Chapter 1

# DISFUNCTION OF THE APOPTOTIC PATHWAY IN CANCER CELLS

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Abstract: Apoptosis is an important physiological process for maintaining homeostasis, remodeling and eliminating abnormal cells in normal tissues. Development of human cancer is a multistage process involving various genetic alternations and cellular abnormalities. Cellular changes should lead to activation of the apoptotic pathway and induction of cell death, which prevents tumor growth and progression. However, during tumorigenesis, some tumor cells develop apoptosis-resistant mechanisms that allow the cancer cells to avoid apoptotic cell death, resulting in the initiation and progression of human cancers. Defects in the apoptotic signaling pathway have been detected in many cancer cells and cancer tissues. A deregulated apoptotic signal pathway confers a high survivability and resistance of the tumor cells to therapeutic reagents. Understanding the alterations in apoptotic signaling in human cancer cells should provide important information for the development of novel cancer therapies directly targeting the apoptotic signal pathway in cancer cells.

Key words: Apoptosis resistance, apoptotic signal pathway, human cancer cell, apoptosis signal defects

## 1. INTRODUCTION

Programmed cell death (apoptosis) is an important cellular process that allows proper development and remodeling of normal tissues, generating immune responses and destroying abnormal cells. A regulated apoptotic pathway ensures homeostasis and integrity of the normal tissues [1,2]. It is well known that malignant transformation of human cancer cells is a multi-stage



*M. Sluyser (ed.), Application of Apoptosis to Cancer Treatment,* 1-28.

process involving mutations or deletions of various tumor suppressor genes, activation of oncogenes and alterations in the levels of expression of key regulatory genes, providing growth advantages and metastatic potential for tumor cells [3]. Those genetic alterations result in abnormalities in cellular and nuclear morphology and signal transduction pathways which would normally activate a suicidal pathway and induce apoptosis in the cells [2,4]. Increasing evidence shows that impairments in apoptotic signaling enable tumor cells to avoid apoptotic cell death and grow into tumor masses that are resistant to apoptosis [5-8]. Defects in regulation of apoptosis have been detected in both upstream and downstream of the apoptotic signal pathway in many types of human tumor cells [6-9]. Recent studies have also revealed molecular targets in the apoptotic pathway that play important roles in the apoptosis resistance of cancer cells. The feasibility of targeting the apoptotic pathway as a novel cancer therapy has also been examined.

Apoptosis is the most common type of cell death, characterized as chromatin condensation, nuclear fragmentation, cell shrinkage and membrane blebbing. Apoptotic cells then break into small membrane-surrounded apoptotic bodies that are removed by phagocytosis [1]. In normal cells, apoptosis is induced under some physiological conditions such as tissue and organ development in fetus, menstrual cycle, and involution of breast ducts after lactation [10-13]. Apoptotic cell death is also induced in the cells with viral infection, DNA damage or other genomic alterations and regulation of cell-mediated immune responses [2,14,15]. Regulated apoptosis therefore maintains tissue integrity as well as a balance between cell proliferation and death in normal tissues.

During the last decade, the identification and characterization of cellular factors in the apoptotic signal pathway have been an intensive research area. Many cellular factors involved in apoptotic signaling were discovered and their roles in the regulation of the apoptotic pathway have been elucidated. Apoptosis is initiated when the cells receive negative signaling, such as growth factor withdrawal, DNA damage by oxidants, ultraviolet light and x-rays, and chemotherapy drug treatment [2,16-18]. Activation of apoptotic signaling is achieved by either an extrinsic or an intrinsic pathway [19]. The extrinsic pathway is triggered by ligation of cell surface death receptors with their specific ligands, such as Fas Ligand, tumor necrosis factor α (TNF-α) and tumor necrosis factor-related apoptosis inducing ligand (TRAIL). Binding of apoptosis inducing ligands to their corresponding receptors activates an intracellular domain (the death domain) of the receptor to attract an adaptor protein, Fas-associated death domain protein (FADD). FADD then recruits inactive caspase 8 to form a death-inducing signaling complex (DISC), resulting in the activation of caspase-8. Active caspase 8 then cleaves and activates caspase-3 and -7 [19]. The intrinsic pathway is activated

when the cells are under severe stresses such as growth factor deprivation, oxidants and DNA-damaging agents by leakage of cytochrome c from mitochondria. This results in the activation of caspase-9 and then caspase-3, -6, and -7 [19-21]. The crosstalk between cell death receptors and mitochondrial pathways is also present in some conditions. Death receptor activated caspase-8 cleaves Bid, which then translocates to the mitochondria to amplify the apoptosis signal by activating the mitochondrial pathway [22].

Caspases can be divided into two groups based on the length of their prodomain and substrate specificity. Caspase-2, -8, -9 and -10 are initiator caspases using their long N-terminal prodomains to interact with adapter molecules and form a death inducing signal complex (DISC). Downstream caspases, including caspase-3, -6, and -7, are executioner caspases that remain dormant until the initiator caspases activate them by proteolysis [22]. Activated executioner caspase-3, -6 and -7 recognize specific substrate sequences in targeting cellular proteins and cleave a number of structural and regulatory proteins such as Poly (ADP-ribose) polymerase (PARP), lamins, DNA fragmentation factor-45 (DFF45/ICA) and cytokeratins, leading to apoptotic cell death [23].

Examination of the levels and activity of apoptotic effectors, inhibitors and regulators in human cancer cells and tissues has demonstrated that deregulation of apoptotic signal pathway is present in most human cancer cells. Human tumor cells escape apoptotic cell death by avoiding the activation of upstream apoptotic signals and/or by upregulation of inhibitory factors in the apoptotic signal pathway [6-9].

# 2. CELL DEATH RECEPTOR-MEDIATED APOPTOSIS

## 2.1 Fas and Fas ligand (Fas L)

Fas (APO-1 or CD 95) is a widely expressed transmembrane protein in the tumor necrosis receptor family. Interaction of Fas with its legend, FasL, initiates the death receptor-mediated cell death pathway [22,24]. However, dysfunction of the Fas-mediated apoptotic signal has been found in several tumor types. It has been shown that many tumor cells are resistant to FasL or Fas antibody induced apoptosis [25,26]. Further studies indicate that human cancer cells have developed resistant mechanisms to avoid Fas-mediated apoptosis. Somatic deletions and mutations of Fas receptor were first discovered in human lymphoid-lineage malignancies [26,27]. Later, Fas mutations were detected in small percentage of solid tumors, such as in

gastric (11.6%), non-small cell lung (7.7-20%), and malignant melanomas (6.8%)[28,29]. Although Fas mutation is not a common phenomenon in solid tumors, a reduced level of expression of cell surface Fas receptor is found in many tumor types either by downregulating Fas gene expression or by decreasing cell surface transportation [30-33]. In addition, some tumor cells also produce a high level of soluble Fas to block interactions between cell surface Fas receptor and FasL [34,35].

Although downregulation of Fas levels or function could explain the insensitivity to Fas-mediated apoptosis in some tumor cells, many tumor cells do not have Fas mutations and an adequate level of Fas expression is detected in tumor cells that are resistant to Fas-mediated apoptosis [36-40]. Interestedly, those tumor cells also co-express a high level of FasL, an activating ligand for Fas receptor. In normal tissues, FasL is only expressed at a low level in cytotoxic T lymphocyte, natural killer cells, sertoli cells of testis, ocular cells and normal breast ductal epithelial cells [41-43]. However, upregulation of FasL has been found in many tumor cells as well as tumor tissues [44-47]. Co-expression of Fas and FasL in tumor cells resistant to Fas-mediated apoptosis suggests the presence of intrinsic anti-apoptotic factors downstream of the death receptor that block the apoptotic signal pathway and prevent apoptosis [31]. Consistent with this notion, it has been shown that tumor cells resistant to Fas-induced cell death also showed a low sensitivity to chemotherapy drugs or to TRAIL induced apoptosis [48-50]. Moreover, upregulation of cell surface FasL provides a growth advantage to the cells by counteracting tumor-infiltration immune cells and/or facilitating the destruction of surrounding tissues to increase the invasiveness of the tumor cells [25,44].

## 2.2 TRAIL and TRAIL receptors

TRAIL is a member of the tumor necrosis factor (TNF) family of cytokines that binds to its death receptors, DR4 and DR5, and activates the apoptotic pathway [51]. Although TRAIL is constitutively expressed in many tissue types, apoptotic cell death is selectively induced in cancer cells but not in normal cells [52-54]. This selectivity may be due to a higher level of TRAIL receptors in cancer cells than in normal cells. In addition, TRAIL also interacts with "decoy" receptors DcR1 and DcR2, which lack functional death domains and do not induce apoptosis [55]. The role of the decoy receptors in protecting normal cells from TRAIL-induced apoptosis has yet to be determined.

Although activation of TRAIL-mediated apoptotic pathway has great potential for developing tumor-specific therapy, further studies of the ant-tumor effects of TRAIL in different tumor cell lines indicate that human

tumor cells have a wide range of sensitivity to TRAIL-induced apoptosis [56]. A large fraction of tumor cells display a low level of TRAIL expression or activity. Some tumor cells have completely lost the expression of TRAIL receptor [57,58]. Additionally, several studies demonstrate that high levels of both TRAIL receptor and ligand are found in some TRAIL-resistant tumor cells, suggesting other downstream anti-apoptotic factors may contribute to lack of TRAIL-induced apoptosis in those cells. However, it has been shown that treatment of TRAIL resistant tumor cell lines with subtoxic concentrations of chemotherapy drugs sensitizes TRAIL-induced apoptosis [57,59,60].

## 2.3 TNF-α and receptors

TNF-α, a cytokine produced by macrophages/monocytes during acute inflammation, regulates inflammation, survival, proliferation and apoptosis of cells. TNF-α binds to cell surface receptor TNFR-1 or TNFR-2 and trimerizes the receptors [61]. The activated receptors further recruit adaptor proteins TRADD and TRAF2, and death effect domain protein FADD to form DISC and then cleaves procaspase 8 to active caspase 8 [62,63]. Unlike other TNF-α family receptors, recruiting TRAF2 to TNF-R1 triggers the activation of cell survival factor NF-κB resulting in the activation of anti-apoptosis factors such as c-FLIP or cIAPs, which are inhibitors for caspase 8 [62,64]. Since the level of TRAF2 is elevated in various human tumors, this may cause the formation of the TNF-R, TRADD and TRAF2 complex and activate the cell survival pathway, resulting in resistance of the tumor cells to TNF-α mediated apoptosis [65,66].

