

MINI REVIEW

Tumor resistance to apoptosis

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One of the hallmarks of human cancers is the intrinsic or acquired resistance to apoptosis. Evasion of apoptosis may contribute to carcinogenesis, tumor progression and also to treatment resistance, since most current anticancer therapies including chemotherapy, radio- and immunotherapy primarily act by activating cell death pathways including apoptosis in cancer cells. Hence, a better understanding of the molecular mechanisms underlying tumor resistance to apoptotic cell death is expected to provide the basis for a rational approach to develop molecular targeted therapies.

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Programmed cell death or apoptosis is the cell's intrinsic death program that is involved in the regulation of many physiological and pathological processes and that is evolutionary highly conserved.¹ Since tissue homeostasis is the result of a subtle balance between proliferation and cell death, too little cell death by apoptosis can promote tumor formation as well as progression.^{2,3} It is therefore not surprising that one of the characteristic features of human cancers is the inability to undergo apoptosis in response to stimuli that otherwise trigger apoptosis in sensitive cells. Hence, a better understanding of the molecular mechanisms that cause tumor resistance to apoptosis is anticipated to provide a molecular basis for the design of new strategies to eventually overcome apoptosis resistance of human cancers.

Signaling to apoptotic cell death

Two principle apoptosis signaling pathways have been delineated, *i.e.*, the death receptor (extrinsic) pathway and the mitochondrial (intrinsic) pathway.¹ Activation of either pathway eventually leads to activation of caspases, a family of cysteine proteases that function as common death effector molecules by cleaving a range of cytoplasmic or nuclear substrates.⁴ Ligation of death receptors of the tumor necrosis factor (TNF) receptor superfamily such as CD95 (APO-1/Fas) or tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors by their corresponding natural ligands, *i.e.*, CD95 ligand or TRAIL, results in the recruitment of caspase-8 into a multimeric complex at the plasma membrane, the death inducing signaling complex (DISC).^{5,6} This in turn leads to caspase-8 activation, which can then directly cleave downstream effector caspases such as caspase-3.⁶ Alternatively, caspase-8 can promote outer mitochondrial membrane permeabilization by cleaving Bid, a BH3-only protein that translocates to mitochondria upon cleavage and causes cytochrome c release.⁷ The mitochondrial pathway is initiated by the release of apoptogenic factors such as cytochrome c, apoptosis inducing factor, second mitochondria-derived activator of caspase (Smac)/direct inhibitor of apoptosis proteins (IAP) Binding protein with Low PI (DIABLO) or Omi/high temperature requirement protein A (HtrA2) from the mitochondrial intermembrane space into the cytosol.⁸ The release of cytochrome c into the cytosol causes activation of caspase-3 *via* formation of a large cytosolic complex, *i.e.*, the cytochrome c/Apaf-1/caspase-9-containing apoptosome complex.⁸ Smac/DIABLO or Omi/HtrA2 enhance caspase activation by binding to IAPs, thereby disrupting the interaction of IAPs with caspase-3 or -9.^{8,9} Activation of apoptosis pathways is tightly controlled by a variety of positive and negative regulators under

normal conditions, since accidental stimulation of the apoptotic machinery potentially has detrimental effects on cell survival. These anti-apoptotic mechanisms are often abnormally upregulated in human cancers, which enables cancer cells to evade apoptotic cell death.¹⁰ Besides caspase-dependent and caspase-independent apoptosis, additional nonapoptotic modes of cell death have also to be taken into consideration, *e.g.*, necrosis, autophagy, mitotic catastrophe and lysosomal cell death.¹¹ Activation of these nonapoptotic cell death modes presents an alternative approach to induce cell death in apoptosis resistant types of human cancers.¹²

The molecular mechanisms that initiate apoptosis or alternative modes of cell death upon treatment with cytotoxic agents have often not exactly been identified and likely depend on the individual stimulus. Following exposure to genotoxic substances, damage to DNA or to other critical molecules is considered to be a common initial event which is then transmitted by the cellular stress response to the activation of cellular effector systems such as the apoptotic machinery.¹³ Various stress-inducible molecules, for example JNK, MAPK/ERK, NFκB or ceramide have been implicated in propagating the apoptotic signal.^{14,15} Further, oncogenic events such as deregulated expression of the *myc* oncogene can trigger apoptosis besides stimulating tumor growth.²

Mechanisms of resistance to apoptosis in human cancers

One hallmark of human cancers is the ability to evade apoptosis. In principle, signaling to cell death can be blocked by an increase in anti-apoptotic molecules and/or by a decrease or defective function of pro-apoptotic proteins. In the following paragraphs examples of alterations in apoptosis signaling pathway often occurring in human cancers will be discussed (Fig. 1).

