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REVIEW

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Current status of the molecular mechanisms of anticancer drug-induced apoptosis

The contribution of molecular-level analysis to cancer chemotherapy

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Abstract Apoptosis is an important phenomenon in cytotoxicity induced by anticancer drugs. Here, we review the current status of the molecular mechanisms of anticancer drug-induced apoptosis in order to assess the contribution of molecular-level analysis to cancer chemotherapy. It is apparent that the molecular mechanisms by which anticancer drugs induce apoptosis are mediated by death receptor-dependent and -independent pathways, which are related to the release of cytochrome *c* through voltage-dependent anion channels in the mitochondrial inner membrane. The release of cytochrome *c* is the central gate in turning on/off apoptosis, and is regulated by the interaction of proapoptotic proteins, including Bid, Bax and Bak, and antiapoptotic proteins including Bcl-2 and Bcl-X_L, and a specific class of inhibitors of apoptosis proteins (IAPs) including Akt, survivin, and heat-shock proteins. The caspase cascade is activated by the release of cytochrome *c*, which is initiated by the formation of apoptosomes consisting of procaspase-9, Apaf-1 and cytochrome *c* in the presence of dATP, and results in the activation of caspase-9 and caspase-3, thereby leading to apoptosis. Drug sensitivity can be enhanced by the introduction of proapoptotic genes and the inhibition of antiapoptotic proteins. The latter process is mediated by antisense oligonucleotides and is associated with apoptosis. The signal transduction pathways that are triggered by the central gate in mitochondria play a critical role in anticancer drug-induced apoptosis. The modulation of signal transduction pathways targeting the proteins involved in these signal transduction pathways using antisense IAPs, and growth factor antibodies may be a good strategy for enhancing therapeutic efficacy of anticancer drugs in cancer chemotherapy.

Keywords Apoptosis · Molecular mechanisms · Drug sensitivity · Anticancer drugs · Cancer chemotherapy

Abbreviations *BSO:* buthionine sulfoximine · *CAD:* caspase-activated DNAase · *DR:* death receptor · *Hsp:* heat shock protein · *IAP:* inhibitor of apoptosis protein · *ICAD:* inhibitor of caspase-activated DNAase · *JNK:* Jun N-terminal protein kinase · *MRP:* multidrug resistance-related protein · *PI 3K:* phosphatidylinositol 3 kinase · *tBid:* truncated Bid · *TNF:* tumor necrosis factor · *TRAIL:* tumor necrosis factor-related apoptosis-inducing ligand · *VDAC:* voltage-dependent anion channel · *XIAP:* X-linked inhibitor of apoptosis protein

Introduction

The phenomenon of apoptosis, which is morphologically distinct from that of necrotic cell death, was first described by Kerr et al. in 1972 [1]. In the cell undergoing apoptosis, extracellular stimuli induce nuclear condensation and cellular shrinkage preserving the cell membrane, whereas necrosis is characterized by destruction of the cell membrane via an increase in osmotic pressure from outside the cell [1, 2, 3]. Apoptosis is therefore known as programmed cell death and follows the activation of signal transduction [4, 5]. In cancer cells, the growth balance, which is determined as the ratio between the rate of cell proliferation and incidence of apoptosis, is uncontrolled, and the increase in abnormal proliferation of cancer cells causes tumor

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invasion and metastatic potential [6]. Cell proliferation and apoptosis in tumor growth is regulated by several oncogenes and tumor suppressor genes associated with the cell cycle, as well as by apoptosis-related genes such as those of the p53 and bcl-2 families [7, 8, 9].

Apoptosis is an important phenomenon in cancer chemotherapy, because anticancer drugs exert their antitumor effect against cancer cells by inducing apoptosis. The apoptotic body, which is characterized by nuclear condensation and cell shrinkage is subsequently dealt with by phagocytosis by tumor associated-macrophages. Although the rate of apoptosis, which is assessed in terms of the apoptotic index, is not necessarily high in tumor tissues, the induction of apoptosis is correlated with tumor response and clinical outcome in cancer patients [10, 11, 12]. Resistance to apoptosis causes a decrease in the sensitivity of cancer cells to drugs, resulting in the failure of chemotherapy. Since the induction of apoptosis following chemotherapy is associated with the activation of proapoptotic genes and the suppression of antiapoptotic genes, attenuation of proapoptotic genes and increases in antiapoptotic genes causes resistance to apoptosis. Thus, to increase the therapeutic effect of cancer chemotherapy, the assessment of molecular mechanisms and targeting apoptosis-related genes may lead to new strategies for the enhancement of the antitumor effect against target organs. We review here recent advances in the elucidation of the molecular mechanisms of apoptosis induced by anticancer drugs, and discuss molecular targeting of apoptosis-related genes for cancer chemotherapy.

General pathway of apoptosis

The general pathway of apoptosis is initiated by various extracellular stimuli, such as DNA damage, heat shock, Fas, and growth factor deprivation, and is known to be an irreversible event. The process is activated by apoptosis genes and the caspase cascade (Fig. 1). The activation of caspase is mediated by two pathways consisting of the initiator caspases, caspase-8 and -9, and effector caspases, caspase-3 and -7 [5, 13]. DNA fragmentation is induced by the activation of endonuclease, which in turn is activated by the cleavage of ICAD, resulting in the activation of CAD [14, 15]. Another pathway leading to apoptosis is the nuclear condensation pathway mediated by acinus [16].

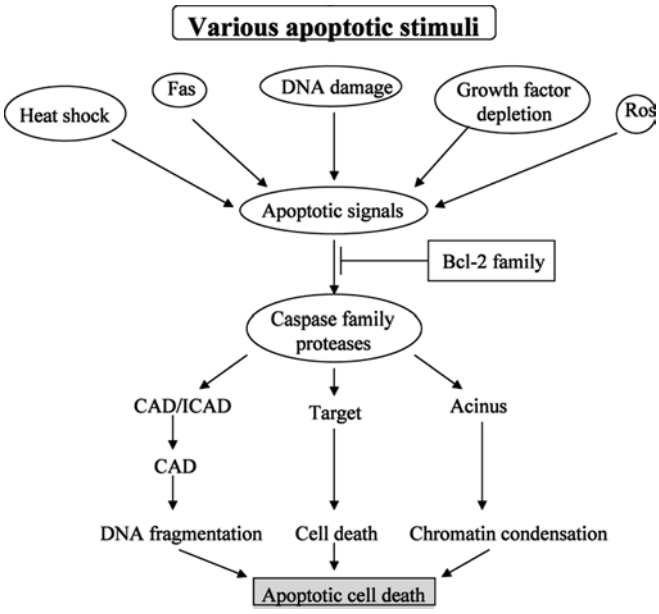

Fig. 1. Common pathways of apoptosis. Apoptosis is an irreversible event initiated by various extracellular stimuli, such as DNA damage, heat shock, Fas, and growth factor deprivation, as well as by activation of apoptosis genes and the caspase cascade. The activation of the apoptotic signals can be blocked by Bcl-2 family proteins. Apoptotic cell death can be subdivided into three types, which are characterized by DNA fragmentation by CAD, cell death without small DNA fragmentation, and chromatin condensation by acinus

Molecular mechanisms of apoptosis induced by anticancer drugs

DR-independent pathway

The critical event in the initiation of apoptosis is mitochondrial dysfunction, which is regulated by bcl-2 family proteins. Bcl-2 family proteins consist of only three subfamilies, the proapoptotic and antiapoptotic proteins, including Bcl-2 and Bax, and the BH-3 subfamily [17]. The antiapoptotic Bcl-2 subfamily includes Bcl-X_L, Bcl-w and Mcl-1, the proapoptotic Bax subfamily includes Bax, Bak and Bok proteins, and the BH-3 subfamily includes Bik, Bim, Bad, and Bid proteins. Sequencing alignment and mutagenesis studies have identified up to four conserved domains in Bcl-2 family proteins, which are termed BH-1, BH-2, BH-3 and BH-4, and which have important functions. The BH-4 domain is found in only Bcl-2 protein and dimerizes with proapoptotic proteins as part of the antiapoptotic function, whereas the BH-3 domain is involved in the proapoptotic function. The BH-1 and BH-2 domains show ion channel activity for regulating the release of cytochrome *c* from mitochondria [18, 19]. The switching on and off of apoptosis is determined by the ratio of proapoptotic to antiapoptotic proteins [20, 21].

