Cancer Immunol Immunother (2004) 53: 153–159
DOI 10.1007/s00262-003-0474-8

REVIEW

Klaus-Michael Debatin

Apoptosis pathways in cancer and cancer therapy

Received: 12 September 2003 / Accepted: 24 October 2003 / Published online: 29 January 2004

Abstract Activation of apoptosis pathways is a key mechanism by which cytotoxic drugs kill tumor cells. Also immunotherapy of tumors requires an apoptosis sensitive phenotype of target cells. Defects in apoptosis signalling contribute to resistance of tumors. Activation of apoptosis signalling following treatment with cytotoxic drugs has been shown to lead to activation of the mitochondrial (intrinsic) pathway of apoptosis. In addition, signalling through the death receptor (extrinsic) pathways, contributes to sensitivity of tumor cells towards cytotoxic treatment. Both pathways converge finally at the level of activation of caspases, the effector molecules in most forms of cell death. In addition to classical apoptosis, non-apoptotic modes of cell death have recently been identified. Mechanisms to overcome apoptosis resistance include direct targeting of antiapoptotic molecules expressed in tumors as well as re-sensitization of previously resistant tumor cells by re-expression of caspases and counteracting apoptotis inhibitory molecules such as Bcl-2 and molecules of the IAP family of endogenous caspase inhibitors. Molecular insights into regulation of apoptosis and defects in apoptosis signalling in tumor cells will provide novel approaches to define sensitivity or resistance of tumor cells towards antitumor therapy and provide new targets for rational therapeutic interventions for future therapeutic strategies.

Keywords Apoptosis · Cancer therapy · Resistance mechanisms · Cytotoxic drugs · Immunotherapy · Apoptosis defects

This work was presented at the first Cancer Immunology and Immunotherapy Summer School, 8–13 September 2003, Ionian Village, Bartholomeio, Peloponnese, Greece.

K.-M. Debatin
Department of Medicine,
University of Ulm,
89069 Ulm, Germany
E-mail: klaus-michael.debatin@medizin.uni-ulm.de
Tel.: +49-731-50027701

Introduction

Killing of tumor cells by diverse cytotoxic approaches such as anticancer drugs, γ-irradiation, suicide genes, or immunotherapy has been shown to be mediated through induction of apoptosis in target cells [1, 2, 3, 4, 5, 6, 7]. Apoptosis or programmed cell death is a distinct, intrinsic cell death program that occurs in various physiological and pathological situations [8]. The underlying mechanism for initiation of an apoptosis response upon cytotoxic therapy may vary for different stimuli and is only partially understood. However, damage to DNA or to other critical molecules and/or subcellular structures appears to be a common early hit by some inducers which is then propagated by the cellular stress response [9]. Multiple stress-inducible molecules—e.g., INK, MAPK/ERK, NFkB, or ceramide—may have a profound impact on apoptosis pathways [2, 10, 11, 12]. On the other hand, cytotoxic T cells or NK cells may release compounds such as granzyme B which directly activates downstream apoptosis effector mechanisms inside the cell [8]. Apoptosis is characterized by typical morphological and biochemical hallmarks including cell shrinkage, nuclear DNA fragmentation, and membrane blebbing [8]. Protelolytic enzymes such as caspases play an important role as effector molecules in apoptosis including cytotoxic therapy-induced cell death [13, 14, 15, 16, 17, 18, 19]. Because of the potentially detrimental effects on cell survival in case of inappropriate caspase activity, activation of caspases has to be tightly controlled. The antiapoptotic mechanisms regulating activation of caspases have also been postulated to be involved in drug resistance to tumor cells [20, 21, 22]. However, the concept that anticancer therapies primarily act by triggering apoptosis has also been challenged, since a consistent link between the ability of tumor cells to undergo apoptosis in vitro and their susceptibility to anticancer therapy in vivo has not always been observed [23]. Therefore, nonapoptotic modes of cell death—e.g., necrosis or some forms of cell

death that cannot be easily classified at present—may mediate the cell death response to cytotoxic therapy [24, 25]. Also, non-caspase-dependent apoptosis has been found to be induced by anticancer drugs in some cells [26, 27, 28]. Thus, a better understanding of these diverse modes of tumor cell death following cytotoxic therapies will provide a molecular basis for new strategies targeting caspase-dependent and independent death pathways in apoptosis-resistant forms of cancer.

Mechanisms of apoptosis

Most signaling pathways activated by anticancer drugs ultimately result in activation of caspases, a family of cysteine proteases that act as common death effector molecules in various forms of cell death [8, 13, 14, 15, 16, 17, 18]. Caspases are synthesized as inactive zymogens and activated by proteolytic cleavage—i.e., cleavage between the large and the small subunit followed by cleavage between the large subunit and the prodomain [13, 14, 15, 16, 17, 18]. Caspases involved in apoptosis signaling are currently categorized into initiator and effector caspases, respectively [13, 14, 15, 16, 17, 18]. Initiator caspases transduce various signals into protease activity and are directly linked to death-inducing signaling complexes (DISCs): caspase-8 or caspase-10 via their death effector domain (DED) interact with adaptor proteins (FADD), recruited and bound to activated death receptors, while caspase-9 is recruited to the apoptosome via its CARD domain [13]. Effector caspases cleave various cytoplasmatic or nuclear substrates, which mark many of the morphologic features of apoptotic cell death [13, 14, 15, 16, 17, 18]. For example, polynucleosomal DNA fragmentation is initiated by cleavage of ICAD (inhibitor of caspase-activated DNase), the inhibitor of the endonuclease CAD (caspase-activated DNase) that cleaves DNA into the characteristic oligomeric fragments [8]. DNA condensation is caused by AIF, a mitochondrial protein that translocates to the nucleus upon death triggering, and by Acinus, which stands for "apoptotic chromation condensation inducer in the nucleus" [29, 30]. AIF may also mediate caspase-independent cleavage of DNA into larger fragments [29, 30]. Likewise, loss of overall cell shape is due to proteolysis of cytoskeletal proteins including fodrin, gelsolin, actin, plectrin, and cytokeratin, while nuclear shrinking and budding occurs after degradation of lamin [8].