# 3. CASPASE ACTIVATION

## 3.1 Downregulation of caspases in tumor cells

Caspases are synthesized as inactive zymogenes with a prodomain followed by a large (p20) and a small (p17) subunit. Activation of the procaspases by a series of cleavage events is a critical process for execution of apoptosis. Deficiency in the levels of expression of procaspase genes is detected in some tumor cell lines and tissues. For example, deletion or silencing of the caspase 8 gene was discovered in neuroblastoma and non-small lung carcinomas [67-69]. Deficiency in caspase 3 was also found in some human tumor cell lines and tissues such as human breast cancer cells, drug resistant human cervical cancer cells, human neuroblastoma, hepatocellular

and renal cell carcinomas tissues [68,70,71]. Results from examination of levels of caspase expression using immunohistochemistry staining further showed that 46% to 85% of human colon cancer tissues have low levels of caspase-7 and -9 [72].

## 3.2 Apoptotic protease activating factor 1

Apoptotic protease activating factor 1 (APAF-1) is a cytoplasm protein that binds to cytochrome C after its release from mitochondria and forms an apoptosome with cytochrome C and procaspase 9. At the apoptosome, procaspase 9 is activated, resulting in the cleavage and activation of caspase 3. Functional of APAF-1 is required for activation of caspase 9 in the intrinsic pathway [73]. However, tumor cells, such as metastatic melanomas have developed a way to avoid the mitochondrial-mediated apoptosis by downregualting expression of the APAF-1 gene through allelic loss or gene methylation [74]. Those APAF 1-negative cells are highly resistant to chemotherapy [4].

## 3.3 Upregulation of levels of gene expression and caspase activity in human tumor cells

Although impairments in the levels of caspases greatly affect the apoptotic response in human tumor cells, recent studies have demonstrated the presence of higher levels of expression of procaspase genes and/or active caspases in some tumor cells and tissues as compared to normal cells [75,76,77 #143,78,79]. Examination of levels of procaspases and active caspases in breast carcinoma tissues from 440 breast cancer patients at different stages of the disease in five independent studies yielded surprising results demonstrating a high level of procaspases and/or active forms of caspases in most human breast cancer tissues [75-79]. A high level of procaspase-3 expression is found in 58% of ductal carcinoma *in situ* (DCIS) and ~90% of invasive breast cancer tissues but is not found in normal breast ductal cells. A strong expression of procaspase-3, -6 and -8 is significantly associated with the extent of apoptosis and high grade of DCIS lesions [76]. It has also been shown that over 80% of breast cancer tissues display high levels of active caspase-3 and -6 detected by immunohistochemical staining using antibodies specific for active forms of caspase -3 and -6. In these patient samples, apoptosis is highly correlated with the level of proliferation but not with the level of active caspases [77].

Overexpression of caspase-3 gene is also detected in pancreatic cancer but not in normal pancreas tissues[80]. High levels of caspase-8, -3 and -6 activity are found in pancreatic and colon cancer cells that are not

undergoing apoptotic cell death [79]. Analysis of the expression of caspase-3, -8, -9 and -10 in 60 advanced gastric adenocarcinomas by immunohistochemistry using a tissue microarray approach showed that over 90% of the gastric cancer tissues express high levels of caspase-3, -8, -9 and -10. However, normal gastric mucosal cells show no or weak expression of caspases. A high level of active caspase-3 in gastric cancer tissues is significantly correlated with lymph node metastasis and a worse prognosis of the patient but not with the extent of apoptosis [81]. At present, the significance of caspase activation in human tumor tissues is still under investigation. Activation of the caspase cascade, especially caspase 3, has been considered as an irreversible process that leads to "point of no-return" apoptotic death in the cells. An important question to be answered is that how those tumor cells with active caspases are still alive and maintaining proliferative ability. Recent studies showed that in addition to their function in apoptosis, limited activation of caspases is required for some normal cell functions such as proliferation of T and B lymphocytes and differentiation of several cell types [82-84]. In tumor cells, activation of caspases may result from the activation of the apoptotic pathway due to the presence of abnormalities in cancer cells. In addition to a high level of caspase and/or active caspase, upregulation of FasL, an important activator for death receptor-mediated apoptosis, is seen in many tumor cells and tissues. Expression of FasL has been associated with counteracting the cytotoxic T cell immune response and the invasiveness of the tumor cells [85]. It seems that those FasL-mediated effects are the results of activation of apoptotic signal in tumor cells. Development of anti-apoptosis mechanisms, especially factors inhibiting caspase activity, allows survival and progression of human tumor cells.

# 4. ANTI-APOPTOTIC FACTORS IN TUMOR CELLS

## 4.1 Upstream inhibitors for cell death receptor-mediated apoptotic pathway

Death receptor activated apoptosis is negatively regulated by FADD-like interleukin-1 β-converting enzyme-like protease (cFLIP) [86]. cFLIP protein has homology with procaspase 8 but lacks the catalytic domain of the enzyme. Binding of cFLIPs to the DISC interferes with the processing and activation of caspase 8, which inhibits initiation of death receptor-mediated apoptosis. It has been shown that cFLIP, potently inhibits death signaling mediated by all known death receptors, including Fas, TNF-R, and TRAIL-Rs. cFLIP is constitutively expressed at a high level in many human tumor

types including heptocellular carcinomas, malignant melanomas, gastric, ovary and prostate cancers [87-89]. The anti-apoptosis function of cFLIP is further demonstrated by the attenuating cisplatin-induced cleavage of caspase-8 and -3 and apoptosis in chemosensitive ovary cells after overexpression of cFLIP, and by increased apoptosis after downregulating cFLIP in chemoresistant cells [90].

## 4.2 Bcl-2 family proteins

The mitochondria-dependent apoptosis pathway is regulated by anti- and pro-apoptotic proteins of the Bcl-2 family. About twenty proteins have been identified as members of the Bcl-2 family [91]. The anti-apoptotic Bcl-2 family includes proteins such as Bcl-2, Bcl-$X_L$, Bcl-w, Mcl-1, A1/BFL1, which contain Bcl-2 homology (BH) domains 1, 2 and 4. The Bcl-2 family proteins with a proapoptotic function can be further divided into Bax subfamily (Bax, Bak and Bok), and BH3 subfamily with such members as Bik, Bim, Bad, HRK/DP5, NOX, Puma, NIP3, Bid and BMF [92].

Overexpression of anti-apoptotic Bcl-2 proteins inhibits apoptosis induced by various apoptosis stimuli including chemotherapy drugs, γ-radiation, FasL and TNF-α [93]. In normal tissues, maintaining homeostasis requires a balance between the anti-apoptotic and proapoptotic Bcl-2 family proteins. When cells are under stress, Bax and Bak translocate from the cytoplasm to the outer mitochondria membrane and undergo oligomeriztion. Oligomerized Bax or Bak then inserts into the membrane to induce cytochrome c release. Bcl-2 selectively binds to Bax and prevents insertion of Bax into the mitochondrial membrane. Therefore, the interaction of pro- and anti-apoptotic Bcl-2 family proteins determines mitochondrial membrane permeability suppression or promotion, which controls the release of cytochrome C and other apoptosis activating proteins from the mitochondria [19,92]. Anti-apoptotic proteins Bcl-2 and Bcl-$X_L$ are overexpressed in many tumor types [6,94]. Upregulation of Bcl-2 or Bcl-$X_L$ has been demonstrated to block the apoptotic response and to be a key factor in tumorigenesis and apoptosis resistance in several tumor types [95,96]. Downrgulation of Bcl-2 function or expression by anti-sense or synthetic BH3 peptides has been shown to induce apoptosis and sensitize tumor cells to chemotherapy [97-99].

Although the role of Bcl-2 in apoptosis resistance has been demonstrated in several tumor types, especially in lymphomas, whether Bcl-2 protein plays an important role in breast cancer has yet to be determined. It is clear that over 80% of breast cancer tissues express a high level of Bcl-2 [94,100]. Overexpression of the BCL-2 protein enhanced resistance to apoptosis in human breast cancer cell lines [96,101]. However, expression of Bcl-2 in human

breast cancer tissues correlates with a favorable prognosis and an overall better survival rate [100,102-104]. This intriguing observation may be interpreted in part by the effect of Bcl-2 prolonging the transition from $G_0$ or $G_1$ to S phase of the cycle [92]. However, further studies are needed to determine the significance of Bcl-2 expression in the apoptosis or survival of breast cancers.

Increasing evidence demonstrates that apoptosis resistance in cancer cells is as a result of impairment of the mitochondria-mediated apoptotic pathway by downregulating the function or levels of proapoptotic Bcl-2 family proteins in cancer cells [105]. It has been shown that transgenic mice deficient in Bax have accelerated onset of tumor growth [106]. Bax frameshift mutations are found in over 50% of colon and gastric cancers of the microsatellite mutator phenotype [107]. Bax deficiency has been shown to promote drug resistant and oncogenic transformation of cells. Results from analysis of the level of Bax expression in breast cancer tissues show that most breast cancer tissues weakly express Bax gene and about one-third of the cancer tissues have lost this gene expression. Moreover, a reduced Bax level is associated with a poor response to therapy, faster tumor progression, and an overall poorer prognosis for the patient [108-110]. On the other hand, overexpression of the BAX gene induces apoptotic cell death and enhances the effect of chemotherapy drugs on cancer cell lines [108].