After induction of the apoptotic stimuli by anticancer drugs, the proapoptotic proteins, including Bax, Bak and Bad, are induced in accordance with their sensitivity to the anticancer drugs. The activation of the bax gene leads to an increase in the amount of protein, which forms Bax homodimers that are translocated from the cytoplasm to the mitochondria [22]. The homodimerized Bax acts on VDAC, which is localized in the outer membrane of mitochondria resulting in the release of cytochrome *c* which activates the caspase cascade. The release of cytochrome *c* is also induced by heterodimers with Bax and Bak in the same way [23]. Bak-deficient

leukemia cells are resistant to apoptosis induced by etoposide and cisplatin, and the resistance to anticancer drugs of Bak-deficient cells cannot be restored by recombinant Bax or truncated Bid [24]. Although Bax is translocated from the cytosol to the mitochondrial membrane following treatment with anticancer drugs, Bax leaves the mitochondrial membrane, Bak colocalizes in the Bax clusters to form Bax and Bak heterodimers resulting in cell death progression [25].

With respect to the mechanism by which the Bax homodimers release cytochrome *c*, three models have been proposed: (1) cytochrome *c* is released through a VDAC in the mitochondrial outer membrane (VDAC-Bax theory) [26], (2) Bax homodimers form another ion channel in the mitochondria leading to the release of cytochrome *c* (Bax ion channel theory) [27], and swollen mitochondria cause destruction of the outer membrane, resulting in the release of cytochrome *c* (destroyed outer membrane theory) [28]. Since it has been shown that microinjection of a VDAC-neutralizing antibody inhibits apoptosis [29], it appears that the release of cytochrome *c* is mediated through the ion channel. On the other hand, it has also been reported that the release of cytochrome *c* is inhibited by the antiapoptotic proteins Bcl-2 and Bcl-X_L, which, like Bax, have ion channel activity [30, 31, 32]. Thus, it is clear that regulation of apoptotic signals is mediated through release of cytochrome *c* in the mitochondrial membrane following treatment with anticancer drugs, and this process is regulated by the proapoptotic proteins Bax and Bak, and the antiapoptotic proteins Bcl-2 and Bcl-X_L. The release of cytochrome *c* from mitochondria has been shown to promote the oligomerization of a cytochrome *c*/Apaf-1/procaspase-9 complex (apoptosome) that activates caspase-9 resulting in the cleavage of downstream effector caspase-3 and -7 in the presence of dATP leading to apoptosis (Fig. 2) [17, 33, 34, 35].

DR-dependent pathway

Another pathway leading to apoptosis following treatment with anticancer drugs is the DR-dependent pathway mediated by Fas, DR4 and DR5 in cancer cells [36, 37]. The DR pathway was initially identified by Fas/Fas L and TRAIL/DR4 and DR5 systems in immunological response to cytotoxic T cell and TNF. Subsequent reports have also indicated that an increase in the expression of Fas and DR5 following treatment with anticancer drugs is involved in the DR-independent apoptotic pathway through activation of caspase-8 [38]. The mechanisms by which the stimulation of Fas induces apoptosis are divided into two subpathways. First, DISC (death-inducing signaling complex) formation, which consists of Fas-associated DR domain, caspase-8 and Fas, resulting from stimulation of a large amount of Fas leads to activation of caspase-8 followed by direct cleavage of caspase-3 and leads in turn to apoptosis (type I, mitochondria-independent pathway). Second, stimulation of Fas activates caspase-8 to cleave Bid protein, resulting in truncated Bid (tBid) and the release of cytochrome *c* from mitochondria, which is mediated by dimerizing with Bak (type II, mitochondria-dependent pathway) [39, 40]. A small amount of activated caspase-8 is sufficient to activate caspase-3 through the activated Bid protein for releasing cytochrome *c* in mitochondria. Only the type II pathway of apoptosis can be blocked by overexpressed Bcl-2 of Bcl-X_L.

Cell survival factors (PI 3K/Akt pathway, Hsp, survivin, and NF-κB)

Because Akt, a serine and threonine kinase, is known to be an important survival factor in signal transduction pathways involved in cell growth, this kinase is a possible target for anticancer drug-induced apoptosis.

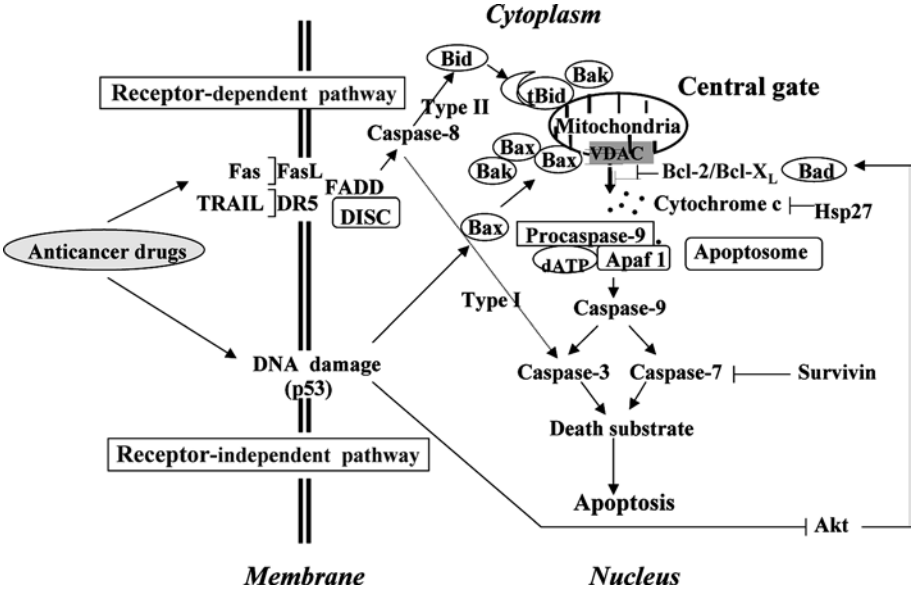

Fig. 2. DR-dependent and -independent pathways in anticancer drug-induced apoptosis. The central gate of cytochrome *c* release in mitochondria is regulated by a balancing of the signals of proapoptotic proteins and IAPs. Drug sensitivity can be modulated by the activation of proapoptotic proteins, including Bax, Bak, and Bid, to release cytochrome *c* leading to apoptosis, and/or the blocking of IAPs, including Bcl-2, Akt, survivin, and Hsp27, in the regulation of premitochondrial and postmitochondrial actions

Overexpression of Akt has been reported to be involved in drug resistance [41], and treatment with anticancer drugs such as CPT-11 and growth factor deprivation have been shown to suppress the activity of Akt leading to loss of cell viability and apoptosis [42]. In this context, it is worth noting that Bad, a BH-3 family protein, is phosphorylated by Akt, which blocks dimerization with Bcl-2 to promote cell survival [43, 44]. The activation of Akt also directly inhibits caspase-9 in apoptosis [45]. Akt activity is regulated by XIAP, and treatment with cisplatin has been shown to decrease XIAP protein levels and to induce Akt cleavage and apoptosis in drug-sensitive ovarian cancer cells, but not in resistant cells [46]. The downregulation of Akt by anticancer drugs can increase susceptibility to apoptosis in the signal transduction pathways by targeting the ion channel and the downstream region of caspase-9, thereby leading to apoptosis.