Alterations in the death receptor pathway in human cancers

Biology of death receptors. Death receptors belong to the TNF receptor gene superfamily, which comprises more than 20 proteins with various biological functions, *e.g.*, regulation of cell death and survival, differentiation or immune regulation.^{6,16} Members of the TNF receptor family share a characteristic cytoplasmic domain called the "death domain", which is pivotal for transducing the death signal from the cell's surface to intracellular signaling pathways.^{6,16} CD95 (APO-1/Fas), TNF receptor 1 and TRAIL receptors are the best-characterized death receptors and their corresponding ligands of the TNF superfamily are CD95 ligand, TNFα and TRAIL.^{6,16}

Impaired death receptor expression or function. Death receptor signaling can be impaired in human cancers at several levels. At the receptor level, surface expression of these receptors can be

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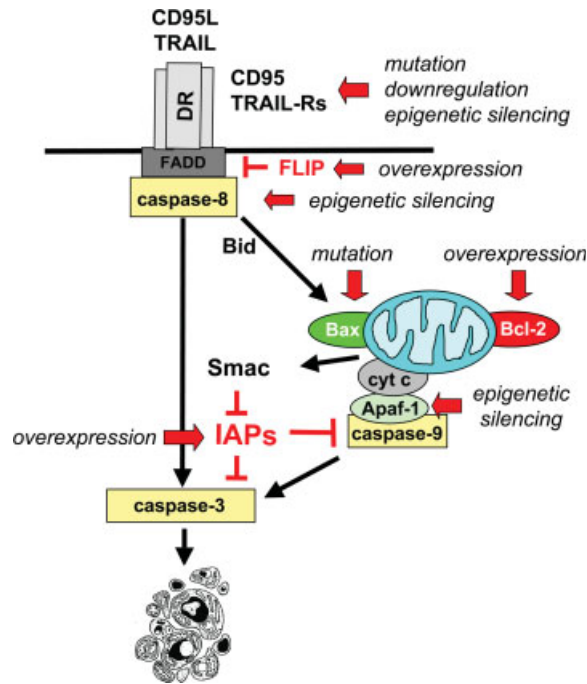


FIGURE 1 – Defects in apoptosis pathways in human cancers. Signaling *via* the death receptor pathway can be inhibited by mutations, downregulation or epigenetic silencing of death receptors (DR) such as CD95 or TRAIL receptors (TRAIL-Rs), overexpression of FLIP or epigenetic silencing of caspase-8. The mitochondrial pathway may be impaired by overexpression of anti-apoptotic Bcl-2 proteins, Bax mutations or epigenetic silencing of Apaf-1. Activation of downstream caspases can be blocked by high levels of “Inhibitor of Apoptosis Proteins” (IAPs). See text for more details. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

downregulated or can even be completely absent, thereby preventing the transmission of the death signal from the cell surface to intracellular signaling cascades (Fig. 1). For example, decreased expression of CD95 has been reported in leukemia or neuroblastoma cells, which are resistant to chemotherapeutic agents suggesting that intact expression of CD95 is critical for drug sensitivity.^{17,18} Furthermore, abnormal transport of the apoptosis-inducing TRAIL receptors TRAIL-R1 and TRAIL-R2 from intracellular stores such as the endoplasmic reticulum to the cell surface was reported to confer resistance to TRAIL-induced cell death in colon carcinoma.¹⁹