## 4.3 Inhibitor of apoptosis protein family

In addition to upstream apoptotic inhibitory factors that control the activation of cell death receptor or mitochondria pathway, the apoptotic signal is also regulated by the inhibitor of apoptosis protein (IAP) family. IAPs are a family of proteins containing one or more conserved, cysteine and histidine-rich baculoviral IAP repeat (BIR) N-terminal domains and a C-terminal RING domain. About seven IAP proteins, including NAIP, XIAP, c-IAP1, c-IAP2, survivin, Livin and Ts-IAP, have been identified and their roles in inhibiting caspase activity have been elucidated [7,9,111-113]. The BIR domains of the IAPs form the zinc-figure-like structures that bind to the surface of caspases to block caspase activity. The RING domain acts as an ubiquitin ligase to facilitate the proteasomal degradation of caspases [114]. Specific interactions of BIR domains with different caspases have been determined by studying the structures of caspases and IAPs. The results from crystallography and mutagenesis studies of XIAP show that the proximal link region of BIR2 binds and blocks the active site of caspase-3 and -7. The interaction of the BIR2 domain with the amino-terminal of the small subunit of caspase 7 further stabilizes the binding. The BIR3 domains of XIAP, c-IAP1 and C-IAP2 are able to bind and inhibit active caspase-9.

Single BIR domain IAP proteins such as livin and Ts-IAP have been demonstrated to bind and inhibit caspase-9. However, the role of another single BIR domain protein, survivin, in the inhibition of caspase-3 and -7 is still controversial. Although physical interactions between survivin and caspases, and inhibition of caspase-3 and -7 activities have been reported, a structural basis for a direct interaction between survivin and caspase-3 has not been defined [115]. Increasing evidence suggests that survivin is closely associated with mitochondria-dependent apoptosis. Downregulation of survivin expression or function results in the activation of caspase-9. A recent study shows that survivin is able to associate with XIAP through the BIR domain and form a survivin-XIAP complex that promotes increased XIAP stability and synergistic inhibition of apoptosis [116].

It has been shown that Smac/DIABLO (second mitochondria activator of caspases), a proapoptotic protein released together with cytochrome C from mitochondria into the cytosol, interacts with all mammalian IAP proteins on both BIR 2 and BIR 3 domains. Binding of Smac to IAPs inactivates the function of IAPs and enhances the apoptotic response by releasing caspases from the IAP-inhibition [117,118].

Upregulation of IAPs is found in many tumor cell lines as well as in primary tumor tissues. Although XIAP is expressed at a low level in normal cells and tissues, a high level of XIAP is detected in many human tumor cells. Increases in XIAP expression have been associated with apoptosis resistance and low sensitivity to chemotherapy drugs in several tumor types. Downregulation of XIAP releases its inhibition on caspase-3 and induces apoptotic cell death in tumor cell lines as well as *in vivo* in a mouse tumor model. In addition to increasing the XIAP level, tumor cells also downregulate cellular factors that inhibit XIAP function. In normal cells, expression of XIAP associated factor 1 (XAF1) counteracts the anti-apoptotic function of XIAP by competing with active caspases for XIAP binding sites and releasing caspases from XIAP inhibition [119]. However, the level of XAF 1 is decreased or lost in many tumor cell types [7,119-121].

Unlike other IAPs, survivin is expressed broadly in embryonic and fetal tissues but is undetectable in most differentiated normal adult tissues, except thymocytes, CD 34+ stem cells and basal colonic epithelial cells [122,123]. However, survivin is expressed in most common tumor types including brain, lung, breast, liver, pancreas, gastric, colon, uterus, ovary, lymphoma, leukemia, melanoma and soft tissue sarcomas [123-125]. For example, survivin is found in over 70% of human breast or pancreatic cancer tissues and in 64% of human colon tissues [80,126,127]. Expression of the survivin gene in human tumor cells is regulated at a transcriptional level through increasing survivin promoter activity, amplification of the survivin locus on 17q25, demethylation of survivin exon 1, and releasing transcriptional repression by

p53 mutation [123,128-131]. Recent studies also demonstrate that survivin is a reliable marker for aggressive disease, resistant to chemo- or radio-therapy and indicative of a poor prognosis for human cancers [132-134]. Overexpression of survivin in human tumor cells reduces the apoptotic response induced by various apoptosis stimuli [115]. Transgenic expression of survivin in the skin inhibits UVB-induced apoptosis in skin epidermal cells in the mice whereas it does not affect Fas-induced cell death [135]. On the other hand, downregulation of survivin function with anti-sense, siRNA, dominant negative mutant or the ribozyme for survivin induces apoptotic cell death and sensitizes cancer cells to chemotherapy drugs [79,135-137].

In addition to its anti-apoptotic function, survivin is also linked to mitotic progression and cell division. Expression of survivin is increased in cells undergoing mitosis. Disrupting survivin function results in cells with centrosome deregulation, multipolar mitotic spindles and multinucleated nuclei [138]. Therefore, survivin has a dual function in regulating the cell cycle progression and blocking apoptotic signaling.

# 5. REGULATION OF APOPTOSIS SIGNAL BY OTHER CELL SIGNAL TRANSDUCTION PATHWAYS

## 5.1 Tumor suppressor gene p53

Mutation of tumor suppressor gene p53 is one of the most common types of genetic alterations in human tumors. p53 suppresses tumor growth through multiple pathways that involve gene transcription, DNA synthesis and repair, cell cycle arrest, senescence and apoptosis. Mutations of p53 gene or loss of p53 function results in tumor progression, genetic instability and apoptosis resistance [139-141]. It has been shown that p53 regulates both extrinsic and intrinsic apoptotic pathways through the transactivating transcription of proapoptotic factors and suppressing expression of anti-apoptotic genes. For example, upregulation of cell death receptors such as Fas and TRAIL-Rs is detected in tumor cells following DNA-damaging or chemotherapy drug-induced p53 expression [142]. Induction of transcription of proapoptotic Bcl-2 family genes including Bid, Bax, and Puma, and APAF-1 by p53 further enhances the mitochondria-mediated apoptosis [4,143,144]. Importantly, p53 also acts as a transrepressor for anti-apoptosis factors. It binds to survivin promoter and inhibits survivin gene transcription [131]. In addition, p53 itself can activate apoptosis without utilization of its transcription function. For example, p53 protein directly localizes to

mitochondria following DNA damage and interacts with anti-apoptotic protein Bcl-2 and Bcl-$X_L$ to promote apoptosis [145,146]. Mutations in p53 have been found in more than half of human tumors [139]. p53 mutations in human cancer cells confer apoptosis resistance and promote survival and progression of the tumors.

## 5.2 PI3 kinase/AKT pathway

The Phosphatidylinositol 3-kinase (PI3K) pathway is a major cell survival pathway activated by growth factors, cytokins, and hormones [147]. PI3K is a heterodimer composed of a p85 regulatory and a p110 catalytic subunit. Active PI3K phosphorylates 3-phosphorylated lipid phosphatidylinositol-3,4,5-trisphophosphate (PtdIns(3,4,5,)P3), which then recruits the phosphoinositide-dependent protein kinases (PDK 1 and PDK 2) and protein kinase B (AKT) to the cellular membrane [148]. In the complex, PDKs activate AKT by phosphorylation [149]. Activation of AKT mediates a series of downstream effects to promote cell survival, such as phosphorylation and inhibition of proapoptotic Bad and caspase 9 and decreasing p53-mediated transcription of proapoptotic genes [150,151]. Phosphorylation of XIAP by Akt protects XIAP from ubiquitination and degradation in response to apoptosis stimuli [152]. Moreover, activation of the PI3K/AKT pathway after VEGF treatment increases the level of IAP protein survivin in endothelial cells [153] .

The role of the PI3K/AKT pathway in the survival, growth and metastasis of tumor cells has been extensively studied. It has been shown that the PI3K/AKT pathway is highly activated in many tumor types [154,155]. The presence of an activated PI3K/AKT signal confers tumor cell resistance to apoptosis induction by growth factor withdrawal or chemotherapy drugs. Further, inhibition of PI3K/AKT activity greatly increases apoptotic cell death and drug sensitivity [156].

PI3K activity is negatively regulated by a tumor suppressor gene PTEN. PTEN antagonizes PI3K function by removing the 3-phosphate from (PtdIns(3,4,5,)P3). PTEN gene is frequently mutated or lost in several human tumor types [157]. Loss of PTEN function releases the inhibition on the PI3K/AKT pathway and increases the cell survival.

## 5.3 NFκB pathway

Nuclear factor κB (NFκB) is a transcriptional factor regulating apoptosis and cell survival. NFκB is present in cytoplasm in an inactive state by binding with its inhibitor protein, IκB. Upon receiving external stimuli, such as stress, cytokines, DNA damaging reagents or pathogens, IκB is

phosphorylated and then degraded by ubiquitinylation, resulting in migration of DNA-bound subunit NFκB into the nucleus and activation of transcription of target genes [158]. It has been shown that NFκB functions as either an anti-apoptotic or a proapoptotic factor [159-162]. Recent findings have provided important insights into the role of the NFκB in regulating life and death decision. In the TNF-α activated cell death pathway, recruiting TRAF2 into TNFR, TRADD and RIP1 complex activates NFκB resulting in transcriptional activation of the caspase 8 inhibitor cFLIP. However, binding of the same complex to FADD activates caspase-8 and -10 and induces apoptosis [163]. NFκB-dependent transcription of anti-apoptotic Bcl-2 family proteins such as Bcl-2 and Bcl-$X_L$ confers protection against hypoxia and nitric oxide-induced apoptosis [114,164,165]. Upregualtion of the expression of the IAP genes such as c-IAP1, c-IAP2 and XIAP further enhances the anti-apoptotic effect of the NFκB [114,166,167]. Although it is clear that NFκB is a critical cell survival factor, there are a number of reports showing that under certain circumstances, activation of NFκB promotes apoptosis. NFκB induces expression of proapoptotic factor genes such as p53, FasL, TRAIL, cell death receptors and proapoptotic Bcl-Xs protein [168].

The anti-apoptotic activity of NFκB has been shown to be an important factor for tumorigenesis [161]. A high level of constitutive nuclear NFκB activity has been found in many human leukemias, lymphomas and solid tumors [169-171]. Suppression of the NFκB function results in apoptosis and/or sensitization of tumor cells to TNF-α or chemotherapy drug-induced apoptosis [166,172].