Activation of caspases can principally be triggered by two different mechanisms. According to the induced proximity model, initiator caspases such as caspase-8 or caspase-9 are activated in a multimeric complex—e.g., caspase-8 in the death-inducing signaling complex (DISC) and caspase-9 in the apoptosome [8, 13, 31, 32, 33]. Alternatively, caspases are activated by catalytic processing of the zymogens at specific cleavage sites [13]. Caspase activation can be initiated through different entry sites—e.g., at the plasma membrane by death receptor mediated signaling (receptor pathway) or at the mitochondria (mitochondrial pathway) [8, 13]. Stimulation of death receptors of the tumor necrosis factor (TNF) receptor superfamily such as CD95 (APO-1/Fas) or TRAIL receptors results in receptor aggregation and recruitment of the adaptor molecule Fas-associated death domain (FADD) and capase-8 [31, 32, 33]. Upon recruitment, caspase-8 becomes activated and initiates apoptosis by direct cleavage of downstream effector caspases [31, 32, 33]. The mitochondrial pathway is initiated by the release of apoptogenic factors such as cytochrome *c*, apoptosis-inducing factor (AIF), Smac/Diablo, Omi/HtrA2, endonuclease G, caspase-2 or caspase-9 from the mitochondrial intermembrane space [34, 35, 36, 37, 38, 39]. The release of cytochrome *c* into the cytosol triggers caspase-3 activation through formation of the cytochrome-*c*/Apaf-1/caspase-9–containing apoptosome complex [40, 41]. Smac/Diablo and Omi/HtrA2 promote caspase activation by neutralizing the inhibitory effects on IAPs, while AIF and endonuclease G cause DNA condensation [30, 38, 39, 42, 43].

The receptor and the mitochondrial pathway can be interconnected at different levels [44]. Following death receptor stimulation, activation of caspase-8 may result in cleavage of Bid, a BH3 domain containing protein of the Bcl-2 family, which assumes cytochrome-*c*–releasing activity upon cleavage, thereby initiating a mitochondrial amplification loop [43, 44]. In addition, mitochondria-triggered caspase-6 cleavage may feed back to the receptor pathway by cleaving caspase-8 [16].

Signaling pathways in cancer therapy

Apoptosis in response to cancer therapy proceeds through activation of the core apoptotic machinery including the receptor and the mitochondrial signaling pathway [2, 3, 4,]. The relative contribution of the receptor and the mitochondrial pathway to drug-induced apoptosis has been a subject of controversial discussion [2, 3, 4,]. While a number of initial studies suggested that cancer therapy–triggered apoptosis involves activation of the CD95 receptor/ligand system, compelling evidence subsequently indicated that the majority of cytotoxic drugs initiate cell death by triggering the cytochrome-*c*/Apaf-1/caspase-9–dependent pathway through the mitochondria [45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68]. To this end, targeted disruption of genes involved in the mitochondrial pathway points to a crucial and indispensable role of this pathway for apoptosis in response to anticancer drug treatment [66, 67]. Caspase-$9^{-/-}$ embryonic stem cells and Apaf-$1^{-/-}$ thymocytes are resistant to cytotoxic drugs but remain sensitive to death receptor triggering [66, 67]. In contrast, $FADD^{-/-}$ and caspase-$8^{-/-}$ embryonic fibroblasts are refractory to death receptor stimulation, but

equally sensitive to cytotoxic drugs suggesting that the death receptor pathway has a dispensable role in drug-induced apoptosis, at least in these particular non-transformed cells [64, 65]. However, the relative contribution of the death receptor versus the mitochondrial pathway may depend on the cytotoxic drug, dose, and kinetics, or on differences between certain cell types similar to the cell type-dependent signaling in the CD95 pathway [52, 68]. Importantly, this amplification of the chemoresponse through activation of the CD95 system may be clinically meaningful, since it may critically affect the time required for execution of the death program. Multiple proapoptotic and antiapoptotic signaling paths as discussed below regulate the net outcome of signaling through the core apoptotic machinery [2, 4].

Proapoptotic signaling in cancer therapy

Caspases

Given the important role of caspases as effector molecules in various forms of cell death including drug-induced apoptosis, the ability of anticancer agents to trigger caspase activation appears to be a critical determinant of sensitivity or resistance to cytotoxic therapies [2, 4]. As a consequence, inhibition of caspase activation may be an important factor in chemoresistance [2, 4].