The Hsps, including Hsp70 and Hsp27, are involved in the inhibition of downstream apoptotic pathways [47, 48]. Hsp27 can block the formation of apoptosome preventing procaspase-9 activation and therefore caspase-9 activation[49], whereas Hsp70 can interact with the antiapoptotic protein, Bcl-2, to inhibit the release of cytochrome *c* from mitochondria. Abrogation of Hsp70 expression by antisense Hsp70 has been shown to induce apoptosis in oral squamous cell cancer cells, leading to a decrease in Bcl-2 protein [50]. In contrast, although treatment with the antisense Hsp70 induces apoptosis in breast cancer cells, overexpression of Bcl-2 and Bcl-X_L cannot protect against apoptosis in cancer cells [51]. It remains to be determined whether Hsp70 and Bcl-2 interact to contribute to the inhibition of apoptosis.

Survivin is a member of the apoptosis-inhibiting family, along with Bcl-2 and Hsps, and is expressed in different cancer tissues, including breast, lung, colon, pancreas, stomach, and soft tissue sarcoma [52, 53, 54, 55, 56, 57]. Increased expression of survivin has been correlated with a reduction in tumor cell apoptosis in gastric and colorectal cancers, and is also associated with shorter patient survival. Survivin can block apoptosis induced by growth factor deprivation, anti-Fas, and anticancer drugs [55, 58]. Interestingly, the survivin gene is overexpressed in the G_2/M phase of the cell cycle, and downregulated following cell cycle arrest, suggesting that it has an antiapoptotic function in the G_2/M cell cycle checkpoint for the progression of transformed cells [58]. It has been hypothesized that survivin blocks apoptosis by binding to and inhibiting the activation of the effector caspases-3 and -7, but not by inhibiting the initiator protease caspase-8 [55, 59]. A more recent report indicates that the loss of phosphorylation on Thr34 results in dissociation of a survivin-caspase-9 complex in mitosis, indicating that phosphorylation of Thr34 by p34(cdc2)-cyclinB1 may be required to inhibit caspase-9 activation for cell viability at cell division [60]. Treatment with cisplatin has been shown to increase the expression levels of mRNA and protein in gastric cancer cells, suggesting that survivin expression may correlate with chemoresistance [61]. In contrast, treatment with antisense survivin induces apoptosis, and sensitizes lung cancer cells and HeLa cells to the chemotherapeutic agent etoposide [62, 63]. Further, the transcriptional regulation of survivin is mediated by wild-type p53, and its overexpression markedly inhibits apoptosis induced by DNA damage [64].

NF-κB is also known to be a member of the IAP family which regulate immune and inflammatory responses as transcription factors [65]. NF-κB induces cellular resistance to apoptosis by its transcriptional activation of cellular IAP1 (cIAP1) and cIAP2, and other specific antiapoptotic proteins including A20 and Mn-SOD [66, 67]. It has been suggested that IAP induced by NF-κB plays a more important role in cancer progression than survivin. Transfection of the dominant negative construct of I-κB (inhibitory factor of NF-κB) has been shown to increase sensitivity to TNF, doxorubicin, and paclitaxel in breast cancer cells [68]. NF-κB also contributes to resistance in CD95 and TRAIL receptor-mediated apoptosis [69]. IAP inhibits the functional activity of proapoptotic proteins, such as Bax and Bak, as well as the activity of caspases by binding to the proteins. NF-κB also induces transcription of the bcl-2 gene, which has been identified as an NF-κB site in the bcl-2 p2 promoter region in prostate cancer cells [70]. Moreover, NF-κB can partially inhibit the transactivation of the bax promoter by p53, which has been identified as one of the three putative binding sites for NF-κB in the human bax gene promoter [71]. In addition, phosphorylated Akt kinase is associated with NF-κB expression in squamous cell carcinoma, and this is mediated through activation of PI 3K-dependent pathways [72]. NF-κB downregulates the JNK cascades, suggesting a link between the NF-κB and the JNK signaling cascades in promoting apoptosis [73, 74].

In contrast, it has been demonstrated that etoposide and doxorubicin induce the NF-κ-dependent activation of both proapoptotic and a specific class of antiapoptotic proteins including TRAIL and its DR, DR5, and IAPs in human lung cancer cells [75]. Further, the inhibition of NF-κB activation by genotoxin induces a loss of cell surface expression of TRAIL and DR5, resulting in chemoresistance of the tumors in vivo. In addition, paclitaxel-induced inhibitor I-κB-alpha degradation and NF-κB activation may contribute to the mediation of paclitaxel-induced apoptosis in breast and ovarian cancer cells [76]. Further, activation of NF-κB is associated with sensitivity to camptothecin in HeLa cells in accordance with the low level of nonphosphorylated I-κB-alpha [77]. These findings suggest that activation of NF-κB in response to DNA damage is involved in both positive and negative regulation of apoptosis, depending on the target genes for transcriptional activation. However, the detailed mechanism by which transcriptional activation of NF-κB leads to apoptosis remains to be elucidated.

Molecular targeting of apoptosis-related proteins that enhance drug sensitivity

Enhancement of antitumor effect by introducing apoptosis genes

Introduction of proapoptotic genes would be expected to enhance drug sensitivity in association with apoptosis, because the activation of proapoptotic genes is required for the critical event of cytochrome *c* release from mitochondria. The introduction of the bax gene into ovarian cancer cells [78] and gastric cancer cells [79, 80] in vitro and in vivo, and of bcl-Xs into breast cancer cells in vitro [81] has been shown to enhance sensitivity to several drugs and apoptosis. Further, the stable transfectant of the BAD protein sensitizes human ovarian cancer cells to the cytotoxic effects of paclitaxel, vincristine, and to a lesser extent etoposide [82]. It is conceivable that the enhancement of apoptosis is also associated with induction of cytochrome *c* release and caspase-3. It has also been reported that introduction of the bax gene into gastric cancer cells coactivates JNK1 and caspase-3, which are more activated following treatment with docetaxel in the bax-transfected cells [79]. Although the involvement of JNK1 in apoptosis has been demonstrated in a transfection experiment [83], the important role of JNK1 in signal transduction pathways leading to apoptosis is the phosphorylation of Bcl-2 protein by treatment with microtubule-damaging agents, which results in the inhibition of the functional ability to dimerize with proapoptotic proteins [84]. These findings indicate that modulation of the signal transduction pathways of apoptosis in the upstream region of mitochondria effectively enhances the sensitivity of cancer cells to drugs.