Besides this functional impairment of death receptor expression on the cell's surface, mutations of the CD95 gene were also reported in hematological malignancies as well as in various solid tumors (Fig. 1).^{20–25} In the TRAIL system, loss of expression of the apoptosis-inducing TRAIL receptors, *i.e.*, TRAIL-R1 and R2, can confer resistance towards TRAIL. Notably, both receptors are located on chromosome 8p, which is often lost due to heterozygosity (LOH) in human cancers.^{26,27} Deletions or mutations resulting in loss of both copies of TRAIL-R1 or TRAIL-R2 were detected in a small percentage of cancers, *e.g.*, nonHodgkin's lymphoma, hepatocellular carcinoma, breast carcinoma, head and neck cancer, osteosarcoma or lung carcinoma.^{28–32} Alternatively, surface expression of death receptors can be impaired by epigenetic changes such as CpG-island hypermethylation of gene promoters (Fig. 1).^{33,34}

Aberrant decoy receptor expression. Aberrant expression of any of the decoy receptors is another mechanism to evade TRAIL- or CD95-induced apoptosis. To this end, overexpression or genetic amplification of decoy receptor 3, which counteracts CD95-mediated apoptosis by competitively binding CD95 ligand, was identi-

fied in lung or colon carcinoma and in glioblastoma.^{35,36} In addition, overexpression of TRAIL-R3, a decoy receptor for TRAIL, was reported in gastric carcinoma.³⁷

Elevated expression of cFLIP or PED/PEA-15. In addition to these genetic and epigenetic mechanisms, signal transduction *via* the death receptor pathway can also be functionally blocked at the receptor level, *e.g.*, by aberrant expression of anti-apoptotic proteins that prevent activation of caspase-8 at death receptors. Cellular FLICE-inhibitory protein (cFLIP) and phosphoprotein enriched in diabetes/phosphoprotein enriched in astrocytes-15 kDa are examples of such inhibitors that interfere with the recruitment of caspase-8 to activated death receptors (Fig. 1).^{38,39} These proteins are recruited into the DISC upon receptor ligation instead of procaspase-8, since they harbor high sequence homology to caspase-8, thereby preventing caspase-8 activation.^{38,39}

Inactivation of caspase-8. Further, caspase-8 expression or function may be impaired by genetic or epigenetic mechanisms in cancer cells (Fig. 1). Despite the essential function of caspase-8 for signal transduction in the death receptor pathway, the frequency of caspase-8 mutations in human tumors is, however, only relatively low. Such mutant variants of caspase-8, which have for example been identified in colorectal and head and neck carcinoma, can act in a dominant-negative manner by preventing the recruitment of the wild-type form of caspase-8 to activated death receptors, thereby inhibiting apoptosis.^{40,41} Further, homo- or heterozygous genomic deletions were found in some neuroblastoma.⁴² In addition, caspase-8 expression can be transcriptionally regulated by splicing, for example in leukemia and neuroblastoma.^{43,44} To this end, alternative splicing of intron 8 of the caspase-8 gene results in the generation of caspase-8L, which lacks the catalytic site while retaining the 2 N-terminal DED repeats.⁴³ By interfering with the recruitment of wild-type caspase-8 to activated death receptors, caspase-8L can act as a dominant-negative inhibitor of apoptosis.⁴⁴ Furthermore, caspase-8 expression is frequently inactivated by hypermethylation of regulatory sequences of the caspase-8 gene in a number of different tumors, *e.g.*, neuroblastoma, malignant brain tumors, Ewing tumor, retinoblastoma, rhabdomyosarcoma or small lung cell carcinoma.^{42,45–48} Also, co-methylation for caspase-8 and FLIP as well as for the pairs of agonistic TRAIL receptors TRAIL-R1/TRAIL-R2 or antagonistic TRAIL receptors TRAIL-R3/TRAIL-R4 has been described in neuroblastoma indicating a nonrandom pattern of epigenetic silencing of these genes.⁴⁹ Furthermore, phosphorylation of caspase-8 on tyrosine 308 by kinases such as Src results in suppression of its pro-apoptotic activity.⁵⁰

Alterations in the mitochondrial pathway in human cancers

Biology of Bcl-2 family proteins. The Bcl-2 family of proteins consists of both anti-apoptotic proteins, for example Bcl-2, Bcl-X_L and Mcl-1, as well as pro-apoptotic molecules such as Bax, Bak and BH3 domain only molecules.⁷ There are currently 2 models to explain the activation of Bax and Bak by BH3-only proteins. The direct activation model holds that BH3-only proteins, which act as direct activators such as Bim and the cleaved form of Bid (tBid), bind directly to Bax and Bak to trigger their activation, whereas BH3-only proteins that act as sensitizers, *e.g.*, Bad, bind to the pro-survival Bcl-2 proteins.⁵¹ According to the indirect activation model, BH3-only proteins activate Bax and Bak in an indirect fashion by engaging the multiple anti-apoptotic Bcl-2 proteins that inhibit Bax and Bak, thereby releasing their inhibition on Bax and Bak.^{52,53} Regardless of the exact mode of Bax and Bak activation, the ratio of anti-apoptotic *versus* pro-apoptotic Bcl-2 proteins rather than the expression levels of one particular molecule of the Bcl-2 family regulates apoptosis sensitivity.