Expression levels of individual caspases may have an impact on their overall activity, since deficient expression levels of caspases may simply impair activation of caspases [69, 70, 71]. For example, MCF-7 breast carcinoma cells completely lack caspase-3 expression due to a frameshift mutation within exon 3 of the caspase-3 gene [70]. These cells can be sensitized by transfection of pro-caspase-3 toward treatment with cytotoxic drugs [71]. Moreover, caspase expression may be impaired by epigenetic alterations such as promoter hypermethylation. Accordingly, caspase-8 expression was found to be frequently inactivated by hypermethylation of regulatory sequences of the caspase-8 gene in a number of different tumor cells derived from neuroblastoma, malignant brain tumors, Ewing tumors, and small lung cell carcinoma both in vitro and also in vivo in primary tumor samples [72, 73]. Importantly, restoration of caspase-8 expression by gene transfer or by demethylation treatment, sensitized resistant tumor cells to death receptor–induced or drug-induced apoptosis [73]. Conversely, enhanced transcription of caspase genes in response to cytotoxic treatment may increase expression levels [74]. Thus, treatment with IFN-γ resulted in enhanced expression of caspase proteins mediated by direct activation of Stat-1, a downstream transcription factor involved in IFN-γ signaling [75]. Moreover, transcriptional upregulation of caspase-3 or caspase-8 was found upon drug treatment independent of Stat-1 [76].

Antiapoptotic signaling in cancer therapy

Bcl-2 proteins

Bcl-2 family proteins play a pivotal role in the regulation of the mitochondrial pathway, since these proteins localize to intracellular membranes, in particular the mitochondrial membrane [77]. They comprise both antiapoptotic members—e.g., Bcl-2, Bcl-X_L, and Mcl-1—as well as proapoptotic molecules such as Bax, Bek, and Bad, and BH3 domain-only molecules which link the death receptor pathway to the mitochondrial pathway (Bid, Bim, Puma, and Noxa) [77]. Upon apoptosis induction, proapoptotic Bcl-2 proteins with multidomains such as Bax translocate from the cytoplasm to the outer mitochondrial membrane, where they oligomerize to form a pore-like structure, thereby promoting cytochrome-*c* release [78]. This translocation to mitochondria can be triggered by Bcl-2 proteins, which have a BH3 domain only [77]. BH3 domain-only proteins include Bid, which is activated by caspase-8–mediated cleavage; Bim, a microtubule-associated protein, or Noxa and PUMA, two p53-induced proteins [77]. Bcl-2 or Bcl-X_L exert their antiapoptotic function, at least in part, by sequestering BH 3 domain-only proteins in stable mitochondrial complexes, thereby preventing activation and translocation of Bax or Bak to mitochondria [78]. In addition, Bcl-2 and Bcl-X_L block apoptosis by preventing cytochrome-*c* release through a direct effect on mitochondrial channels such as the voltage-dependent anion channel (VDAC) or the permeability transition pore complex (PTPC) [35, 36]. Mutations or altered expression of proapoptotic or antiapoptotic Bcl-2 family proteins can drastically alter drug response in experimental systems [79]. In addition, several clinical correlative studies have provided support that high level expression of antiapoptotic Bcl-2 proteins confers a clinically important chemoresistant phenotype on cancer cells, including AML, ALL, CLL, multiple myeloma, prostate carcinoma, malignant brain tumors, and neuroblastoma [22, 80, 81, 82]. Likewise, reduced Bax levels have been associated with poor responses to chemotherapy and shorter overall survival in breast or colorectal carcinoma [83]. Conversely, enhanced Bax levels correlated in several cell types with response to chemotherapy in vivo [84].

Inhibitor of apoptosis proteins (IAPs)

Inhibitor of apoptosis proteins (IAPs) have been reported to directly inhibit active caspase-3 and caspase-7 and to block caspase-9 activation [85, 86, 87]. In addition to regulation of apoptosis, IAP members such as survivin have been found to be involved in the regulation of mitosis [87]. The activity of IAPs are controlled at various levels—e.g., by the transcription factor NFkB that has been reported to stimulate expression of cIAP1, cIAP0, and XIAP [85]. IAPs are negatively regulated by

caspase-mediated cleavage [85]. In addition, Smac/Diablo and Omi, two proteins released from mitochondria upon apoptosis induction, neutralize IAPs through binding to IAPs thereby displacing them from their caspase partners [86]. Likewise, XAF1 has been found to displace IAPs from bound caspases in the nucleus [86]. Inhibition of apoptosis by IAPs in response to cytotoxic therapy has been suggested by several experimental studies [88, 89, 90, 91]. XIAP, cIAP1, or cIAP2 suppressed apoptosis in vitro following treatment with cisplatin, cytarabine, TRAIL, or staurosporine, or after γ-irradiation [92, 93]. Also, increased IAP expression correlated with poor treatment response in myeloid leukemia cells, and elevated survivin expression predicted adverse prognosis in several tumors—e.g., neuroblastoma, AML, colon, lung and esophagus carcinoma [88, 89, 90, 91].