Another means of enhancing apoptosis is to enhance sensitivity to drugs by introducing apoptosis genes into the downstream region of mitochondria. The introduction of Apaf-1 enhances sensitivity to several drugs associated with apoptosis [85], and the introduction of caspase-3 into MCF-7 breast cancer cells, which are deficient in caspase-3, reconstitutes the caspase-3-dependent pathway leading to apoptosis [86, 87, 88]. Although apoptosis in MCF-7 breast cancer cells can be induced by treatment with anticancer drugs, the apoptotic pathway is mediated by a caspase-3-independent pathway, which is characterized by activation of caspase-7 and -6 leading to apoptosis [87, 88]. The involvement of serine protease as well as cysteine protease (caspases) in apoptosis has been reported, and treatment with these inhibitors can partially block anticancer drug-induced apoptosis [89].

Enhancement of antitumor effect by inhibiting antiapoptotic genes

Another way to enhance apoptosis at the molecular level is by targeting inhibition of antiapoptotic genes using antisense oligonucleotides of bcl-2 and bcl-X_L. Modulation of drug sensitivity by cotreatment with antisense phosphorothioate oligonucleotides of bcl-2 has been reported in lymphoma and melanoma cells in vitro and in vivo, and this treatment is currently undergoing phase II and III study [90, 91]. Further, the specific dual inhibition of bcl-2 and bcl-X_L in colon cancer cells markedly enhances apoptosis without cotreatment with anticancer drugs [92]. These findings indicate that antiapoptotic proteins such as Bcl-2 and Bcl-X_L are good targets to modulate drug sensitivity by affecting apoptotic pathways.

Overexpression of Bcl-2 is associated with a poor response to chemotherapy and a poor prognosis in various human cancers [8]. In the case of breast cancer patients, however, a paradoxical role of Bcl-2 protein has been observed [93], since the overexpression of Bcl-2 protein has been correlated with estrogen receptor-positive tumors, the prognosis of which is better than that of estrogen receptor-negative tumors. The overexpression of Bcl-2 protein in estrogen receptor-positive tumors is mediated by estrogen receptor response elements in the promoter region of the bcl-2 gene [94]. Nevertheless, the above study also showed that overexpression of Bcl-2 in breast cancer is correlated with drug resistance, and that downregulation of Bcl-2 by antisense oligonucleotides modulates drug sensitivity in association with apoptosis. We have also observed that the introduction of Bcl-2 antisense with lipofectamine enhances the sensitivity of MDA-MB-231 breast cancer cells to mitomycin C and paclitaxel (unpublished results). Although the difference between the role played by Bcl-2 and that played by Bcl-X_L in the inhibition of apoptosis remains uncertain, overexpression of Bcl-X_L protein may play a more dominant role in drug resistance in epithelial cancer cells than overexpression of Bcl-2.

On the other hand, in breast cancer, the high expression of survivin is associated with a poorer prognosis than low expression, indicating the existence of drug resistance in the chemotherapy. Treatment with antisense survivin and mutant survivin is also a good strategy for enhancing drug sensitivity in the promotion of apoptosis. Treatment with antisense survivin has been shown to induce apoptosis, and to sensitize lung cancer cells to the chemotherapeutic agent etoposide [62], and in HeLa and PtK1 cells transfection with, or microinjection of, survivin antisense oligonucleotides has been shown to induce apoptosis characterized by micronucleated progeny [95]. Expression of a phosphorylation-defective inhibitor survivin mutant enhances apoptosis induced by cisplatin in human melanoma cell lines [96]. Similarly, a survivin mutant adenovirus induces spontaneous apoptosis in breast, lung and colorectal cancer cells, and also enhances sensitivity to chemotherapeutic drugs such as paclitaxel and doxorubicin in breast cancer cells in vivo [97], indicating the potential for a selective cancer gene therapy.

The role of modulation of apoptosis signals in cellular drug resistance

Because anticancer drugs at low and high concentrations cause apoptosis and necrosis, respectively, cancer can be killed apoptotically by anticancer drugs, and this cell death is dependent on activation of the internal constituents to induce apoptosis. Alteration of the apoptotic pathways is closely associated with drug resistance. As described above, the apoptotic pathways of anticancer drug-induced cell death involve mitochondria-dependent activation through Bax and mitochondria-independent activation through Fas activation. The mitochondria-independent pathway involving Fas crosstalks with the mitochondria-dependent pathway, which is mediated by the low-grade activation of tBid protein by cleavage by caspase-8. The activation of tBid results in allosteric activation of Bak through its oligomerization into pores leading to the release of cytochrome *c* [98]. It is important to note that the proapoptotic protein, Bax, which is highly homologous with Bak, can also enter mitochondria and induce cytochrome *c* efflux by posttranslational modulation by caspase-8. The significance of apoptosis in releasing cytochrome *c* in the signal transduction pathways is indicated by the finding that activation of the multidomain of Bax or Bak in mitochondria is required as an essential gateway in anticancer drug-induced apoptosis [99]. Alternatively, in anticancer drug-induced apoptosis, Bax insertion into mitochondria may be mediated by Bid-dependent and Bid-independent pathways [100].

It is well known that there are several drug-resistance factors, including drug transport, detoxification, and apoptotic pathways, and these factors cooperate to achieve multidrug resistance in cancer cells. It is not yet clear which factor is the most clinically relevant, or how these factors interact to confer drug resistance. The relationship between the membrane drug efflux ABC transporters, such as P-gp and MRP, and apoptosis-related proteins and their possible role in inducing drug resistance has been reported in subgroups coexpressing other resistant proteins [101, 102, 103]. With respect to the modulation of drug sensitivity of multidrug-resistant cells overexpressing P-gp, there are two possible ways to overcome drug resistance. One is to increase the concentration of P-gp inhibitors in resistant cells. In a study using multidrug-resistant MCF-7 breast cancer cells, which are cross-resistant to doxorubicin and paclitaxel due to the overexpression of P-gp, the P-gp inhibitor valspodar has been shown to restore the accumulation of paclitaxel, resulting in increased cytotoxicity and apoptosis [104]. Another way to overcome multidrug resistance is by modulating the signal transduction pathways in apoptosis by targeting the central gate of mitochondria to release cytochrome *c*. Combination treatment with TRAIL sensitizes doxorubicin-resistant melanoma cells to doxorubicin by inducing mitochondrial membrane depolarization for cytochrome *c* release and activation of caspase-9 and caspase-3, thereby leading to apoptosis [105].

Regarding modulation of apoptosis signals, our previous study using MRP-overexpressing multidrug-resistant KB cells, which are resistant to anthracyclines and vinca alkaloids, has demonstrated that introduction of bcl-Xs results in a more than 50% recovery of the sensitivity to vinca alkaloids of vincristine- and vindesine-resistant cells compared to parent cells [106]. In contrast to the finding that modulation of the membrane transporter of MRP by BSO causes a functional reduction to less than 50% in sensitivity to vinca alkaloids, the dual modulation by BSO in bcl-Xs-transfected cells leads to complete recovery in sensitivity to vinca alkaloids (unpublished results). Further, introduction of the bcl-Xs gene increases mRNA expression of bax, suggesting that the modulation of apoptosis pathways is mediated through the critical gateway of Bax in mitochondrial action leading to apoptosis. Interaction and functional cooperation between proapoptotic bax and bcl-Xs genes has been reported [107]. Thus, signal transduction of apoptosis pathways may be more dominantly involved in multidrug resistance than the membrane transporter, and the dual modulation of apoptotic signals and intracellular drug concentration may overcome multidrug resistance in cancer cells. From the above model, it appears that the signal transduction pathways involved in the activation of proapoptotic genes play a crucial role in overcoming cellular drug resistance in cancer cells, although the drug efflux pump involved in control of intracellular drug concentration still contributes in part to drug sensitivity. Regarding the interaction among drug-resistance factors, there have been no reports indicating which is the predominant factor affecting drug sensitivity in terms of promoting cell death.