Abnormal expression of anti-apoptotic Bcl-2 family proteins. An increase in the ratio of anti- to pro-apoptotic Bcl-2 proteins has been detected in various cancers and has been correlated to tumor cell survival and apoptosis resistance (Fig. 1).

For example, overexpression of Bcl-2 is a characteristic feature of human follicular lymphoma and is caused by chromosomal translocation of the *bcl-2* oncogene into the immunoglobulin heavy chain gene locus.⁵⁴ Generation of Bcl-2 transgenic mice revealed that Bcl-2 overexpression promotes neoplastic transformation of B and T lymphocytes and also of myeloid cells.^{55,56} In chronic lymphocytic leukemia, Mcl-1 expression was recently identified as an important regulator of disease progression and outcome.⁵⁷

Inactivation of Bax or BH3-only proteins. Moreover, somatic mutations resulting in the inactivation of the pro-apoptotic *bax* gene have been identified in certain solid tumors and hematological malignancies (Fig. 1). For example, single nucleotide substitution or frameshift mutations of the *bax* gene can occur in mismatch repair-deficient colon cancer or hematopoietic malignancies.^{58,59}

Furthermore, BH3-only proteins may act as *bona fide* tumor suppressors. In a mouse model of B cell lymphoma, loss of a single allele of Bim was shown to accelerate B cell lymphomagenesis induced by expression of a *c-myc* transgene.⁶⁰ Another example is *bid* deficient mice, which spontaneously develop a myeloproliferative disorder that may eventually progress to a chronic myelomonocytic form of leukemia.⁶¹ In human hematological malignancies, homozygous deletion of *bim* were detected in mantle cell lymphoma and promoter hypermethylation of *bim* were identified in Burkitt lymphoma.^{62,63} Further, the pro-apoptotic BH3-only protein Noxa was reported to be mutated or epigenetically silenced in diffuse large B-cell lymphoma.^{62,63} In renal carcinoma, loss of Bik expression was recently found to be caused by deletion of the *bik* gene at 22q13.2 or DNA methylation-mediated transcriptional silencing.⁶⁴

Defects at the postmitochondrial level: loss of Apaf-1. In addition to these genetic alterations in Bcl-2 family molecules, defects in the mitochondrial pathway of apoptosis may also occur at the postmitochondrial level. To give an example, decreased or absent expression or activity of Apaf-1 has been reported to impair the assembly of a functional apoptosome in melanoma, leukemia, glioblastoma and gastric cancer and may be caused by promoter hypermethylation or loss of heterozygosity at chromosome 12q22-23 (Fig. 1).⁶⁴⁻⁶⁸

Aberrant expression of inhibitor of apoptosis proteins in human cancers

Biology of inhibitor of apoptosis proteins. Moreover, tumor resistance to apoptosis may be caused by aberrant expression or function of IAPs (Fig. 1). IAPs are a family of endogenous caspase inhibitors with 8 human members, *i.e.*, XIAP, cIAP1, cIAP2, survivin, livin (ML-IAP), NAIP, Bruce (apollon) and ILP-2.^{9,69} All IAP proteins have at least one baculovirus IAP repeat (BIR) domain that is required for classification as IAP family protein. This domain is also the region of the protein that mediates the interaction with caspases.⁷⁰ Among the IAP family proteins, XIAP exhibits the strongest anti-apoptotic properties and inhibits apoptosis signaling by binding to active caspase-3 and -7 and by preventing caspase-9 activation.⁷¹ XIAP also regulates other signaling cascades, for example the NF- κ B pathway. To this end, XIAP has been described to activate NF- κ B by enhancing degradation of I κ B proteins, by promoting nuclear translocation of NF- κ B and *via* complex formation with TAK1 and TAB1.⁷²⁻⁷⁶ Survivin is an IAP family protein that controls also mitosis in addition to its role in the regulation of apoptosis.⁷⁷