The discovery that the IAP-inhibitor Smac/Diablo, one of the photogenic factors released from mitochondria, is required to fully activate caspases in many instances, has prompted interest in expressing Smac/Diablo in the erythroplasma of tumor cells to overcome IAP-mediated inhibition of apoptosis induction in tumors. Thus, cytoplasmic localization of Smac by transfection-enforced overexpression or by using cell permeable peptides as Smac agonists has shown efficacy in sensitizing previously resistant tumors to apoptosis induction [92, 93].

NFkB

The ability of the transcription factor NFkB to suppress apoptosis is thought to confer resistance to cytotoxic therapies [96]. As outlined above, NFkB may be constitutively active in certain tumor types such as pancreatic carcinoma [96]. In addition, NFkB activity is induced in response to a variety of stimuli—e.g., in response to cellular stress and anticancer agents [96]. NFkB is composed of heterodimers or homodimers of the NFkB/Rel family of proteins, which mediate protein dimerization, nuclear import, and specific DNA binding [96]. In most cell types, NFkB is sequestered in the cytoplasm by its interaction with IkB proteins and therefore remains inactive [96]. Upon stimulation, IkB becomes phosphorylated following activation of the IKK complex and is degraded via the proteasome, thereby releasing NFkB to translocate into the nucleus for transcription of target genes [96]. NFkB target genes include several antiapoptotic proteins—e.g., cIAP1, cIAP2, TRAF1, TRAF2, Bfl-1/A1, Bcl-X_L, and FLIP [96]. Interestingly, NFkB also controls promoter activation of certain proapoptotic factors such as CD95L, CD95, TRAIL-R1, and TRAIL-R2, an observation consistent with reports that NFkB can promote apoptosis under certain circumstances [96]. The NFkB-signaling pathway has been linked to death receptor signaling because RIP, which serves as an adaptor molecule for the TNFRI receptor to the NFkB pathway, can be cleaved by caspases, thereby modulating the balance between proapoptotic and antiapoptotic signals upon TNF receptor signaling and may even stimulate an autocrine "death loop" [96]. Since certain types of anticancer treatments result in induction of NFkB transcriptional activity, inhibition of NFkB in parallel with chemotherapy strongly enhanced the cytotoxic effect of chemotherapy [96]. Thus, NFkB may play an important role in inducible chemoresistance, and inhibition of NFkB may serve as a potential new adjuvant approach to chemotherapy [2, 4, 96].

Caspase-independent and nonapoptotic modes of cell death

Although a large body of data points to an essential role of caspase-dependent apoptosis in mediating tumor cell death upon cytotoxic therapy, this concept has also been challenged [1, 2, 3, 4, 5, 6, 7, 23]. Thus, a clear, consistent link between the cells' ability to undergo apoptosis and their susceptibility to anticancer therapy could not be observed [23]. In addition, p53 status did not always correlate with the ability of a tumor cell to respond to treatment [97]. Cells harboring wild-type p53 may fail to respond, and those lacking functional p53 may even respond better [97]. Thus, "nonclassical apoptosis," or nonapoptotic modes of cell death—e.g., necrosis or some forms of cell death that cannot be easily classified at present—have also been taken into consideration as a response to cytotoxic therapy [23, 24, 25, 26, 27, 28]. Also, delayed suppression of tumors upon irradiation, for example, has been taken as evidence against a predominant apoptotic mode of cell death, since apoptosis appears to be induced fairly rapidly in vitro and in vivo upon appropriate stimulation [23]. Although the signaling pathways and molecules involved in these alternative forms of cell death have not yet been exactly defined, noncaspase proteases such as calpains and cathepsins, Bax or Bax-like molecules, and AIF or endonuclease G may be involved [8, 23, 24, 25, 26, 27, 28]. The relative contribution of these different modes of cell death for chemoresponses in vitro and in vivo remains to be defined.

Conclusion

Numerous studies over the last years have indicated that anticancer therapies primarily act by activating the apoptosis response pathway in tumor cells [1, 2, 3, 4, 5, 6, 7]. However, several points remain to be addressed in future studies: Firstly, most of the apoptosis signaling components have not been studied in clinical samples [2, 4, 6, 7, 22]. Secondly, while many experimental studies indicate that alterations in components of the apoptotic machinery have an impact on sensitivity of tumor cells toward cytotoxic therapy, this premise remains to be tested in clinical settings [1, 2, 3, 4, 5, 22]. Moreover, the

biology that determines the individual responses of different tumors to cytotoxic therapies warrants further investigation to provide the basis for more specific therapeutic interventions. In this context, the multiple connections between death receptor signaling and the cellular stress response and even cytokine mediated modulation of pathways may be particularly important in vivo [98, 99, 100].

Finally, the concept that "classical" apoptosis represents the major mechanism by which tumor cells are eliminated by cytotoxic therapies may not universally apply and thus caspase-independent modes of cell death should also be considered [23, 24, 25, 26, 27, 28]. Nonetheless, studies on the regulation of apoptosis signaling pathways triggered by anticancer therapies have provided substantial insights into the molecular mechanisms regulating the response of tumor cells toward current therapies. Future studies on the role of apoptosis signaling molecules in individual tumors both in vitro and in vivo in tumor cells of patients undergoing chemotherapy—e.g., using DNA microarrays or proteomic studies—may provide the basis for "tailored" tumor therapy and may identify new targets for therapeutic intervention.