# 6. TUMOR ENVIRONMENT AND APOPTOSIS RESISTANCE

## 6.1 Hypoxia

It is well known that human tumors contain regions that are deficient in oxygen due to a rapid growth rate of the tumor cells and the presence of an abnormal vasculature [173]. Studies have shown that there are significant associations between intratumoral hypoxia and tumor metastasis, response to chemotherapy or radiotherapy, and prognosis of cancer patients [174-177]. Hypoxia induces upregulation of a key transcription factor, HIF-1 α, which mediates transcription of hypoxia-inducible genes in the cells [173]. It has been shown that hypoxia upregulates either anti-apoptotic or proapoptotic factors in cancer cells depending on the cell types and experimental conditions [178,179].

Evidence indicates that hypoxia suppresses the apoptosis induced by chemotherapy drugs or γ-irradiation. A recent study demonstrates that hypoxia-induced HIF-1 α expression protects HepG2 cells from apoptosis induction [180]. Resistance to staurosporine-induced apoptosis in hypoxic cells is mediated by an HIF-1 α independent upregulation of c-IAP2 [181]. Treatment of cancer cells with chronic hypoxia results in selective growth of apoptosis resistant cells that express a high level of anti-apoptotic Bcl-2 family protein BCL-$X_L$ [182]. It has also been shown that hypoxia activates the PI3K/Akt/NFκB and the MAPK(Erk) signaling pathways, resulting in the resistance of pancreatic cancer cells to gemcitabine treatment [183]. Hypoxia also increases the level of survivin expression in human tumor cells through HIF-1 α– dependent transcription. However, survivin is not expressed in normal cells either under normoxic or hypoxic conditions [184]; unpublished results, Lily Yang).

Despite its anti-apoptotic effects, hypoxia also activates proapoptotic factors and induces apoptosis in cancer cells. It has been demonstrated that hypoxia-induced apoptosis mainly relies on mitochondrial pathways. In human tumors, hypoxia may lead to the selection of hypoxia-resistant cells with defects in mitochondrial apoptosis signaling pathways [179]. Expression of a proapoptotic Bcl-2 family protein, BNIP3, is increased in hypoxia through transcriptional activation of BNIP 3 by HIF-1 α. The presence of a hypoxia-responsive element in the BNip3 promoter that activates the level of BNIP 3 gene transcription by HIF-1 α has been demonstrated in many types of human cancer cell lines [185]. In pancreatic cancer tissues, methylation of BNIP 3 promoter inhibits the expression of the BNIP 3 gene despite the upregulation of other hypoxia-inducible genes, resulting in resistance to hypoxia-induced apoptosis. Restoration of BNIP 3 expression increases the sensitivity of the pancreatic cancer cells to hypoxia-induced cell death [186].

Moreover, hypoxia also induces the stabilization of p53 protein, which is a key transcription factor for promoting apoptosis [187]. It is possible that the dual effects of hypoxia on apoptosis are influenced by the severity of hypoxia in the cells. The proapoptotic function of HIF-1 α is activated in the cells under extreme hypoxia when the cellular protective function is not sufficient to protect cells from hypoxia damage [178].

## 6.2 Extracellular matrix

It is well established that extracellar matrix (ECM) is a critical regulator for signal transduction pathways. Interactions between cancer cells and ECM also contribute to the survival and apoptosis resistance in the cells [188,189]. Loss of contact between ECM and cells has been associated with apoptosis induction and lumen formation during normal tissue development [12]. A

special form of apoptotic cell death, anoikis, is induced in the cells that have lost contact with ECM and surrounding cells [190]. A recent study further demonstrates that ECM increases expression of antiapoptotic proteins Bcl-2 and Bcl-$X_L$ and reduced drug-induced apoptosis in small lung cancer cells, myelomas and gliomas [188,189,191]. Additionally, upregulation of matrix metalloproteinase (MMP) is a common phenomenon in human tumors and has been associated with tumor progression, metastasis and angiogenesis [191,192]. It has been shown that MMP-7, which is produced by tumor cells, specifically cleaves Fas and FasL, resulting in inhibition of Fas-mediated apoptosis [188,193,194]. Overexpression of the MMP-7 gene in the mouse mammary gland promotes mammary hyperplasia and accelerates the onset of oncogene-induced mammary tumors [195]. Cell-ECM interactions are mediated by adhesion receptors such as integrins on the cell surface. Studies have shown that integrins are expressed in some human tumor cells as well as in angiogenic tumor endothelial cells. Interaction of β1 integrin with ECM in breast cancer cells significantly inhibits apoptosis induced by chemotherapy drugs paclitaxel and vincristine [196]. A recent study reports that loss of cell attachment to ECM induces caspase independent apoptosis through releasing a mitochondria protein Bit-1 into the cytosol and inducing apoptosis in the cell. Tumor cells expressing the αvβ5 integrin initiates signals capable of blocking Bit-induced apoptosis [197].

## 7. MOLECULAR TARGETS IN APOPTOSIS SIGNAL PATHWAY FOR CANCER THERAPY

Understanding molecular alterations in apoptosis signal pathway helps to identify novel therapeutic targets. Results from the examination of apoptotic effectors and regulators in the apoptotic signal pathway in various tumor types demonstrate the presence of a deregulated apoptosis signal pathway in human cancer cells. Those defects confer apoptosis resistance and provide growth advantage for the tumor cells.

Strategies for targeting upstream defects in apoptosis pathways are developed and the feasibility of those approaches has been evaluated in human tumor cell lines and animal tumor models. For example, it has been shown that overexpression of death receptor-ligands, such as Fas L and TRAIL, with adenoviral vectors or delivery of recombinant FasL and TRAIL induces apoptotic cell death and sensitizes the response to chemotherapy drugs in some tumor cells [198]. However, extensive investigations of this approach on various human tumor cells reveals that many human tumor cells are resistant to FasL or TRAIL-induced apoptosis despite the expression of cell-death receptors on the cells [25,26,56].

Since activation of caspases is a hallmark for apoptosis induction, a logical approach for activating apoptosis is to express procaspase or active caspase genes in tumor cell. The feasibility of apoptosis induction by overexpression of procaspase-3, -7, -8 and -9, and an engineered autocatalytic caspase-3 have been examined in several laboratories using various tumor cells [199-201]. The results of those studies have shown that expression of procaspase or active caspase gene is able to induce apoptotic cell death in many human tumor cell lines. However, as compared to apoptosis induction in normal cells, tumor cell lines are less sensitive to caspase-induced apoptosis than normal cells[79].

Deregulation of Bcl-2 family proteins is found in many cancer types. Strategies downregulating anti-apoptotic or increasing the levels of proapoptotic Bcl-2 family proteins have been developed and some of them are already in clinical trails to determine the toxicity and efficiency. It has been shown that a decrease in Bcl-2 expression using Bcl-2 antisense induces apoptosis and sensitizing the cells to chemotherapy drugs [98]. Expression of Bax or Bak genes from adenoviral vectors shows anti-tumor effects both *in vitro* and in animal tumor models [202,203]. Small peptides targeting Bcl-2 and Bcl-$X_L$ are capable of inhibiting activity of Bcl-2 and Bcl-$X_L$ and have shown therapeutic potential as anticancer drugs for treating cancers overexpressing Bcl-2 and/or Bcl-X(L) proteins [97].

Results from dissecting deregulated apoptotic signals in human tumor cells further show that although different upstream deficiencies, such as Fas mutation and defects in caspase expression, are found in tumor cell lines and tissues, they are limited to small percentage of tumor cells in several cancer types [29,67,68,204]. On the other hand, upregulation of IAPs is a common feature for the majority tumor types [7,123]. Novel approaches targeting the IAP proteins should provide new ways to treat most human cancers.

A recent study has shown that inhibition of XIAP with small molecular antagonists stimulates an increase in the level of caspase activity and induces apoptotic cell death both in tumor cell lines and in established animal tumor models. Interestedly, apoptosis induction through inhibition of XIAP is tumor specific and there is very litter toxicity in normal cell lines as well as in normal tissues [205]. Inhibition of XIAP function could also be achieved through expression of a XIAP-counteracting protein gene, XAF1. Overexpression of XAF1 using an adenoviral vector selectively increases caspase 3 activity and induces apoptotic cell death in human breast and pancreatic cancer cells but not in normal cells [79].

Survivin is not expressed in normal cells but it is highly expressed in most tumor cells. Direct inhibition of survivin expression or function may have greater impact on the survival of tumor cells than for normal cells. Several reports have shown that inhibition of survivin function with

expression of a dominant negative mutant survivin (T34A), survivin antisense or siRNA increases caspase 9 activity and results in apoptotic cell death in human tumor cells and xenografted tumor models [123,136,206]. Importantly, downregulation of survivin specifically induces apoptotic cell death in tumor cells without obvious toxic effects on various normal cell lines [79,136]. Downregulation of survivin function also enhances the effects of chemotherapy drugs on the tumor cells [136,137]. Therefore targeting IAP proteins is a promising approach for the development of cancer-cell specific therapy.

At present, the mechanisms for tumor-specific induction of apoptosis by inhibiting IAP function are still under investigation. As discussed above, apoptosis is the physiological cell death process for the removal of abnormal cells. Human tumor cells are generated from multiple genetic alterations and have enormous abnormalities. These should cause activation of the apoptotic signal and induction of apoptosis. Evaluation of the process of tumor development suggests that this is the case for most transformed pre-neoplastic cells. Induction of apoptotic cell death in pre-neoplastic lesions has been found in early stage of human cancers such as breast ductal carcinoma *in situ* (DCIS) [207]. It has been shown that many DCIS lesions keep a balance between cell proliferation and apoptosis for many years without developing into invasive breast cancers. It is well established that treating rats with chemical carcinogens initiates many pre-neoplastic nodules in the liver but only a few of these develop into hepatocellular carcinomas [208]. It is possible that most pre-neoplastic cells are destroyed by apoptosis and only a small fraction of transformed cells that have upregulated their anti-apoptotic mechanisms, such as IAPs and Bcl-2, are able to survive and develop into a tumor mass. The selective growth of tumor cells with a high level of anti-apoptotic factors confers apoptosis resistance and a poor response to therapeutic reagents (Figure). Co-existence of high levels of active caspase 3 and IAP proteins, survivin and XIAP, has been demonstrated in human pancreas, colon and breast cancer cell lines that are not apoptotic [79]. The results from analysis of human breast cancer tissues further supported the presence of activated apoptotic signals and upregulated anti-apoptotic factors in cancer tissues. A positive correlation between the levels of active caspase-3 and -6, and the IAP proteins survivin and XIAP has been established using either immunostaining or Western blots with antibodies specific for active caspase-3, caspase 6, XIAP and survivin [77,79].