Future perspectives

Combination therapy to modulate signal transduction pathways of apoptosis

Over the past decade, the molecular mechanism(s) by which anticancer drugs induce apoptosis have been clarified with a focus on mitochondrial action. It has been shown that this mechanism is characterized by the release of cytochrome *c* followed by activation of the caspase cascade. It is clear that the release of cytochrome *c* from mitochondria plays a central role in apoptosis induced by anticancer drugs, and several proapoptotic and antiapoptotic genes are involved in the regulation of apoptotic signals in the upstream and downstream regions of mitochondria. Since the modulation of signal transduction pathways leading to apoptosis is critical for modulation of drug sensitivity, the introduction of proapoptotic genes and the inhibition of antiapoptotic genes are effective in inducing apoptosis as described above. Interestingly, recent reports indicate that treatment with growth factor antibodies such as HER-2 and EGFR increases the sensitivity of breast and colon cancers to anthracyclines, taxanes and 5-FU [108, 109,

110]. Although the precise mechanisms by which the combination therapy with anti-HER-2 and anti-EGFR antibodies enhance drug sensitivity remains to be elucidated, it appears that modulation of the signal transduction pathways of apoptosis by these antibodies may facilitate cell death. In addition, the mechanism of this combination therapy might involve resensitization to the anticancer drugs through modulation of the apoptotic pathways. Regarding the modulation of apoptosis signals by anti-HER-2 antibody (Herceptin), it has been reported that treatment with Herceptin downregulates the expression of HER-2 protein thus enhancing apoptosis [111]. Treatment with antisense HER-2 also downregulates the Bcl-2 protein in association with apoptosis [112]. Furthermore, the tyrosine kinase inhibitors targeting the EGFR receptor and the VEGF receptor effectively induce apoptosis in drug-resistant tumors following treatment [113, 114, 115]. These findings imply that modulation of the signal transduction pathways of apoptosis by treatment with antibodies to HER-2, EGFR, and VEGF proteins and the tyrosine kinase inhibitors may provide new insights into the enhancement of drug sensitivity and overcoming drug resistance in cancer chemotherapy. Further investigations are needed to clarify the detailed molecular mechanisms involved in enhancing drug sensitivity in combination with molecular-targeting drugs.

Individualized chemotherapy using biomarkers of apoptosis-related proteins

Our improved understanding of the antitumor effects of anticancer drugs at the molecular level suggests the possibility of individualizing chemotherapy to the individual cancer patient. Assessment of the expression levels of proapoptotic and antiapoptotic proteins might be used to categorize patients as responders or nonresponders prior to chemotherapy. However, while there have been many retrospective analyses of the response to chemotherapy and the tumor expression of apoptosis-related proteins and other biomarkers, no definitive biomarkers have been established for distinguishing between responders and nonresponders prior to chemotherapy. Possible reasons for the difficulty in predicting tumor response include: (1) the interactions and predominant relationships between the many factors affecting drug sensitivity have not been clarified; (2) using DNA microarray analysis, candidate genes controlling the response to drug treatment have been identified, but it has not yet been established how the information can be used to identify responders; (3) in considering individualized chemotherapy, it may be more important to predict which patients will be intermediate responders and which nonresponders or to predict which patients will show poor survival and which will be responders, because responders with good survival can be treated by standard chemotherapy. Therefore, the top priorities in developing individualized chemotherapy should be to find a means of identifying nonresponders, and then to establish an appropriate chemotherapy targeting apoptosis-related proteins.

Development of new drugs and effective regimens targeting apoptosis-related proteins and signal transduction pathways is currently underway. Although the targeting of apoptosis-related genes using adenovirus or antisense strategies may be a useful method to enhance drug sensitivity, gene therapy cannot move beyond localized therapy unless the adverse effects on normal cells are overcome by producing greater tumor specificity. Elucidation of the molecular mechanisms of anticancer drug-induced cell death has provided several new strategies to enhance drug sensitivity in human cancers, and the complex regulation of apoptosis by the signal transduction pathways is being revealed. Further insights will be needed to fully utilize the apoptotic signaling pathways to stem the uncontrollable proliferation of cancer cells in patients with poor prognosis.

References

1. Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic phenomenon with wide-raging implications in tissue kinetics. Br J Cancer 26:239
2. Green DR, Reed JC (1998) Mitochondria and apoptosis. Science 281:1309
3. Gross A, McDonnell JM, Korsmeyer SJ (1999) BCL-2 family members and mitochondria in apoptosis. Genes Dev 13:1899
4. Evan G, Littlewood T (1998) A matter of life and cell death. Science 281:1317
5. Thornberry NA, Lazebnik Y (1998) Caspases: enemies within. Science 281:1312
6. Olopade OI, Adeyanju MO, Safa AR, Hagos F, Mick R, Thompson CB, Recant WM (1997) Overexpression of BCL-x protein in primary breast cancer is associated with high tumor grade and nodal metastases. Cancer J Sci Am 3:230
7. Ferreira CG, Tolis C, Giaccone G (1999) p53 and chemosensitivity. Ann Oncol 10:1011
8. Reed JC (1998) Bcl-2 family proteins. Oncogene 17:3225
9. Cao DT, Korsmeyer SJ (1998) Bcl-2 family: regulators of cell death. Annu Rev Immunol 16:395
10. Salomons GS, Smets LA, Verwijs-Janssen M, Hart AA, Haarman EG, Kaspers GJ, Wering EV, Der Does-Van Den Berg AV, Kamps WA (1999) Bcl-2 family members in childhood acute lymphoblastic leukemia: relationships with features at presentation, in vitro and in vivo drug response and long-term clinical outcome. Leukemia 13:1574
11. Cassinelli G, Supino R, Perego P, Polizzi D, Lanzi C, Pratesi G (2001) A role for loss of p53 function in sensitivity of ovarian carcinoma cells to taxanes. Int J Cancer 92:738
12. Kim R, Nishimoto N, Inoue H, Toge T (2000) An analysis of the therapeutic efficacy of protracted infusion of low-dose 5-fluorouracil and cisplatin in advanced gastric cancer. J Infect Chemother 6:222
13. Riedl SJ, Fuentes-Prior P, Renatus M, Kairies N, Krapp S, Huber R, Salvesen GS, Bode W (2001) Structural basis for the activation of human procaspase-7. Proc Natl Acad Sci U S A 98:14790
14. Nagata S (2000) Apoptotic DNA fragmentation. Exp Cell Res 256:12
15. Lechardeur D, Drzymala L, Sharma M, Zylka D, Kinach R, Pacia J, Hicks C, Usmani N, Rommens JM, Lukacs GL (2000) Determinants of the nuclear localization of the heterodimeric DNA fragmentation factor (ICAD/CAD). J Cell Biol 150:321

