock proteins (Hsps) constitute powerful modulators of both the mitochondrial and the receptor-mediated apoptotic cascades. Hsps represent a network of chaperone molecules, some of which are constitutively expressed but others rapidly induced in response to a variety of stress impulses from the environment or from within the cell. Hsps serve to disaggregate, refold and renature misfolded or aggregated proteins, which protects cells from several forms of death, including apoptosis (Hartl and Hayer-Hartl, 2002; Beere, 2005). In normal cells, the expression of most Hsps is low, but can be induced by stress due to misfolded or denatured proteins (e.g. heat shock, mutated proteins) (Mosser et al., 2000). Various members of the Hsp family (Hsp70, Hsp90) are abundantly expressed in tumours, where they contribute to cancer cell survival by stabilizing diverse aberrant proteins with direct or indirect anti-apoptotic activities. Hsp70 binds adaptor protein Apaf-1, which blocks the assembly of apoptosome. Hsp27 can successfully block the Smac/DIABLO pro-apoptotic enhancer. Hsp90 takes active part in the NFκB signalling pathway.

Table 1. Summary of the TNF-α superfamily death and decoy receptors with corresponding ligands and outline of their functions

Death / decoy receptor*	Death ligand/ alias(es)	Function
Death receptor family		
TNF-R1	TNF (-α)	Corresponds to DR1. Death receptor for TNF (tumour necrosis factor-α) and LTA (lymphotoxin-α). Binds only LTA homotrimers. Exerts pleiotropic functions in immune system regulation, inflammation, proliferation, apoptosis, etc.
	LTA/TNF-β	
Fas/Apo1	FasL/Apo1L	Corresponds to DR2. Death receptor for Fas ligand. Involved in peripheral deletion of autoreactive lymphocytes. Plays an important role in the tumour immune surveillance.
DR3/Apo3	TL1A	Death receptor for TL1A. Interaction with another TNF-family ligand APRIL has not been confirmed. Preferentially expressed by lymphocytes. Efficiently induced after T-cell activation.
DR4/TRAIL-R1/Apo2	TRAIL/Apo2L	Death receptor for TRAIL. Plays an important role in tumour immune surveillance.
DR5/TRAIL-R2	TRAIL/Apo2L	Death receptor for TRAIL. Plays an important role in tumour immune surveillance.
DR6	?	Orphan death receptor up to the present. Ligand still to be identified.
Other death-inducing receptors (that do not contain death domains)**		
TNF-R2	TNF (-α)	Receptor for TNF and LTA. Binds only LTA homotrimers. Exerts pleiotropic functions in immune system regulation, inflammation, proliferation, apoptosis, etc.
	LTA/TNF-β	
LTbetaR	LTB	Receptor for LTB (lymphotoxin-β) and LIGHT (homologous to Lymphotoxins, Inducible expression, competes with HSV Glycoprotein D for HVEM, a receptor expressed on T cells). Membrane-bound LTB forms on the cell surface heterotrimers with LTA, which otherwise exists only as a secreted cytokine. LTA/B heterotrimers cannot bind TNF-R1/2. LTB homotrimers have not been identified up to now.
	LIGHT	
Fn14	TWEAK	Receptor for TWEAK (TNF-like WEAK inducer of apoptosis). Besides inducing apoptosis in tumours it belongs to the principal regulators of angiogenesis.
Decoy receptor family		
TRAIL-R3/ DcR1	TRAIL	Decoy receptor for TRAIL. Lacks completely the cytoplasmic portion: tethered to the membrane by GPI anchor. Counters effects of TRAIL.
TRAIL-R4/ DcR2	TRAIL	Decoy receptor for TRAIL. Contains truncated (not functional) death domain. Counters effects of TRAIL.
DcR3	FasL	Soluble decoy receptor for FasL, LIGHT, and TL1A. Counters the effects elicited by FasL, LIGHT, and TL1A. Overexpressed in a variety of tumour cell lines and primary cells. Implicated in the progression and immune evasion of tumours.
	LIGHT	
	TL1A	
Other decoy-like receptors		
OPG	TRAIL	Soluble decoy ligand ("receptor") for TRAIL and RANKL (receptor-activator of NF κB ligand). Binds and neutralizes all activities elicited by RANKL-RANK and TRAIL-DR4/5 interactions.
	RANKL/OPGL/ TRANCE	

* DR = death receptor, DcR = decoy receptor.

** Death receptors induce death via death domains, other death-inducing TNF-R superfamily members induce death via recruiting the TRAF family of adaptor proteins.

Inhibitor of apoptosis proteins comprise a family of at least eight proteins (cIAP1, cIAP2, NAIP, XIAP, survivin, etc.) that function as caspase inhibitors, thereby blocking the effector phases of apoptosis (Deveraux and Reed, 1999; Yang and Li, 2000; Clem, 2001; Liston et al., 2003; Shi, 2004). In many *in vitro* studies overexpression of IAPs correlated tightly with the multiresistant phenotype. IAPs are capable of both inhibiting the cleavage of pro caspases and blocking the activated caspases. The natural inhibitor of IAPs, the Smac/DIABLO protein, is released together with cytochrome c from the mitochondria during intrinsic apoptosis induction, which leads to substantial enhancement of the death signal (Du et al., 2000).

The ubiquitin/proteasome system comprises a large proteinase complex that is responsible for the turnover of various intracellular proteins. Many of the key regulators of apoptosis are substrates of the proteasome, e.g. p53, NFκB, Bcl-2 family members Bax, Bad, and others. Hence, ubiquitin/proteasome belongs among indirect, yet extremely important players that interfere with death signals (Voorhees et al., 2003; Zhang et al., 2004).

Apart from the above-mentioned groups and families of proteins, a wide range of other molecules take part in direct or indirect modulation of the apoptotic process. Diverse cytokine receptors, protein kinases, transcription factors, cell cycle regulators and other diverse proteins form a complex net of interdependent signalling cascades with a myriad of mutual interconnections, feedbacks and loops with direct or indirect interference with programmed cell death. Several key pro-survival signalling pathways, such as PI3K (phosphatidylinositol-3 kinase)-Akt/PKB (protein kinase B), Ras-Raf-Mek-Erk MAPK, NFκB, or protein kinase C (PKC) pathways belong to extremely powerful inhibitors of programmed cell death machinery, while stress-related signaling pathways, e.g. JNK/p38 stress MAPK, or ROS/ceramide-mediated signalling, act to promote it (Kennedy et al., 1999; Suhara et al., 2002).

Conclusion

Through basic research, the major players of apoptosis have been identified. Classical chemotherapeutic agents induce cell death mainly through the mitochondrial apoptotic pathway, employing a variety of mechanisms. Treatment failure of leukaemia represents a failure of the malignant clone to undergo apoptosis in response to chemotherapeutic agents. The molecular basis of apoptosis resistance and treatment failure in leukaemia is only partially understood and is often caused by chromosomal translocations that result in aberrant gene expression. Attempts to develop novel therapeutic compounds focus on both the receptor-mediated and mitochondria-mediated pathways of apoptosis and on the development of molecules that specifically overcome blocks in the apoptosis pathway.

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