It has been shown that overexpression of procaspase 3 gene in ovary cancer cells increases survivin gene expression. It is possible that one of the cellular responses to a high level of caspase or caspase activity is to upregulate cell survival factors such as survivin and XIAP (Figure). The presence of a high balance between pro- and anti-apoptotic factors in human

tumor cells but not in normal cells suggests that targeting IAP proteins provides a selective advantage, inducing apoptotic cell death in human tumor cells while minimizing the effects on normal cells (Figure 1).

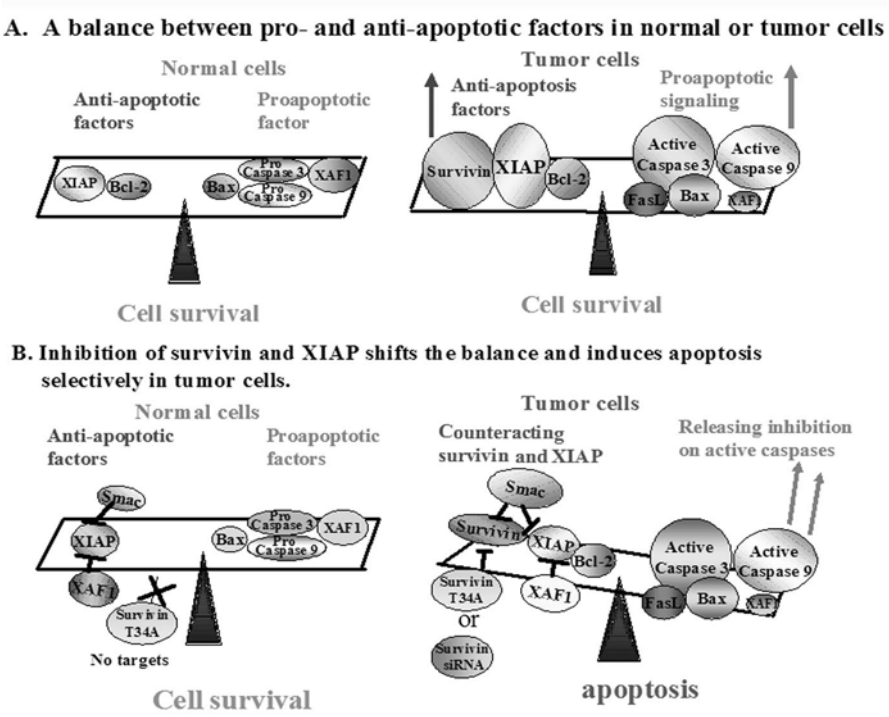


*Figure 1.* Targeting IAP proteins for cancer specific therapy. In normal cells, the absence of an apoptotic signal keeps a low balance between pro- and anti-apoptotic factors. However, molecular changes associated with malignant transformation of human tumor cells lead to activation of the apoptotic signals such as expression of FasL and activation of caspases. The tumor cells are able to block apoptosis by upregulating IAPs that inhibit active caspases. Therefore, cancer cells have high levels of both pro- and anti-apoptosis factors. The apoptotic process could be restored selectively in tumor cells by inhibiting IAP functions such as the expression of dominant negative survivinT34A gene and survivin siRNA, XIAP counteracting protein XAF1 and active Smac protein or peptides.

## REFERENCES


1. Wyllie AH. Apoptosis: cell death under homeostatic control. *Arch Toxicol Suppl* 1987; **11:** 3-10.
2. Rich T, Allen RL, Wyllie AH. Defying death after DNA damage. *Nature* 2000; **407:** 777-783.
3. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100:** 57-70.
4. Hickman ES, Helin K. The regulation of APAF1 expression during development and tumourigenesis. *Apoptosis* 2002; **7:** 167-171.
5. Wyllie AH *et al.* Apoptosis and carcinogenesis. *Br J Cancer* 1999; **80 Suppl 1:** 34-37.
6. Reed JC. Mechanisms of apoptosis avoidance in cancer. *Curr Opin Oncol* 1999; **11:** 68-75.
7. Holcik M, Gibson H, Korneluk RG. XIAP: apoptotic brake and promising therapeutic target. *Apoptosis* 2001; **6:** 253-261.
8. Igney FH, Krammer PH. Death and anti-death: tumour resistance to apoptosis. *Nat Rev Cancer* 2002; **2:** 277-288.
9. Altieri DC. Survivin and apoptosis control. *Adv Cancer Res* 2003; **88:** 31-52.
10. Zakeri Z, Lockshin RA. Cell death during development. *J Immunol Methods* 2002; **265:** 3-20.
11. Spencer SJ, Cataldo NA, Jaffe RB. Apoptosis in the human female reproductive tract. *Obstet Gynecol Surv* 1996; **51:** 314-323.
12. Debnath J *et al.* The role of apoptosis in creating and maintaining luminal space within normal and oncogene-expressing mammary acini. *Cell* 2002; **111:** 29-40.
13. Hahm HaD, NE. Apoptosis in the mammary gland and breast cancer. *Endocrine-Related Cancer* 1998; **5:** 199-211.
14. Collins M. Potential roles of apoptosis in viral pathogenesis. *Am J Respir Crit Care Med* 1995; **152:** S20-24.
15. Winoto A. Cell death in the regulation of immune responses. *Curr Opin Immunol* 1997; **9:** 365-370.
16. Kamarajan P, Chao CC. UV-induced apoptosis in resistant HeLa cells. *Biosci Rep* 2000; **20:** 99-108.
17. Park IC *et al.* Ionizing radiation and nitric oxide donor sensitize Fas-induced apoptosis via up-regulation of Fas in human cervical cancer cells. *Oncol Rep* 2003; **10:** 629-633.
18. Kaufmann SH, Earnshaw WC. Induction of apoptosis by cancer chemotherapy. *Exp Cell Res* 2000; **256:** 42-49.
19. Reed JC. Mechanisms of apoptosis. *Am J Pathol* 2000; **157:** 1415-1430.
20. Suzuki A *et al.* Mitochondrial regulation of cell death: mitochondria are essential for procaspase 3-p21 complex formation to resist Fas-mediated cell death. *Mol Cell Biol* 1999; **19:** 3842-3847.
21. Henry-Mowatt J, Dive C, Martinou JC, James D. Role of mitochondrial membrane permeabilization in apoptosis and cancer. *Oncogene* 2004; **23:** 2850-2860.
22. Budihardjo I *et al.* Biochemical pathways of caspase activation during apoptosis. *Annu Rev Cell Dev Biol* 1999; **15:** 269-290.
23. Stroh C, Schulze-Osthoff K. Death by a thousand cuts: an ever increasing list of caspase substrates. *Cell Death Differ* 1998; **5:** 997-1000.
24. Peter ME, Krammer PH. The CD95(APO-1/Fas) DISC and beyond. *Cell Death Differ* 2003; **10:** 26-35.
25. Barnhart BC *et al.* CD95 ligand induces motility and invasiveness of apoptosis-resistant tumor cells. *Embo J* 2004; **23:** 3175-3185.

26. Landowski TH *et al.* CD95 antigen mutations in hematopoietic malignancies. *Leuk Lymphoma* 2001; **42:** 835-846.
27. Beltinger C *et al.* CD95 (APO-1/Fas) mutations in childhood T-lineage acute lymphoblastic leukemia. *Blood* 1998; **91:** 3943-3951.
28. Lee SH *et al.* Alterations of Fas (Apo-1/CD95) gene in non-small cell lung cancer. *Oncogene* 1999; **18:** 3754-3760.
29. Boldrini L *et al.* Identification of Fas (APO-1/CD95) and p53 gene mutations in non-small cell lung cancer. *Int J Oncol* 2002; **20:** 155-159.
30. Bullani RR *et al.* Frequent downregulation of Fas (CD95) expression and function in melanoma. *Melanoma Res* 2002; **12:** 263-270.
31. Lee SH *et al.* Expression of Fas and Fas-related molecules in human hepatocellular carcinoma. *Hum Pathol* 2001; **32:** 250-256.
32. Nambu Y *et al.* Lack of cell surface Fas/APO-1 expression in pulmonary adenocarcinomas. *J Clin Invest* 1998; **101:** 1102-1110.
33. Viard-Leveugle I *et al.* Frequent loss of Fas expression and function in human lung tumours with overexpression of FasL in small cell lung carcinoma. *J Pathol* 2003; **201:** 268-277.
34. Lee SH *et al.* In vivo expression of soluble Fas and FAP-1: possible mechanisms of Fas resistance in human hepatoblastomas. *J Pathol* 1999; **188:** 207-212.
35. Liu JH *et al.* Blockade of Fas-dependent apoptosis by soluble Fas in LGL leukemia. *Blood* 2002; **100:** 1449-1453.
36. Elnemr A *et al.* Human pancreatic cancer cells disable function of Fas receptors at several levels in Fas signal transduction pathway. *Int J Oncol* 2001; **18:** 311-316.
37. Gerharz CD *et al.* Resistance to CD95 (APO-1/Fas)-mediated apoptosis in human renal cell carcinomas: an important factor for evasion from negative growth control. *Lab Invest* 1999; **79:** 1521-1534.
38. Muschen M *et al.* Resistance to CD95-mediated apoptosis in breast cancer is not due to somatic mutation of the CD95 gene. *Int J Cancer* 2001; **92:** 309-310.
39. Keane MM *et al.* Fas expression and function in normal and malignant breast cell lines. *Cancer Res* 1996; **56:** 4791-4798.
40. Ungefroren H *et al.* Human pancreatic adenocarcinomas express Fas and Fas ligand yet are resistant to Fas-mediated apoptosis. *Cancer Res* 1998; **58:** 1741-1749.
41. Suda T, Takahashi T, Golstein P, Nagata S. Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. *Cell* 1993; **75:** 1169-1178.
42. Bellgrau D *et al.* A role for CD95 ligand in preventing graft rejection. *Nature* 1995; **377:** 630-632.
43. Mullauer L *et al.* Fas ligand is expressed in normal breast epithelial cells and is frequently up-regulated in breast cancer. *J Pathol* 2000; **190:** 20-30.
44. Abrahams VM, Kamsteeg M, Mor G. The Fas/Fas ligand system and cancer: immune privilege and apoptosis. *Mol Biotechnol* 2003; **25:** 19-30.
45. Bennett MW *et al.* Fas ligand upregulation is an early event in colonic carcinogenesis. *J Clin Pathol* 2001; **54:** 598-604.
46. Herrnring C *et al.* Expression of the apoptosis-inducing ligands FasL and TRAIL in malignant and benign human breast tumors. *Histochem Cell Biol* 2000; **113:** 189-194.
47. Sheehan KM *et al.* Prognostic relevance of Fas (APO-1/CD95) ligand in human colorectal cancer. *Eur J Gastroenterol Hepatol* 2003; **15:** 375-380.
48. Boesen-de Cock JG *et al.* Common regulation of apoptosis signaling induced by CD95 and the DNA-damaging stimuli etoposide and gamma-radiation downstream from caspase-8 activation. *J Biol Chem* 1999; **274:** 14255-14261.