16. Sahara S, Aoto M, Eguchi Y, Imamoto N, Yoneda Y, Tsujimoto Y (1999) Acinus is a caspase-3-activated protein required for apoptotic chromatin condensation. Nature 401:168
17. Adams JM, Cory S (1998) The Bcl-2 protein family: arbiters of cell survival. Science 281:1322
18. Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng T-I, Jones DP, Wang X (1997) Prevention of apoptosis by Bcl-2: release of cytochrome *c* from mitochondria blocked. Science 275:1129
19. Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD (1997) The release of cytochrome *c* from mitochondria: a primary site for Bcl-2 regulation of apoptosis. Science 275:1132
20. Krajewski S, Blomqvist C, Franssila K, Krajewska M, Wasenius VM, Niskanen E, Nordling S, Reed JC (1995) Reduced expression of proapoptotic gene BAX is associated with poor response rates to combination chemotherapy and shorter survival in woman with metastatic breast adenocarcinoma. Cancer Res 55:4471
21. Ogura E, Senzaki H, Yamamoto D, Yoshida R, Takada H, Hioki K, Tsubura A (1999) Prognostic significance of Bcl-2, Bcl-XL/S, Bax and Bak expressions in colorectal carcinomas. Oncol Rep 6:365
22. Nomura M, Shimizu S, Ito T, Narita M, Matsuda H, Tsujimoto Y (1999) Apoptotic cytosol facilitates Bax translocation to mitochondria that involves cytosolic factor regulated by Bcl-2. Cancer Res 59:5542
23. Tsujimoto Y, Shimizu S (2000) VDAC regulation by the Bcl-2 family of proteins. Cell Death Differ 7:1174
24. Wang GQ, Gastman BR, Wieckowski E, Golstein LA, Gambotto A, Kim TH, Fang B, Rabinovitz A, Yin XM, Rabinowich H (2001) A role for mitochondrial Bak in apoptotic response to anticancer drugs: J Biol Chem 276:34307
25. Nechushtan A, Smith CL, Lamensdorf I, Yoon SH, Youle RJ (2001) Bax and Bak coalesce into novel mitochondria-associated clusters during apoptosis. J Cell Biol 153:1265
26. Shimizu S, Narita M, Tsujimoto Y (1999) Bcl-2 family proteins regulate the release of apoptogenic cytochrome *c* by the mitochondrial channel VDAC. Nature 399:483
27. Saito M, Korsmeyer SJ, Schlesinger PH (2000) BAX-dependent transport of cytochrome *c* reconstituted in pure liposomes. Nat Cell Biol 2:553
28. Vander Heiden MG, Chandel NS, Williamson EK, Schumacker PT, Thompson CB (1997) Bcl-XL regulates the membrane potential and volume homeostasis of mitochondria. Cell 91:627
29. Shimizu S, Matsuoka Y, Shinohara Y, Yoneda Y, Tsujimoto Y (2001) Essential role of voltage-dependent anion channel in various forms of apoptosis in mammalian cells. J Cell Biol 152:237
30. Schendel SL, Xie Z, Montal MO, Matsuyama S, Montal M, Reed JC (1997) Channel formation by antiapoptotic protein Bcl-2. Proc Natl Acad Sci U S A 94:5113
31. Minn AJ, Velez P, Schendel SL, Liang H, Muchmore SW, Fesik SW, Fill M, Thompson CB (1997) Bcl-XL forms an ion channel in synthetic lipid membranes. Nature 385:353
32. Narita M, Shimizu S, Ito T, Chittenden T, Lutz RJ, Matsuda H, Tsujimoto Y (1998) Bax interacts with the permeability transition pore to induce permeability transition and cytochrome *c* release in isolated mitochondria. Proc Natl Acad Sci U S A 95:14681
33. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemeri ES, Wang X (1997) Cytochrome *c* and dATP-dependent formation of Apaf-1/caspase-9 complex initiates apoptotic protease cascade. Cell 91:479
34. Hu Y, Benedict MA, Wu D, Inohara N, Nunez G (1998) Caspase 9, Bcl-XL interacts with Apaf-1 and inhibits Apaf-1-dependent caspase 9 activation. Proc Natl Acad Sci U S A 95:4386
35. Green DR (1998) Apoptotic pathways: the roads to ruin. Cell 94:695
36. Boldin MP, Goncharow TM, Goltsev YV, Wallach D (1996) Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1 and TNF receptor-induced cell death. Cell 85:803
37. Mizuo M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J, Scaffidi C, Bretz JD, Zhang M, Gentz R, Mann M, Krammer PH, Peter ME, Dixit VM (1996) FLICE, a noel FADD-homologous ICE/CED-3 like protease, is required to CD95 (Fas/APO-1) death-inducing signaling complex. Cell 85:817
38. Suliman A, Lam A, Datta R, Srivastava RK (2001) Intracellular mechanisms of TRAIL: apoptosis through mitochondrial-dependent and -independent pathways. Oncogene 20:2122
39. Scaffidi C, Schmitz I, Zha J, Korsmeyer SJ, Krammer PH, Peter ME (1999) Differential modulation of apoptosis sensitivity in CD95 type I and type II cells. J Biol Chem 274:22532
40. Fulda S, Meyer E, Friesen C, Susin SA, Kroemer G, Debatin KM (2001) Cell type specific involvement of death receptor and mitochondrial pathways in drug-induced apoptosis. Oncogene 20:1063
41. Page C, Lin HJ, Jin Y, Castle VP, Nunez G, Huang M, Lin J (2000) Overexpression of Akt/AKT can modulate chemotherapy-induced apoptosis. Anticancer Res 20:407
42. Nakashio A, Fujita N, Rokudai S, Sato S, Tsuruo T (2000) Prevention of phosphatidylinositol 3′-kinase-Akt survival signaling pathway during topotecan-induced apoptosis. Cancer Res 60:5303
43. Zha J, Harada H, Yang E, Jockel J, Korsmeyer SJ (1996) Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-XL. Cell 87:619
44. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME (1997) Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91:231
45. Zhou H, Li XM, Meinkoth J, Pittman RN (2000) Akt regulates cell survival and apoptosis at a postmitochondrial level. J Cell Biol 151:483
46. Asselin E, Mills GB, Tsang BK (2001) XIAP regulates Akt activity and caspase-3-dependent cleavage during cisplatin-induced apoptosis in human ovarian epithelial cancer cells. Cancer Res 61:1862
47. Jaattela M, Wissing D, Kokholm K, Kallunki T, Egeblad M (1998) Hsp70 exerts its anti-apoptotic function down-stream of caspase-3-like proteases. EMBO J 17:6124
48. Mairesse N, Horman S, Mosselmans R, Galand P (1996) Antisense inhibition of the 27 kDa heat shock protein production affects growth rate and cytoskeletal organization in MCF-7 cells. Cell Biol Int 20:205
49. Garrido C, Bruey J-M, Fromentin A, Hammann A, Arrigo AP, Solary E (1999) HSP27 inhibits cytochrome *c*-dependent activation of procaspase-9. FASEB J 13:2061
50. Kaur J, Kaur J, Ralhan R (2000) Induction of apoptosis by abrogation of HSP70 expression in human oral cancer cells. Int J Cancer 85:1
51. Nylandsted J, Rohde M, Brand K, Bastholm L, Elling F, Jaattela M (2000) Selective depletion of heat shock protein 70 (Hsp70) activates a tumor-specific death program that is independent of caspases and bypasses Bcl-2. Proc Natl Acad Sci U S A 87:7871
52. Ambrosini G, Adida C, Altieri DC (1997) A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 3:917
53. Kawasaki H, Altieri DC, Lu CD, Toyoda M, Tenjo T, Tanigawa N (1998) Inhibition of apoptosis by survivin predicts shorter survival rates in colorectal cancer. Cancer Res 58:5071
54. Lu CD, Altieri DC, Tanigawa N (1998) Expression of a novel antiapoptosis gene, survivin, correlated with tumor cell apoptosis and p53 accumulation in gastric carcinomas. Cancer Res 58:1808
55. Tamm I, Wang Y, Sausville E, Scudiero DA, Vigna N, Oltersdorf T, Reed JC (1998) IAP-family protein survivin

inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. Cancer Res 58:5315
56. Tanaka K, Iwamoto S, Gon G, Nohara T, Iwamoto M, Tanigawa N (2000) Expression of survivin and its relationship to loss of apoptosis in breast carcinoma. Clin Cancer Res 6:127
57. Kappler M, Kohler T, Kampf C, Diestelkotter P, Wurl P, Schmitz M, Bartel F, Lautenschlager C, Rieber EP, Schmidt H, Bache M, Taubert H, Meye A (2001) Increased survivin transcript levels: an independent negative predictor of survival in soft tissue sarcoma patients. Int J Cancer 95:360
58. Li F, Ambrosini G, Chu EY, Plescia J, Tognin S, Marchisio PC, Altieri DC (1998) Control of apoptosis and mitotic spindle checkpoint by survivin. Nature 396:580
59. Shin S, Sung BJ, Cho YS, Kim HJ, Ha NC, Hwang JI, Chung CW, Jung YK, Oh BH (2001) An antiapoptotic protein human survivin is a direct inhibitor of caspase-3 and -7. Biochemistry 40:1117
60. O'Connor DS, Grossman D, Plescia J, Li F, Zhang H, Villa A, Tognin S, Marchisio PC, Altieri DC (2000) Regulation of apoptosis at cell division by p34cdc2 phosphorylation of survivin. Proc Natl Acad Sci U S A 97:13103
61. Ikeguchi M, Liu J, Kaibara N (2002) Expression of survivin mRNA and protein in gastric cancer cell line (MKN-45) during cisplatin treatment. Apoptosis 7:23
62. Olie RA, Simoes-Wust AP, Baumann B, Leech SH, Fabbro D, Stahel RA, Zangemeister-Wittke U (2000) A novel antisense oligonucleotide targeting survivin expression induces apoptosis and sensitizes lung cancer cells to chemotherapy. Cancer Res 60:2805
63. Jiang X, Wilford C, Duensing S, Munger K, Jones G, Jones D (2001) Participation of survivin in mitotic and apoptotic activities of normal and tumor-derived cells. J Cell Biochem 83:342
64. Hoffman WH, Biade S, Zilfou JT, Chen J, Murphy M (2002) Transcriptional repression of the anti-apoptotic survivin gene by wild type p53. J Biol Chem 277:3247
65. LaCasse EC, Baird S, Korneluk RG, MacKenzie AE (1998) The inhibitors of apoptosis (IAPs) and their emerging role in cancer. Oncogene 17:3247
66. Jaattela M, Mourizen H, Elling F, Bastholm L (1996) A20 zinc finger protein inhibitors TNF and IL-1 signaling. J Immunol 156:1166
67. Wong GHW, Goeddel DV (1998) Induction of manganous superoxide dismutase by tumor necrosis factor: possible protective mechanism. Science 242:941
68. Weldon CB, Burow ME, Rolfe KW, Clayton JL, Jaffe BM, Beckman BS (2001) NF-kappa B-mediated chemoresistance in breast cancer cells. Surgery 130:143
69. Trauzold A, Wermann H, Arlt A, Schutze S, Schafer H, Oestern S, Roder C, Ungefroren H, Lampe E, Heinrich M, Walczak H, Kalthoff H (2001) CD95 and TRAIL receptor-mediated activation of protein kinase C and NF-kappaB contributes to apoptosis resistance in ductal pancreatic adenocarcinoma cells. Oncogene 20:4258
70. Catz SD, Johnson JL (2001) Transcriptional regulation of bcl-2 by nuclear factor kappa B and its significance in prostate cancer. Oncogene 20:7342
71. Bentires-Alj M, Dejardin E, Viatour P, Van Lint C, Froesch B, Reed JC, Merville MP, Bours V (2001) Inhibition of the NF-kappa B transcription factor increases Bax expression in cancer cell lines. Oncogene 20:2805
72. Nakayama H, Ikebe T, Beppu M, Shirasuna K (2001) High expression levels of nuclear factor kappaB, IkappaB kinase alpha and Akt kinase in squamous cell carcinoma of the oral cavity. Cancer 92:3037
73. De Smaele E, Zazzeroni F, Papa S, Nguyen DU, Jin R, Jones J, Cong R, Franzoso G (2001) Induction of gadd45beta by NF-kappaB downregulates pro-apoptotic JNK signalling. Nature 414:308
74. Javelaud D, Besancon F (2001) NF-kappa B activation results in rapid inactivation of JNK in TNF alpha-treated Ewing sarcoma cells: a mechanism for the anti-apoptotic effect of NF-kappa B. Oncogene 20:4365
75. Spalding AC, Jotte RM, Scheinman RI, Geraci MW, Clarke P, Tyler KL, Johnson GL (2002) TRAIL and inhibitors of apoptosis are opposing determinants for NF-kappaB-dependent, genotoxin-induced apoptosis of cancer cells. Oncogene 21:260
76. Huang Y, Fan W (2002) IkappaB kinase activation is involved in regulation of paclitaxel-induced apoptosis in human tumor cell lines. Mol Pharmacol 61:105
77. Habraken Y, Piret B, Piette J (2001) S phase dependence and involvement of NF-kappaB activating kinase to NF-kappaB activation by camptothecin. Biochem Pharmacol 62:603
78. Strobel T, Swanson L, Korsmeyer S, Cannistra SA (1996) BAX enhances paclitaxel-induced apoptosis through a p53-independent pathway. Proc Natl Acad Sci U S A 93:14094
79. Kim R, Ohi Y, Inoue H, Toge T (2000) Enhancement of chemotherapeutic agent induced-apoptosis associated with activation of c-Jun N-terminal kinase 1 and caspase 3 (CPP32) in bax-transfected gastric cancer cells. Anticancer Res 20:439
80. Kim R, Minami K, Nishimoto N, Toge T (2001) Enhancement of antitumor effect by intratumoral administration of bax gene in combination with anticancer drugs in gastric cancer. Int J Oncol 18:363
81. Sumantran VN, Ealovega MW, Nunez G, Clarke MF, Wicha MS (1995) Overexpression of bcl-Xs sensitizes MCF-7 cells to chemotherapy-induced apoptosis. Cancer Res 55:2507
82. Strobel T, Tai YT, Korsmeyer S, Cannistra SA (1998) BAD partly reverses paclitaxel resistance in human ovarian cancer cells. Oncogene 17:2419
83. Semiya H, Mashima T, Toho M, Tsuruo T (1997) c-Jun NH_2-terminal kinase-mediated activation of interleukin-1β converting enzyme/CED-3-like protease during anticancer drug-induced apoptosis. J Biol Chem 272:4631
84. Yamamoto K, Ichijo H, Korsmeyer SJ (1999) BCL-2 phosphorylated and inactivated by an ASK/Jun N-terminal protein kinase pathway normally activated at G(2)/M. Mol Cell Biol 19:8469
85. Perkins CL, Fang G, Kim CN, Bhalla KN (2000) The role of Apaf-1, caspase-9, and bid proteins in etoposide- or paclitaxel-induced mitochondrial events during apoptosis. Cancer Res 60:1645
86. Friedrich K, Wieder T, Von Haefen C, Radetzki S, Janicke R, Schulze-Osthoff K, Dorken B, Daniel PT (2001) Overexpression of caspase-3 restores sensitivity for drug-induced apoptosis in breast cancer cell lines with acquired drug resistance. Oncogene 20:2749
87. Yang XH, Sladek TL, Liu X, Butler BR, Froelich CJ, Thor AD (2001) Reconstitution of caspase 3 sensitizes MCF-7 breast cancer cells to doxorubicin- and etoposide-induced apoptosis. Cancer Res 61:348
88. Liang Y, Yan C, Schor NF (2001) Apoptosis in the absence of caspase 3. Oncogene 20:6570
89. Kim R, Inoue H, Tanabe K, Toge T (2001) Effect of inhibitors of cysteine and serine proteases in anticancer drug-induced apoptosis in gastric cancer cells. Int J Oncol 18:1227
90. Webb A, Cunningham D, Cotter F, Clarke PA, di Stefano F, Ross P, Corbo M, Dziewanowska Z (1997) BCL-2 anti-sense therapy in patients with non-Hodgkin lymphoma. Lancet 349:1137
91. Jansen B, Wacheck V, Heere-Ress E, Schlagbauer-Wadl H, Hoeller C, Lucas T, Hoermann M, Hollenstein U, Wolff K, Pehamberger H (2000) Chemosensitisation of malignant melanoma by BCL2 antisense therapy. Lancet 356:1728
92. Zangermeister-Wittke U, Leech SH, Olie RA, Simoes-Wust AP, Gautschi O, Luedke GH, Natt F, Haner R, Martin P, Hall J, Nalin CM, Stahel RA (2000) A novel bispecific antisense oligonucleotide inhibiting both bcl-2 and bcl-XL expression efficiently induces apoptosis in tumor cells. Clin Cancer Res 6:2547
93. Kim R, Osaki A, Toge T (2001) A pitfall in the survival benefit of adjuvant chemotherapy for node- and hormone