In addition to the identification of novel therapeutic strategies, it is expected that a more in-depth understanding of apoptosis signaling will help us to molecularly guide established therapies using chemotherapeutical drugs and irradiation. Altering the thresholds of tumor cells to apoptosis induction by interference with apoptosis resistance to sensitize tumor cells to established therapy protocols is expected to contribute to progress in cancer therapy in the future.

References

1. Debatin KM (1999) The role of the CD95 system in chemotherapy. In: Broxterman HJA (ed) Drug resistance updates. Churchill Livingstone, Edinburgh, pp 85–90
2. Herr I, Debatin KM (2001) Cellular stress response and apoptosis in cancer therapy. Blood 98:2603–2614
3. Debatin, KM (1997) Anticancer drugs, programmed cell death and the immune system: defining new roles in an old play. J Natl Cancer Inst 89:750–753
4. Kaufmann SH, Earnshaw WC (2000) Induction of apoptosis by cancer chemotherapy. Exp Cell Res 256:42–49
5. Solary E, Droin N, Bettaieb A, Corcos L, Dimanche-Boitrel MT, Garrido C (2000) Positive and negative regulation of apoptotic pathways by cytotoxic agents in hematological malignancies. Leukemia 14:1833–1849
6. Lowe SW, Lin AW (2000) Apoptosis in cancer. Carcinogenesis 21:485–495
7. Kaufmann SH, Gores GJ (2000) Apoptosis in cancer: cause and cure. Bioessays 22:1007–1017
8. Hengartner MO (2000) The biochemistry of apoptosis. Nature 407:770–777
9. Rich T, Allen RL, Wyllie AH (2000) Defying death after DNA damage. Nature 407:777–783
10. Leppa S, Bohmann D (1999) Diverse functions of JNK signaling and c-Jun in stress response and apoptosis. Oncogene 18:6158–6162
11. Davis RJ (2000) Signal transduction by the JNK group of MAP kinases. Cell 103:239–252
12. Mayo MW, Baldwin AS (2000) The transcription factor NF-kB: control of oncogenesis and cancer therapy resistance. Biochim Biophys Acta 1470:M55–M62
13. Thornberry N, Lazebnik Y (1998) Caspases: enemies within. Science 281:1312–1316
14. Los M, Wesselborg S, Schulze-Osthoff K (1999) The role of caspases in development, immunity, and apoptotic signal transduction: lessons from knockout mice. Immunity 10:629–639
15. Degen WGJ, Pruijn GJM, Raats JMH, van Venrooij WJ (2000) Caspase-dependent cleavage of nucleic acids. Cell Death Differ 7:616–627
16. Slee EA, Adrain C, Martin SJ (1999) Serial killers: ordering caspase activation events in apoptosis. Cell Death Differ 6:1067–1074
17. Utz PJ, Anderson P (2000) Life and death decisions: regulation of apoptosis by proteolysis of signaling molecules. Cell Death Differ 7:589–602
18. Earnshaw WC, Martins LM, Kaufmann SH (1999) Mammalian caspases: structure, activation, substrates, and functions during apoptosis. Annu Rev Biochem 68:383–424
19. Kaufmann SH (1989) Induction of endonucleolytic DNA cleavage in human acute myelogenous leukemia cells by etoposide, camptothecin, and other cytotoxic anticancer drugs: a cautionary note. Cancer Res 49:5870–5878
20. Los M, Herr I, Friesen C, Fulda S, Schulze-Osthoff K, Debatin KM (1997) Cross-resistance of CD95- and drug-induced apoptosis as a consequence of deficient activation of caspases (ICE/Ced-3 proteases). Blood 90:3118–3129
21. Goyal L (2001) Cell death inhibition: keeping caspases in check. Cell 104:805–808
22. Reed JC (1999) Dysregulation of apoptosis in cancer. J Clin Oncol 17:2941–2953
23. Finkel E (1999) Does cancer therapy trigger cell suicide? Science 286:2256–2258
24. Sperandio S, de Belle I, Bredesen DE (2001) An alternative, nonapoptotic form of programmed cell death. Proc Natl Acad Sci U S A 7:14376–14381
25. Wyllie AH, Golstein P (2000) More than one way to go. Proc Natl Acad Sci U S A 93:11–13
26. Borner C, Monney L (1999) Apoptosis without caspases: an inefficient molecular guillotine? Cell Death Differ 6:497–507
27. Johnson DE (2000) Noncaspase proteases in apoptosis. Leukemia 14:1695–1703
28. Leist M, Jaattela M (2001) Four deaths and a funeral: from caspases to alternative mechanisms. Nat Rev Mol Cell Biol 2:589–598
29. Ferri KF, Kroemer G (2000) Control of apoptotic DNA degradation. Nat Cell Biol 2:E63–E64
30. Daugas E, Nochy D, Ravagnan L, Loeffler M, Susin SA, Zamzami N, Kroemer G (2000) Apoptosis-inducing factor (AIF): a ubiquitous mitochondrial oxidoreductase involved in apoptosis. FEBS Lett 476:118–123
31. Schulze-Osthoff K, Ferrari D, Los M, Wesselborg S, Peter ME (1998) Apoptosis signaling by death receptors. Eur J Biochem 4:439–459
32. Krammer PH (2000) CD95's deadly mission in the immune system. Nature 407:789–95
33. Walczak H, Krammer PH (2000) The CD95 (APO-1/Fas) and the TRAIL (APO-2L) apoptosis systems. Exp Cell Res 256:58–66
34. Costantini P, Jacotot E, Decaudin D, Kroemer G (2000) Mitochondrion as a novel target of anticancer chemotherapy. J Natl Cancer Inst 92:1042–1053
35. Kroemer G, Reed JC (2000) Mitochondrial control of cell death. Nat Med 6:513–519
36. Martinou JC, Green DR (2001) Breaking the mitochondrial barrier. Nat Rev Mol Cell Biol 2:63–67
37. Suzuki Y, Imai Y, Nakayama H, Takahashi K, Takio K, Takahashi R (2001) A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. Mol Cell 8:613–621