49. Fulda S *et al.* The CD95 (APO-1/Fas) system mediates drug-induced apoptosis in neuroblastoma cells. *Cancer Res* 1997; **57:** 3823-3829.
50. Landowski TH, Gleason-Guzman MC, Dalton WS. Selection for drug resistance results in resistance to Fas-mediated apoptosis. *Blood* 1997; **89:** 1854-1861.
51. Wang S, El-Deiry WS. TRAIL and apoptosis induction by TNF-family death receptors. *Oncogene* 2003; **22:** 8628-8633.
52. Kelley SK, Ashkenazi A. Targeting death receptors in cancer with Apo2L/TRAIL. *Curr Opin Pharmacol* 2004; **4:** 333-339.
53. LeBlanc HN, Ashkenazi A. Apo2L/TRAIL and its death and decoy receptors. *Cell Death Differ* 2003; **10:** 66-75.
54. Marsters SA, Pitti RA, Sheridan JP, Ashkenazi A. Control of apoptosis signaling by Apo2 ligand. *Recent Prog Horm Res* 1999; **54:** 225-234.
55. Ashkenazi A, Dixit VM. Apoptosis control by death and decoy receptors. *Curr Opin Cell Biol* 1999; **11:** 255-260.
56. Ibrahim SM *et al.* Pancreatic adenocarcinoma cell lines show variable susceptibility to TRAIL-mediated cell death. *Pancreas* 2001; **23:** 72-79.
57. Singh TR *et al.* Synergistic interactions of chemotherapeutic drugs and tumor necrosis factor-related apoptosis-inducing ligand/Apo-2 ligand on apoptosis and on regression of breast carcinoma in vivo. *Cancer Res* 2003; **63:** 5390-5400.
58. Jin Z, McDonald ER, 3rd, Dicker DT, El-Deiry WS. Deficient TRAIL death receptor transport to the cell surface in human colon cancer cells selected for resistance to TRAIL-induced apoptosis. *J Biol Chem* 2004.
59. Odoux C *et al.* TRAIL, FasL and a blocking anti-DR5 antibody augment paclitaxel-induced apoptosis in human non-small-cell lung cancer. *Int J Cancer* 2002; **97:** 458-465.
60. Shankar S, Srivastava RK. Enhancement of therapeutic potential of TRAIL by cancer chemotherapy and irradiation: mechanisms and clinical implications. *Drug Resist Updat* 2004; **7:** 139-156.
61. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. *Science* 1998; **281:** 1305-1308.
62. Morgan M, Thorburn J, Pandolfi PP, Thorburn A. Nuclear and cytoplasmic shuttling of TRADD induces apoptosis via different mechanisms. *J Cell Biol* 2002; **157:** 975-984.
63. Varfolomeev EE, Ashkenazi A. Tumor necrosis factor: an apoptosis JuNKie? *Cell* 2004; **116:** 491-497.
64. Micheau O *et al.* NF-kappaB signals induce the expression of c-FLIP. *Mol Cell Biol* 2001; **21:** 5299-5305.
65. Murray PG *et al.* Expression of the tumour necrosis factor receptor-associated factors 1 and 2 in Hodgkin's disease. *J Pathol* 2001; **194:** 158-164.
66. Zapata JM *et al.* TNFR-associated factor family protein expression in normal tissues and lymphoid malignancies. *J Immunol* 2000; **165:** 5084-5096.
67. Hopkins-Donaldson S *et al.* Silencing of death receptor and caspase-8 expression in small cell lung carcinoma cell lines and tumors by DNA methylation. *Cell Death Differ* 2003; **10:** 356-364.
68. Iolascon A *et al.* Caspase 3 and 8 deficiency in human neuroblastoma. *Cancer Genet Cytogenet* 2003; **146:** 41-47.
69. Teitz T *et al.* Caspase 8 is deleted or silenced preferentially in childhood neuroblastomas with amplification of MYCN. *Nat Med* 2000; **6:** 529-535.
70. Fujikawa K *et al.* Reduced expression of ICE/caspase1 and CPP32/caspase3 in human hepatocellular carcinoma. *Anticancer Res* 2000; **20:** 1927-1932.

71. Kolenko V *et al.* Dead or dying: necrosis versus apoptosis in caspase-deficient human renal cell carcinoma. *Cancer Res* 1999; **59:** 2838-2842.
72. Palmerini F *et al.* Caspase 7 downregulation as an immunohistochemical marker of colonic carcinoma. *Hum Pathol* 2001; **32:** 461-467.
73. Chu ZL *et al.* A novel enhancer of the Apaf1 apoptosome involved in cytochrome c-dependent caspase activation and apoptosis. *J Biol Chem* 2001; **276:** 9239-9245.
74. Soengas MS *et al.* Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. *Nature* 2001; **409:** 207-211.
75. Nakopoulou L *et al.* Immunohistochemical expression of caspase-3 as an adverse indicator of the clinical outcome in human breast cancer. *Pathobiology* 2001; **69:** 266-273.
76. Vakkala M, Paakko P, Soini Y. Expression of caspases 3, 6 and 8 is increased in parallel with apoptosis and histological aggressiveness of the breast lesion. *Br J Cancer* 1999; **81:** 592-599.
77. Parton M *et al.* Coordinate Expression of Apoptosis-associated Proteins in Human Breast Cancer before and during Chemotherapy. *Clin Cancer Res* 2002; **8:** 2100-2108.
78. O'Donovan N *et al.* Caspase 3 in breast cancer. *Clin Cancer Res* 2003; **9:** 738-742.
79. Yang L, Cao Z, Yan H, Wood WC. Coexistence of high levels of apoptotic signaling and inhibitor of apoptosis proteins in human tumor cells: implication for cancer specific therapy. *Cancer Res* 2003; **63:** 6815-6824.
80. Satoh K *et al.* Expression of survivin is correlated with cancer cell apoptosis and is involved in the development of human pancreatic duct cell tumors. *Cancer* 2001; **92:** 271-278.
81. Yoo NJ *et al.* Stomach cancer highly expresses both initiator and effector caspases; an immunohistochemical study. *Apmis* 2002; **110:** 825-832.
82. Kennedy NJ, Kataoka T, Tschopp J, Budd RC. Caspase activation is required for T cell proliferation. *J Exp Med* 1999; **190:** 1891-1896.
83. Mouhamad S *et al.* Differential modulation of interleukin-2-and interleukin-4-mediated early activation of normal human B lymphocytes by the caspase inhibitor zVAD-fmk. *Eur Cytokine Netw* 2002; **13:** 439-445.
84. Rendl M *et al.* Caspase-14 expression by epidermal keratinocytes is regulated by retinoids in a differentiation-associated manner. *J Invest Dermatol* 2002; **119:** 1150-1155.
85. Reichmann E. The biological role of the Fas/FasL system during tumor formation and progression. *Semin Cancer Biol* 2002; **12:** 309-315.
86. Scaffidi C, Schmitz I, Krammer PH, Peter ME. The role of c-FLIP in modulation of CD95-induced apoptosis. *J Biol Chem* 1999; **274:** 1541-1548.
87. Abedini MR, Qiu Q, Yan X, Tsang BK. Possible role of FLICE-like inhibitory protein (FLIP) in chemoresistant ovarian cancer cells in vitro. *Oncogene* 2004.
88. Lee SH *et al.* Increased expression of FLIP, an inhibitor of Fas-mediated apoptosis, in stomach cancer. *Apmis* 2003; **111:** 309-314.
89. Griffith TS *et al.* Intracellular regulation of TRAIL-induced apoptosis in human melanoma cells. *J Immunol* 1998; **161:** 2833-2840.
90. Kamarajan P, Sun NK, Chao CC. Up-regulation of FLIP in cisplatin-selected HeLa cells causes cross-resistance to CD95/Fas death signalling. *Biochem J* 2003; **376:** 253-260.
91. Reed JC. Bcl-2 family proteins. *Oncogene* 1998; **17:** 3225-3236.
92. Cory S, Huang DC, Adams JM. The Bcl-2 family: roles in cell survival and oncogenesis. *Oncogene* 2003; **22:** 8590-8607.