The expression and function of IAPs is regulated at several levels. For example, mitochondrial proteins such as Smac/DIABLO or Omi/HtrA2 or nuclear proteins such as XIAP-associated factor 1 (XAF-1) function as endogenous IAP antagonists.⁹ Upon induction of apoptosis, Smac/DIABLO translocates from the mitochondria into the cytosol and antagonize IAPs *via* physical interactions.⁷⁸ XAF-1 acts as an endogenous

IAP antagonist by sequestering XIAP in the nucleus.^{79,80} Further, IAPs can be negatively regulated by proteolytic cleavage, *e.g.*, mediated by caspases or by serine proteases such as Omi/HtrA2.⁹ Moreover, expression levels of IAPs are tightly controlled at both the transcriptional and posttranscriptional level, for example by auto- and heteroubiquitination through the RING domain of IAPs.⁸¹ In addition, phosphorylation of XIAP by Akt promotes its protein stability.⁸²

Deregulation of survivin. Expression and/or function of IAP proteins are deregulated in many human cancers and their expression levels in tumor samples have been correlated to clinical parameters and prognosis. For example, survivin was identified as the fourth most common transcriptome of the human genome in human cancers in large scale gene profiling studies.⁸³ In contrast to its high expression levels in the majority of cancers, survivin was found to be expressed at low or undetectable levels in most normal adult tissues.⁸³ Further, survivin is particularly relevant for neuroblastoma, since the *survivin* gene maps to chromosome 17q25, a region which is frequently gained in advanced stages of the disease.⁸⁴ High expression levels of survivin in primary neuroblastoma significantly correlated with high risk tumors and poor prognosis.⁸⁵ In addition, high survivin levels predicted unfavorable prognosis in acute myeloid leukemia (AML) or nonHodgkin lymphoma.^{86,87}

Abnormal expression of cIAP1 and cIAP2. Abnormal expression of cIAP2 may be caused by chromosomal translocations. For example, the t(11;18)(q21;q21) translocation affects the *cIAP2* gene in a large proportion of mucosa-associated lymphoid tissue (MALT) lymphoma.⁸⁸ This translocation fuses the BIR domains of cIAP2 with the MALT1 protein, a paracaspase and critical mediator of T cell receptor-triggered NF- κ B activation. Interestingly, recent evidence suggests that the cIAP2-MALT1 fusion protein constitutively activates the NF- κ B pathway independently of signaling adaptors that otherwise regulate its activity such as TRAF proteins.⁸⁹ Gene expression profiling of IAP genes in hematological malignancies unraveled a specific pattern of expression of cIAP1, cIAP2 and survivin in CLL, B-ALL and follicular lymphoma samples.⁹⁰

Deregulation of XIAP. As far as the prognostic significance of XIAP expression is concerned, the data are conflicting. Although it was reported in one study that AML patients with lower levels have a significantly better survival,⁸⁶ another study showed no prognostic impact of XIAP expression in this malignancy.⁹¹ In childhood *de novo* AML, high expression levels of XIAP and survivin correlated with poor overall survival and an immature M0/1 subtypes according to the French-American-British morphology.⁹² In clear-cell renal carcinoma, XIAP was identified as an independent poor prognostic factor,⁹³ while high XIAP expression was associated with a longer overall survival in nonsmall cell lung cancer.⁹⁴

Conclusions

Evasion of apoptosis is one of the hallmarks of human cancers that promote tumor formation and progression as well as treatment resistance. Studies over the last decade that aimed at identifying the underlying molecular mechanisms of apoptosis resistance have delineated multiple defects at various levels of the apoptosis signal transduction machinery. The discovery of apoptosis escape mechanisms has a great potential for translational medicine, since such defects in apoptosis molecules may serve as targets for the design of novel therapeutic strategies as well as molecular markers to predict treatment response and prognosis. The enormous progress in apoptosis research has started to be translated into the development of innovative cancer diagnostics and therapeutics. Thus, further insights into the mechanisms of tumor resistance to apoptosis are expected to turn into benefit for patients suffering from cancer.

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