38. Du C, Fang M, Li Y, Li L, Wang X (2000) Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell 102:33–42
39. Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE, Moritz RL, Simpson RJ, Vaux DL (2000) Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell 102:43–53
40. Bratton SB, MacFarlane M, Cain K, Cohen GM (2000) Protein complexes activate distinct caspase cascades in death receptor and stress-induced apoptosis. Exp Cell Res 256:27–33
41. Adrain C, Martin SJ (2001) The mitochondrial apoptosome: a killer unleashed by the cytochrome seas. Trends Biochem Sci 26:390–397
42. Li LY, Luo X, Wang X (2001) Endonuclease G is an apoptotic DNase when released from mitochondria. Nature 412:95–99
43. Schimmer AD, Hedley DW, Penn LZ, Minden MD (2001) Receptor- and mitochondrial-mediated apoptosis in acute leukemia: a translational view. Blood 98:3541–3553
44. Roy S, Nicholson DW (2000) Cross-talk in cell death signaling. J Exp Med 192:21–26
45. Friesen C, Herr I, Krammer PH, Debatin KM (1996) Involvement of the CD95 (APO-1/FAS) receptor/ligand system in drug-induced apoptosis in leukemia cells. Nat Med 2:574–577
46. Friesen C, Fulda S, Debatin KM (1999) Drugs and the CD95 pathway. Leukemia 13:1854–1858
47. Fulda S, Sieverts H, Friesen C, Herr I, Debatin KM (1997) The CD95 (APO-1/Fas) system mediates drug-induced apoptosis in neuroblastoma cells. Cancer Res 7:3823–3829
48. Fulda S, Los M, Friesen C, Debatin KM (1998) Chemosensitivity of solid tumor cells is associated with activation of the CD95 system. Int J Cancer 76:105–114
49. Fulda S, Scaffidi C, Pietsch T, Krammer PH, Peter ME, Debatin KM (1998) Activation of the CD95 (APO-1/Fas) pathway in drug- and γ-irradiation-induced apoptosis of brain tumor cells. Cell Death Differ 5:884–893
50. Fulda S, Susin SA, Kroemer G, Debatin KM (1998) Molecular ordering of apoptosis induced by anticancer drugs in neuroblastoma cells. Cancer Res 58:4453–4460
51. Fulda S, Strauss G, Meyer E, Debatin KM (2000) Functional CD95 ligand and CD95 DISC in activation-induced cell death and doxorubicin-induced apoptosis in leukemic T cells. Blood 95:301–308
52. Fulda S, Meyer E, Susin SA, Kroemer G, Debatin KM (2001) Cell type specific activation of death receptor and mitochondrial pathways in drug-induced apoptosis. Oncogene 20:1063–1075
53. Herr I, Wilhelm D, Bohler T, Angel P, Debatin KM (1997) Activation of CD95 (APO-1/Fas) signaling by ceramide mediates cancer therapy-induced apoptosis. EMBO J 16:6200–6208
54. Houghton JA, Harwood FG, Tillman DM (1997) Thymineless death in colon carcinoma cells is mediated via fas signaling. Proc Natl Acad Sci U S A 94:8144–8149
55. Muller M, Wilder S, Bannasch D, Israeli D, Lehlbach K, Li-Weber M, Friedman SL, Galle PR, Stremmel W, Oren M, Krammer PH (1998) p53 activates the CD95 (APO-1/Fas) gene in response to DNA damage by anticancer drugs. J Exp Med 188:2033–2045
56. Muller M, Strand S, Hug H, Heinemann EM, Walczak H, Hofmann WJ, Stremmel W, Krammer PH, Galle PR (1997) Drug-induced apoptosis in hepatoma cells is mediated by the CD95 (APO-1/Fas) receptor/ligand system and involves activation of wild-type p53. J Clin Invest 99:403–413
57. Kasibhatla S, Brunner T, Genestier L, Echeverri F, Mahboubi A, Green DR (1998) DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-kappa B and AP-1. Mol Cell 1:543–551
58. Eichhorst ST, Muller M, Li-Weber M, Schulze-Bergkamen H, Angel P, Krammer PH (2000) A novel AP-1 element in the CD95 ligand promoter is required for induction of apoptosis in hepatocellular carcinoma cells upon treatment with anticancer drugs. Mol Cell Biol 20:7826–7837