93. Reed JC. Bcl-2: prevention of apoptosis as a mechanism of drug resistance. *Hematol Oncol Clin North Am* 1995; **9:** 451-473.
94. Gee JM *et al.* Immunocytochemical localization of BCL-2 protein in human breast cancers and its relationship to a series of prognostic markers and response to endocrine therapy. *Int J Cancer* 1994; **59:** 619-628.
95. Tu Y *et al.* Upregulated expression of BCL-2 in multiple myeloma cells induced by exposure to doxorubicin, etoposide, and hydrogen peroxide. *Blood* 1996; **88:** 1805-1812.
96. Fulda S, Meyer E, Debatin KM. Inhibition of TRAIL-induced apoptosis by Bcl-2 overexpression. *Oncogene* 2002; **21:** 2283-2294.
97. Wang S, Yang D, Lippman ME. Targeting Bcl-2 and Bcl-XL with nonpeptidic small-molecule antagonists. *Semin Oncol* 2003; **30:** 133-142.
98. Reed JC. Promise and problems of Bcl-2 antisense therapy. *J Natl Cancer Inst* 1997; **89:** 988-990.
99. Reed JC. Apoptosis-based therapies. *Nat Rev Drug Discov* 2002; **1:** 111-121.
100. Krajewski S *et al.* Prognostic significance of apoptosis regulators in breast cancer. *Endocr Relat Cancer* 1999; **6:** 29-40.
101. Feuerhake F *et al.* Immunohistochemical analysis of Bcl-2 and Bax expression in relation to cell turnover and epithelial differentiation markers in the non-lactating human mammary gland epithelium. *Cell Tissue Res* 2000; **299:** 47-58.
102. Joensuu H, Pylkkanen L, Toikkanen S. Bcl-2 protein expression and long-term survival in breast cancer. *Am J Pathol* 1994; **145:** 1191-1198.
103. Zhang GJ *et al.* Apoptotic index correlates to bcl-2 and p53 protein expression, histological grade and prognosis in invasive breast cancers. *Anticancer Res* 1998; **18:** 1989-1998.
104. Villar E *et al.* bcl-2 Expression and apoptosis in primary and metastatic breast carcinomas. *Tumour Biol* 2001; **22:** 137-145.
105. Gutierrez MI *et al.* Bax is frequently compromised in Burkitt's lymphomas with irreversible resistance to Fas-induced apoptosis. *Cancer Res* 1999; **59:** 696-703.
106. Knudson CM, Johnson GM, Lin Y, Korsmeyer SJ. Bax accelerates tumorigenesis in p53-deficient mice. *Cancer Res* 2001; **61:** 659-665.
107. Rampino N *et al.* Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. *Science* 1997; **275:** 967-969.
108. Bargou RC *et al.* 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* 1995; **60:** 854-859.
109. Krajewski S *et al.* Reduced expression of proapoptotic gene BAX is associated with poor response rates to combination chemotherapy and shorter survival in women with metastatic breast adenocarcinoma. *Cancer Res* 1995; **55:** 4471-4478.
110. Redondo M *et al.* Expression of bax and p53 proteins in the tumorigenesis and progression of breast carcinomas. *Tumour Biol* 2003; **24:** 23-31.
111. Roy N *et al.* The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. *Embo J* 1997; **16:** 6914-6925.
112. Suzuki Y *et al.* X-linked inhibitor of apoptosis protein (XIAP) inhibits caspase-3 and -7 in distinct modes. *J Biol Chem* 2001; **276:** 27058-27063.
113. Kasof GM, Gomes BC. Livin, a novel inhibitor of apoptosis protein family member. *J Biol Chem* 2001; **276:** 3238-3246.
114. Zhang HG *et al.* Regulation of apoptosis proteins in cancer cells by ubiquitin. *Oncogene* 2004; **23:** 2009-2015.

115. Tamm I *et al.* IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. *Cancer Res* 1998; **58:** 5315-5320.
116. Dohi T *et al.* An IAP-IAP complex inhibits apoptosis. *J Biol Chem* 2004; **279:** 34087-34090.
117. Du C *et al.* Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. *Cell* 2000; **102:** 33-42.
118. Liu Z *et al.* Structural basis for binding of Smac/DIABLO to the XIAP BIR3 domain. *Nature* 2000; **408:** 1004-1008.
119. Liston P *et al.* Identification of XAF1 as an antagonist of XIAP anti-Caspase activity. *Nat Cell Biol* 2001; **3:** 128-133.
120. Fong WG *et al.* Expression and genetic analysis of XIAP-associated factor 1 (XAF1) in cancer cell lines. *Genomics* 2000; **70:** 113-122.
121. Byun DS *et al.* Hypermethylation of XIAP-associated factor 1, a putative tumor suppressor gene from the 17p13.2 locus, in human gastric adenocarcinomas. *Cancer Res* 2003; **63:** 7068-7075.
122. Adida C *et al.* Developmentally regulated expression of the novel cancer anti-apoptosis gene survivin in human and mouse differentiation. *Am J Pathol* 1998; **152:** 43-49.
123. Altieri DC. Survivin, versatile modulation of cell division and apoptosis in cancer. *Oncogene* 2003; **22:** 8581-8589.
124. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med* 1997; **3:** 917-921.
125. Li F. Survivin study: what is the next wave? *J Cell Physiol* 2003; **197:** 8-29.
126. Tanaka K *et al.* Expression of survivin and its relationship to loss of apoptosis in breast carcinomas. *Clin Cancer Res* 2000; **6:** 127-134.
127. Sarela AI *et al.* Expression of survivin, a novel inhibitor of apoptosis and cell cycle regulatory protein, in pancreatic adenocarcinoma. *Br J Cancer* 2002; **86:** 886-892.
128. Islam A *et al.* Role of survivin, whose gene is mapped to 17q25, in human neuroblastoma and identification of a novel dominant-negative isoform, survivin-beta/2B. *Med Pediatr Oncol* 2000; **35:** 550-553.
129. Hattori M, Sakamoto H, Satoh K, Yamamoto T. DNA demethylase is expressed in ovarian cancers and the expression correlates with demethylation of CpG sites in the promoter region of c-erbB-2 and survivin genes. *Cancer Lett* 2001; **169:** 155-164.
130. Bao R *et al.* Activation of cancer-specific gene expression by the survivin promoter. *J Natl Cancer Inst* 2002; **94:** 522-528.
131. Hoffman WH *et al.* Transcriptional repression of the anti-apoptotic survivin gene by wild type p53. *J Biol Chem* 2002; **277:** 3247-3257.
132. Asanuma K *et al.* A role for survivin in radioresistance of pancreatic cancer cells. *Jpn J Cancer Res* 2002; **93:** 1057-1062.
133. Kawasaki H *et al.* Inhibition of apoptosis by survivin predicts shorter survival rates in colorectal cancer. *Cancer Res* 1998; **58:** 5071-5074.
134. Kami K *et al.* Survivin expression is a prognostic marker in pancreatic cancer patients. *Surgery* 2004; **136:** 443-448.
135. Grossman D *et al.* Transgenic expression of survivin in keratinocytes counteracts UVB-induced apoptosis and cooperates with loss of p53. *J Clin Invest* 2001; **108:** 991-999.
136. Mesri M *et al.* Cancer gene therapy using a survivin mutant adenovirus. *J Clin Invest* 2001; **108:** 981-990.
137. Zangemeister-Wittke U. Antisense to apoptosis inhibitors facilitates chemotherapy and TRAIL-induced death signaling. *Ann N Y Acad Sci* 2003; **1002:** 90-94.

138. Li F *et al.* Pleiotropic cell-division defects and apoptosis induced by interference with survivin function. *Nat Cell Biol* 1999; **1:** 461-466.
139. Vogelstein B, Kinzler KW. p53 function and dysfunction. *Cell* 1992; **70:** 523-526.
140. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature* 2000; **408:** 307-310.
141. Polyak K *et al.* A model for p53-induced apoptosis. *Nature* 1997; **389:** 300-305.
142. Muller M *et al.* p53 activates the CD95 (APO-1/Fas) gene in response to DNA damage by anticancer drugs. *J Exp Med* 1998; **188:** 2033-2045.
143. Yin C, Knudson CM, Korsmeyer SJ, Van Dyke T. Bax suppresses tumorigenesis and stimulates apoptosis in vivo. *Nature* 1997; **385:** 637-640.
144. Yu J *et al.* PUMA mediates the apoptotic response to p53 in colorectal cancer cells. *Proc Natl Acad Sci U S A* 2003; **100:** 1931-1936.
145. Marchenko ND, Zaika A, Moll UM. Death signal-induced localization of p53 protein to mitochondria. A potential role in apoptotic signaling. *J Biol Chem* 2000; **275:** 16202-16212.
146. Mihara M *et al.* p53 has a direct apoptogenic role at the mitochondria. *Mol Cell* 2003; **11:** 577-590.
147. Danielsen AJ, Maihle NJ. The EGF/ErbB receptor family and apoptosis. *Growth Factors* 2002; **20:** 1-15.
148. Ram TG, Ethier SP. Phosphatidylinositol 3-kinase recruitment by p185erbB-2 and erbB-3 is potently induced by neu differentiation factor/heregulin during mitogenesis and is constitutively elevated in growth factor-independent breast carcinoma cells with c-erbB-2 gene amplification. *Cell Growth Differ* 1996; **7:** 551-561.
149. Toker A, Newton AC. Akt/protein kinase B is regulated by autophosphorylation at the hypothetical PDK-2 site. *J Biol Chem* 2000; **275:** 8271-8274.
150. Datta SR *et al.* Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* 1997; **91:** 231-241.
151. Sabbatini P, McCormick F. Phosphoinositide 3-OH kinase (PI3K) and PKB/Akt delay the onset of p53-mediated, transcriptionally dependent apoptosis. *J Biol Chem* 1999; **274:** 24263-24269.
152. Dan HC *et al.* Akt phosphorylation and stabilization of X-linked inhibitor of apoptosis protein (XIAP). *J Biol Chem* 2004; **279:** 5405-5412.
153. Tran J *et al.* Marked induction of the IAP family antiapoptotic proteins survivin and XIAP by VEGF in vascular endothelial cells. *Biochem Biophys Res Commun* 1999; **264:** 781-788.
154. Fry MJ. Phosphoinositide 3-kinase signalling in breast cancer: how big a role might it play? *Breast Cancer Res* 2001; **3:** 304-312.
155. Hutchinson J *et al.* Activation of Akt (protein kinase B) in mammary epithelium provides a critical cell survival signal required for tumor progression. *Mol Cell Biol* 2001; **21:** 2203-2212.
156. O'Gorman DM, McKenna SL, McGahon AJ, Cotter TG. Inhibition of PI3-kinase sensitises HL60 human leukaemia cells to both chemotherapeutic drug- and Fas-induced apoptosis by a JNK independent pathway. *Leuk Res* 2001; **25:** 801-811.
157. Haas-Kogan D *et al.* Protein kinase B (PKB/Akt) activity is elevated in glioblastoma cells due to mutation of the tumor suppressor PTEN/MMAC. *Curr Biol* 1998; **8:** 1195-1198.
158. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. *Annu Rev Immunol* 2000; **18:** 621-663.
159. Kimura K, Gelmann EP. Propapoptotic effects of NF-kappaB in LNCaP prostate cancer cells lead to serine protease activation. *Cell Death Differ* 2002; **9:** 972-980.