receptor-positive patients with breast cancer: the paradoxical role of Bcl-2 oncoprotein. Int J Oncol 19:1075
94. Dong L, Wang W, Wang F, Stoner M, Reed JC, Harigai M, Samudio I, Kladde MP, Vyhlidal C, Safe S (1999) Mechanisms of transcriptional activation of bcl-2 gene expression by 17beta-estradiol in breast cancer cells. J Biol Chem 274:32099
95. Kallio MJ, Nieminen M, Eriksson JE (2001) Human inhibitor of apoptosis protein (IAP) survivin participates in regulation of chromosome segregation and mitotic exit. FASEB J 15:2721
96. Grossman D, Kim PJ, Schechner JS, Altieri DC (2001) Inhibition of melanoma tumor growth in vivo by survivin targeting. Proc Natl Acad Sci U S A 98:635
97. Mesri M, Wall NR, Li J, Kim RW, Altieri DC (2001) Cancer gene therapy using a survivin mutant adenovirus. J Clin Invest 108:981
98. Korsmeyer SJ, Wei MC, Saito M, Weiler S, Oh KJ, Schlesinger PH (2000) Pro-apoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that results in the release of cytochrome *c*. Cell Death Differ 7:1166
99. Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, Roth KA, MacGregor GR, Thompson CB, Korsmeyer SJ (2001) Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 292:727
100. Ruffolo SC, Breckenridge DG, Nguyen M, Goping IS, Gross A, Korsmeyer SJ, Li H, Yuan J, Shore GC (2000) BID-dependent and BID-independent pathways for BAX insertion into mitochondria. Cell Death Differ 7:1101
101. Bosanquet AG, Bell PB, Burlton AR, Amos TA (1996) Correlation of bcl-2 with P-glycoprotein expression in chronic lymphocytic leukemia and other hematological neoplasms but of neither marker with ex vivo chemosensitivity or patient survival. Leuk Lymphoma 24:141
102. Kasimir-Bauer S, Ottinger H, Mousors P, Beelen DW, Brittinger G, Seeber S, Scheulen ME (1998) In acute myeloid leukemia, coexpression of at least two proteins, including P-glycoprotein, the multidrug resistance-related protein, bcl-2, mutant p53, and heat-shock protein 27, is predictive of the response to induction chemotherapy. Exp Hematol 26:1111
103. Eid H, Gulyas M, Geczi L, Bodrogi I, Institoris E, Bak M (1998) Expression of bcl-2 in testicular carcinoma: correlation with tumor progression and MDR1/Pgp. Cancer 83:331
104. Chadderton A, Villeneuve DJ, Gluck S, Kirwan-Rhude AF, Gannon BR, Blais DE, Parissenti AM (2000) Role of specific apoptotic pathways in the restoration of paclitaxel-induced apoptosis by valspodar in doxorubicin-resistant MCF-7 breast cancer cells. Breast Cancer Res Treat 59:231
105. Jazirehi AR, Ng CP, Gan XH, Schiller G, Bonavida B (2001) Adriamycin sensitizes the adriamycin-resistant 8226/Dox40 human multiple myeloma cells to apo2L/tumor necrosis factor-related apoptosis-inducing ligand-mediated (TRAIL) apoptosis. Clin Cancer Res 7:3874
106. Ohi, Y, Kim R, Toge T (2000) Overcoming of multidrug resistance by introducing the apoptosis gene, bcl-Xs, into MRP-overexpressing drug-resistant cells. Int J Oncol 16:959
107. Kim R, Ohi Y, Inoue H, Toge T (1999) Activation and the interaction of proapoptotic genes in modulating sensitivity to anticancer drugs in gastric cancer cells. Int J Oncol 15:751
108. Nabboltz JM, Slamon D (2001) New adjuvant strategies for breast cancer: meeting the challenge of integrating chemotherapy and trastuzumab (Herceptin). Semin Oncol 28:1
109. Baselga J (2001) The EGFR as a target for anticancer therapy – focus on cetuximab. Eur J Cancer 37:16
110. Saltz L, Rubin M, Hochster H, Tchekmeydian NS, Waksal H, Needle M (2001) Cetuximab (IMC-C225) plus irinotecan (CPT-11) is active in CPT-11 refractory colorectal cancer (CRC) that express epidermal growth factor receptor (EGFR) (abstract no. 7). Proc ASCO 20:3a
111. Cuello M, Ettenberg SA, Clark AS, Keane MM, Posner RH, Nau MM, Dennis PA, Lipkowitz S (2001) Down-regulation of the erbB-2 receptor by trastuzumab (Herceptin) enhances tumor necrosis-related apoptosis-inducing ligand-mediated apoptosis in breast and ovarian cancer cell lines that overexpress erbB-2. Cancer Res 61:4892
112. Roh H, Pippin JA, Green DW, Boswell CB, Hirose CT, Mokadam N, Drebin JA (2000) HER-2/neu antisense targeting of human breast carcinoma. Oncogene 19:6138
113. Anderson NG, Ahmad T, Chan K, Dobson R, Bundred NJ (2001) ZD1839 (Iressa), a novel epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, potently inhibits the growth of EGFR-positive cancer cell lines with or without erbB2 overexpression. Int J Cancer 94:774
114. Moasser MM, Basso A, Averbuch SD, Rosen N (2001) The tyrosine kinase inhibitor ZD1839 (''Iressa'') inhibits HER2-driven signaling and suppresses the growth of HER2-overexpressing tumor cells. Cancer Res 61:7184
115. Chen HX, Gore-Langton RE, Cheson BD (2001) Clinical trials referral resource: current clinical trials of the antiVEGF monoclonal antibody bevacizumab. Oncology (Huntingt) 15:1023