59. Eichhorst ST, Muerkoster S, Weigand MA, Krammer PH (2001) The chemotherapeutic drug 5-fluorouracil induces apoptosis in mouse thymocytes in *vivo* via activation of the CD95 (APO-1/Fas) system. Cancer Res 61:243–248
60. Beltinger C, Fulda S, Kammertoens T, Meyer E, Uckert W, Debatin KM (1999) Herpes simplex virus thymidine kinase/ganciclovir-induced apoptosis involves ligand-independent death receptor aggregation and activation of caspases. Proc Natl Acad Sci U S A 96:8699–8704
61. Landowski TH, Shain KH, Oshiro MM, Buyuksal I, Painter JS, Dalton WS (1999) Myeloma cells selected for resistance to CD95-mediated apoptosis are not cross-resistant to cytotoxic drugs: evidence for independent mechanisms of caspase activation. Blood 94:265–274
62. Eischen CM, Kottke TJ, Martins LM, Basi GS, Tung JS, Earnshaw WC, Leibson PJ, Kaufmann SH (1997) Comparison of apoptosis in wild-type and Fas-resistant cells: chemotherapy-induced apoptosis is not dependent on Fas/Fas ligand interactions. Blood 90:935–943
63. Villunger A, Egle A, Kos M, Hartmann BL, Geley S, Kofler R, Greil R (1997) Drug-induced apoptosis is associated with enhanced Fas (Apo-1/CD95) ligand expression but occurs independently of Fas (Apo-1/CD95) signaling in human T-acute lymphatic leukemia cells. Cancer Res 57:3331–3334
64. Yeh WC, Pompa JL, McCurrach ME, Shu HB, Elia AJ, Shahinian A, Ng M, Wakeham A, Khoo W, Mitchell K, El-Deiry WS, Lowe SW, Goeddel DV, Mak TW (1998) FADD: essential for embryo development and signaling from some, but not all, inducers of apoptosis. Science 279:1954–1958
65. Varfolomeev EE, Schuchmann M, Luria V, Chiannilkulchai N, Beckmann JS, Mett IL, Rebrikov D, Brodianski VM, Kemper OC, Kollet O, Lapidot T, Soffer D, Sobe T, Avraham KB, Goncharov T, Holtmann H, Lonai P, Wallach D (1998) Targeted disruption of the mouse Caspase 8 gene ablates cell death induction by the TNF receptors, Fas/Apo1, and DR3 and is lethal prenatally. Immunity 9:267–276
66. Hakem R, Hakem A, Duncan GS, Henderson JT, Woo M, Soengas MS, Elia A, de la Pompa JL, Kagi D, Khoo W, Potter J, Yoshida R, Kaufman SA, Lowe SW, Penninger JM, Mak TW (1998) Differential requirement for caspase 9 in apoptotic pathways in vivo. Cell 94:339–352
67. Yoshida H, Kong YY, Yoshida R, Elia AJ, Hakem A, Hakem R, Penninger JM, Mak TW (1998) Apaf1 is required for mitochondrial pathways of apoptosis and brain development. Cell 94:739–750
68. Scaffidi C, Fulda S, Srinivasan A, Friesen C, Li F, Tomaselli KJ, Debatin KM, Krammer PH, Peter ME (1998) Two CD95 (APO-1/Fas) signaling pathways. EMBO J 17:1675–1687
69. Joseph B, Ekedahl J, Sirzen F, Lewensohn R, Zhivotovsky B (1999) Differences in expression of pro-caspases in small cell and non-small cell lung carcinoma. Biochem Biophys Res Commun 262:381–387
70. Janicke RU, Sprengart ML, Wati MR, Porter AG (1998) Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. J Biol Chem 273:9357–9360
71. Yang XH, Sladek TL, Liu X, Butler BR, Froelich CJ, Thor AD (2001) Reconstitution of caspase 3 sensitizes MCF-7 breast cancer cells to doxorubicin- and etoposide-induced apoptosis. Cancer Res 61:348–354
72. Teitz T, Wei T, Valentine MB, Vanin EF, Grenet J, Valentine VA, Behm FG, Look AT, Lahti JM, Kidd VJ (2000) Caspase 8 is deleted or silenced preferentially in childhood neuroblastomas with amplification of MYCN. Nat Med 6:529–535
73. Fulda S, Kufer MU, Meyer E, van Valen F, Dockhorn-Dworniczak B, Debatin KM (2001) Sensitization for death receptor- or drug-induced apoptosis by re-expression of cas-