160. Dudley E *et al.* NF-kappaB regulates Fas/APO-1/CD95- and TCR- mediated apoptosis of T lymphocytes. *Eur J Immunol* 1999; **29:** 878-886.
161. Karin M, Cao Y, Greten FR, Li ZW. NF-kappaB in cancer: from innocent bystander to major culprit. *Nat Rev Cancer* 2002; **2:** 301-310.
162. Kucharczak J, Simmons MJ, Fan Y, Gelinas C. To be, or not to be: NF-kappaB is the answer--role of Rel/NF-kappaB in the regulation of apoptosis. *Oncogene* 2003; **22:** 8961-8982.
163. Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell* 2003; **114:** 181-190.
164. Grumont RJ, Rourke IJ, Gerondakis S. Rel-dependent induction of A1 transcription is required to protect B cells from antigen receptor ligation-induced apoptosis. *Genes Dev* 1999; **13:** 400-411.
165. Viatour P *et al.* NF- kappa B2/p100 induces Bcl-2 expression. *Leukemia* 2003; **17:** 1349-1356.
166. Notarbartolo M *et al.* Expression of the IAPs in multidrug resistant tumor cells. *Oncol Rep* 2004; **11:** 133-136.
167. Wang Q, Wang X, Evers BM. Induction of cIAP-2 in human colon cancer cells through PKC delta/NF-kappa B. *J Biol Chem* 2003; **278:** 51091-51099.
168. Harwood FG *et al.* Regulation of FasL by NF-kappaB and AP-1 in Fas-dependent thymineless death of human colon carcinoma cells. *J Biol Chem* 2000; **275:** 10023-10029.
169. Lin A, Karin M. NF-kappaB in cancer: a marked target. *Semin Cancer Biol* 2003; **13:** 107-114.
170. Cao Y, Karin M. NF-kappaB in mammary gland development and breast cancer. *J Mammary Gland Biol Neoplasia* 2003; **8:** 215-223.
171. Eid MA, Lewis RW, Abdel-Mageed AB, Kumar MV. Reduced response of prostate cancer cells to TRAIL is modulated by NFkappaB-mediated inhibition of caspases and Bid activation. *Int J Oncol* 2002; **21:** 111-117.
172. Meli M *et al.* NF-kappaB inhibition restores sensitivity to Fas-mediated apoptosis in lymphoma cell lines. *Ann N Y Acad Sci* 2003; **1010:** 232-236.
173. Semenza GL. Involvement of hypoxia-inducible factor 1 in human cancer. *Intern Med* 2002; **41:** 79-83.
174. Shibaji T *et al.* Prognostic significance of HIF-1 alpha overexpression in human pancreatic cancer. *Anticancer Res* 2003; **23:** 4721-4727.
175. Akakura N *et al.* Constitutive expression of hypoxia-inducible factor-1alpha renders pancreatic cancer cells resistant to apoptosis induced by hypoxia and nutrient deprivation. *Cancer Res* 2001; **61:** 6548-6554.
176. Bardos JI, Ashcroft M. Hypoxia-inducible factor-1 and oncogenic signalling. *Bioessays* 2004; **26:** 262-269.
177. Weinmann M, Belka C, Plasswilm L. Tumour hypoxia: impact on biology, prognosis and treatment of solid malignant tumours. *Onkologie* 2004; **27:** 83-90.
178. Piret JP, Mottet D, Raes M, Michiels C. Is HIF-1alpha a pro- or an anti-apoptotic protein? *Biochem Pharmacol* 2002; **64:** 889-892.
179. Weinmann M *et al.* Molecular ordering of hypoxia-induced apoptosis: critical involvement of the mitochondrial death pathway in a FADD/caspase-8 independent manner. *Oncogene* 2004; **23:** 3757-3769.
180. Piret JP *et al.* Hypoxia and CoCl2 protect HepG2 cells against serum deprivation- and t-BHP-induced apoptosis: a possible anti-apoptotic role for HIF-1. *Exp Cell Res* 2004; **295:** 340-349.

181. Dong Z, Wang JZ, Yu F, Venkatachalam MA. Apoptosis-resistance of hypoxic cells: multiple factors involved and a role for IAP-2. *Am J Pathol* 2003; **163:** 663-671.
182. Dong Z, Wang J. Hypoxia selection of death-resistant cells. A role for Bcl-X(L). *J Biol Chem* 2004; **279:** 9215-9221.
183. Yokoi K, Fidler IJ. Hypoxia increases resistance of human pancreatic cancer cells to apoptosis induced by gemcitabine. *Clin Cancer Res* 2004; **10:** 2299-2306.
184. Yang L *et al.* Tumor-specific gene expression using the survivin promoter is further increased by hypoxia. *Gene Ther* 2004; **11:** 1215-1223.
185. Sowter HM *et al.* HIF-1-dependent regulation of hypoxic induction of the cell death factors BNIP3 and NIX in human tumors. *Cancer Res* 2001; **61:** 6669-6673.
186. Okami J, Simeone DM, Logsdon CD. Silencing of the hypoxia-inducible cell death protein BNIP3 in pancreatic cancer. *Cancer Res* 2004; **64:** 5338-5346.
187. An WG *et al.* Stabilization of wild-type p53 by hypoxia-inducible factor 1alpha. *Nature* 1998; **392:** 405-408.
188. Mitsiades N, Poulaki V, Mitsiades CS, Anderson KC. Induction of tumour cell apoptosis by matrix metalloproteinase inhibitors: new tricks from a (not so) old drug. *Expert Opin Investig Drugs* 2001; **10:** 1075-1084.
189. Sethi T *et al.* Extracellular matrix proteins protect small cell lung cancer cells against apoptosis: a mechanism for small cell lung cancer growth and drug resistance in vivo. *Nat Med* 1999; **5:** 662-668.
190. Valentijn AJ, Zouq N, Gilmore AP. Anoikis. *Biochem Soc Trans* 2004; **32:** 421-425.
191. Hojilla CV, Mohammed FF, Khokha R. Matrix metalloproteinases and their tissue inhibitors direct cell fate during cancer development. *Br J Cancer* 2003; **89:** 1817-1821.
192. Duffy MJ *et al.* Metalloproteinases: role in breast carcinogenesis, invasion and metastasis. *Breast Cancer Res* 2000; **2:** 252-257.
193. Strand S *et al.* Cleavage of CD95 by matrix metalloproteinase-7 induces apoptosis resistance in tumour cells. *Oncogene* 2004; **23:** 3732-3736.
194. Mitsiades N *et al.* Matrix metalloproteinase-7-mediated cleavage of Fas ligand protects tumor cells from chemotherapeutic drug cytotoxicity. *Cancer Res* 2001; **61:** 577-581.
195. Vargo-Gogola T, Fingleton B, Crawford HC, Matrisian LM. Matrilysin (matrix metalloproteinase-7) selects for apoptosis-resistant mammary cells in vivo. *Cancer Res* 2002; **62:** 5559-5563.
196. Aoudjit F, Vuori K. Integrin signaling inhibits paclitaxel-induced apoptosis in breast cancer cells. *Oncogene* 2001; **20:** 4995-5004.
197. Jan Y *et al.* A mitochondrial protein, Bit1, mediates apoptosis regulated by integrins and Groucho/TLE corepressors. *Cell* 2004; **116:** 751-762.
198. Ambar BB *et al.* Treatment of experimental glioma by administration of adenoviral vectors expressing Fas ligand. *Hum Gene Ther* 1999; **10:** 1641-1648.
199. Komata T *et al.* Caspase-8 gene therapy using the human telomerase reverse transcriptase promoter for malignant glioma cells. *Hum Gene Ther* 2002; **13:** 1015-1025.
200. Shinoura N *et al.* Transduction of Apaf-1 or caspase-9 induces apoptosis in A-172 cells that are resistant to p53-mediated apoptosis. *Biochem Biophys Res Commun* 2000; **272:** 667-673.
201. Shinoura N *et al.* Adenovirus-mediated transfer of caspase-3 with Fas ligand induces drastic apoptosis in U-373MG glioma cells. *Exp Cell Res* 2000; **256:** 423-433.
202. Li X *et al.* Adenovirus-mediated Bax overexpression for the induction of therapeutic apoptosis in prostate cancer. *Cancer Res* 2001; **61:** 186-191.
203. Pataer A *et al.* Adenovirus-mediated Bak gene transfer induces apoptosis in mesothelioma cell lines. *J Thorac Cardiovasc Surg* 2001; **121:** 61-67.

204. Park WS *et al.* Somatic mutations in the death domain of the Fas (Apo-1/CD95) gene in gastric cancer. *J Pathol* 2001; **193:** 162-168.
205. Schimmer AD *et al.* Small-molecule antagonists of apoptosis suppressor XIAP exhibit broad antitumor activity. *Cancer Cell* 2004; **5:** 25-35.
206. Grossman D, Kim PJ, Schechner JS, Altieri DC. Inhibition of melanoma tumor growth in vivo by survivin targeting. *Proc Natl Acad Sci U S A* 2001; **98:** 635-640.
207. Bodis S *et al.* Extensive apoptosis in ductal carcinoma in situ of the breast. *Cancer* 1996; **77:** 1831-1835.
208. Pitot HC *et al.* Quantitation of multistage carcinogenesis in rat liver. *Toxicol Pathol* 1996; **24:** 119-128.