pase-8 through demethylation or gene transfer. Oncogene 20:5865–5877
74. Droin N, Dubrez L, Eymin B, Renvoize C, Breard J, Dimanche-Boitrel MT, Solary E (1998) Upregulation of CASP genes in human tumor cells undergoing etoposide-induced apoptosis. Oncogene 16:2885–2894
75. Fulda S, Debatin KM (2003) IFN γ sensitizes for apoptosis by upregulating caspase-8 expression through the Stat1 pathway. Oncogene (in press)
76. Micheau O, Hammann A, Solary E, Dimanche-Boitrel MT (1999) STAT-1-independent upregulation of FADD and procaspase-3 and -8 in cancer cells treated with cytotoxic drugs. Biochem Biophys Res Commun 256:603–607
77. Antonsson B, Martinou JC (2000) The Bcl-2 protein family. Exp Cell Res 256:50–57
78. Cheng EH, Wei MC, Weiler S, Flavell RA, Mak TW, Lindsten T, Korsmeyer SJ (2001) BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. Mol Cell 8:705–711
79. Minn AJ, Rudin CM, Boise LH, Thompson CB (1995) Expression of bcl-xL can confer a multidrug resistance phenotype. Blood 86:1903–1910
80. Campos L, Rouault JP, Sabido O, Oriol P, Roubi N, Vasselon C, Archimbaud E, Magaud JP, Guyotat D (1993) High expression of bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. Blood 81:3091–3096
81. Bargou RC, Daniel PT, Mapara MY, Bommert K, Wagener C, Kallinich B, Royer HD, Dorken B (1995) Expression of the bcl-2 gene family in normal and malignant breast tissue: low bax-alpha expression in tumor cells correlates with resistance towards apoptosis. Int J Cancer 60:854–859
82. Prokop A, Wieder T, Sturm I, Essmann F, Seeger K, Wuchter C, Ludwig WD, Henze G, Dorken B, Daniel PT (2000) Relapse in childhood acute lymphoblastic leukemia is associated with a decrease of the Bax/Bcl-2 ratio and loss of spontaneous caspase-3 processing in vivo. Leukemia 14:1606–1613
83. Sturm I, Kohne CH, Wolff G, Petrowsky H, Hillebrand T, Hauptmann S, Lorenz M, Dorken B, Daniel PT (1999) Analysis of the p53/BAX pathway in colorectal cancer: low BAX is a negative prognostic factor in patients with resected liver metastases. J Clin Oncol 17:1364–1374
84. Sturm I, Petrowsky H, Volz R, Lorenz M, Radetzki S, Hillebrand T, Wolff G, Hauptmann S, Dorken B, Daniel PT (2001) Analysis of p53/BAX/p16(ink4a/CDKN2) in esophageal squamous cell carcinoma: high BAX and p16(ink4a/CDKN2) identifies patients with good prognosis. J Clin Oncol 19:2272–2281
85. Deveraux QL, Reed JC (1999) IAP family proteins-suppressors of apoptosis. Genes Dev 13:239–252
86. Holcik M, Korneluk RG (2001) XIAP, the guardian angel. Nat Rev Mol Cell Biol 7:550–556
87. Reed JC, Bischoff JR (2000) BIRinging chromosomes through cell division–and survivin' the experience. Cell 102:545–548
88. Tamm I, Kornblau SM, Segall H, Krajewski S, Welsh K, Kitada S, Scudiero DA, Tudor G, Qui YH, Monks A, Andreeff M, Reed JC (2000) Expression and prognostic significance of IAP-family genes in human cancers and myeloid leukemias. Clin Cancer Res 6:1796–1803
89. Adida C, Recher C, Raffoux E, Daniel MT, Taksin AL, Rousselot P, Sigaux F, Degos L, Altieri DC, Dombret H (2000) Expression and prognostic significance of survivin in de novo acute myeloid leukaemia. Br J Haematol 111:196–203
90. Adida C, Berrebi D, Peuchmaur M, Reyes-Mugica M, Altieri DC (1998) Anti-apoptosis gene, survivin, and prognosis of neuroblastoma. Lancet 351:882–883
91. Li J, Feng Q, Kim JM, Schneiderman D, Liston P, Li M, Vanderhyden B, Faught W, Fung MF, Senterman M, Korneluk RG, Tsang BK (2001) Human ovarian cancer and cisplatin resistance: possible role of inhibitor of apoptosis proteins. Endocrinology 142:370–380
92. Datta R, Oki E, Endo K, Biedermann V, Ren J, Kufe D (2000) XIAP regulates DNA damage-induced apoptosis downstream of caspase-9 cleavage. J Biol Chem 2000275:31733–31738
93. Suliman A, Lam A, Datta R, Srivastava RK (2000) Intracellular mechanisms of TRAIL: apoptosis through mitochondrial-dependent and -independent pathways. Oncogene 20:2122–2133
94. Fulda S, Wick W, Debatin K-M (2002) Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma in vivo. Nat Med 8(8):808–815
95. Tamm I, Trepel M, Cardó-Vila M, Sun Y, Welsh K, Cabezas E, Swatterthwait A, Arap W, Reed JC (2003) Peptides teargeting caspase inhibitors. J Bio Chem 278(16):14401–14405
96. Mayo MW, Baldwin AS (2000) The transcription factor NF-kB: control of oncogenesis and cancer therapy resistance. Biochim Biophys Acta 1470:M55–M62
97. Herr I, Posovszky C, Di Marzio L, Cifone MG, Böhler T, Debatin K-M (2000) Autoamplification of apoptosis following ligation of CD95L, TRAIL- and TNF-α. Oncogene 19(37):4255–4262
98. Herr I, Wilhelm D, Böhler T, Angel P, Debatin K-M (1999) JNK/SAPK activity is not sufficient for anticancer therapy-induced apoptosis involving CD95L, TRAIL, and TNF-α. Int J Cancer 80:417–424
99. Herr I, Wilhelm D, Meyer E, Jeremias I, Angel P, Debatin K-M (1999) JNK/SAPK activity contributes to TRAIL-induced apoptosis. Cell Death Differ 6:130–135
100. Herr I, Böhler T, Wilhelm D, Angel P, Debatin K-M (1997) Activation of CD95 (APO-1/Fas) signaling by ceramide mediates cancer therapy-induced apoptosis. EMBO J 16:6200–6208