Inducing cancer cell death by targeting transcription factors

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We review the biological significance of transcription factors such as p53, Myc, E2F family and AP-1 (Jun/Fos) in anticancer drug-induced apoptosis. It is likely that the functional role of these transcription factors is complex in response to DNA damage depending on cancer cell type. Regulation of apoptosis following DNA damage is mediated by cell cycle arrest for DNA repair and subsequent signal transduction pathways leading to apoptosis, which is associated with mitochondrial dysfunction. Activation of transcription factors following anticancer drugs is located upstream of signal transduction pathways, thereby the downstream pathway is promoted, which is connected to activation or suppression of apoptosis-related proteins. Switching on apoptotic signals by anticancer drugs is amplified in mitochondria by releasing cytochrome *c* from the ion channel to activate the caspase cascade, which is regulated by Bcl-2 families in the central gate for drug-induced apoptosis. Activation of transcription factors targeting downstream genes, some of which are apoptosis-related genes, can play a critical role in promoting apoptosis following treatment with anticancer drugs. The strategy of identification of downstream target proteins or transcription factors involved in apoptosis will be necessary for the development of an effective transcription factor-targeted chemotherapy for cancer. *Anti-Cancer Drugs* 14:3–11 © 2003 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2003, **14**:3–11

Keywords: anticancer drug, apoptosis, transcription factor

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Received 15 May 2002 **Revised form accepted** 11 September 2002

Introduction

Apoptosis plays a pivotal role in the homeostasis of human cell proliferation [1]. Dysregulation of cell proliferation due to genetic alteration of oncogenes and tumor suppressor genes is involved in carcinogenesis and an increase in the metastatic potential of cancer cells [2–4]. Despite abnormal proliferation of cancer cells caused by an imbalance between cell division and apoptosis, extracellular apoptotic stimuli such as anticancer drugs, heat shock and radiation can still induce apoptosis in cancer cells. In the general apoptotic pathway, various signals activate caspase cascades that lead to apoptosis, which is marked by DNA fragmentation due to activation of caspase-activated DNase, chromatin condensation by acinus and degradation of target proteins [5,6]. Thus, apoptotic cell death is an important factor in the therapeutic efficacies of drugs used to treat human cancers [7–9].

Current anticancer drugs induce apoptosis via several different molecular mechanisms. Apoptotic pathways induced by anticancer drugs are either death receptor dependent or -independent. The activities of both types are mediated by release from mitochondria of cytochrome *c*, which activates caspase cascades [10]. The death receptor-dependent pathways include the Fas ligand (FasL)–Fas system and TNF-related apoptosis inducible ligand (TRAIL)–death receptor (DR) 5, both of which are mediated by truncated Bid (tBid) through recruitment and activation of caspase-8 via an adaptor protein, Fas-associated death domain protein (FADD). In contrast, the receptor-independent pathways involve translocation of Bax and Bak from the cytoplasm to the mitochondria to form heterodimers and homodimers with each other or with tBid, and then to interact with a voltage-dependent anion channel or cause depolarization at a mitochondrial pore [11]. In both cases, cytochrome *c* is released, and it oligomerizes with procaspase-9 and Apaf-1 in the presence of dATP to activate caspase-9, resulting in the activation of caspase-3, which then leads to apoptosis [12]. Apoptosis is inhibited by anti-apoptosis proteins such as Bcl-2 and Bcl-X_L, which possess the ion channel activity required to block the release of cytochrome *c* [13]. In fact, anticancer drug-induced release of cytochrome *c* can be inhibited by transfection of cells with the *bcl-2* gene, resulting in attenuation of apoptosis [14–16]. Death receptor-dependent pathways induced by Fas and DR5 through tBid can be inhibited by transfection of cells with the *bcl-2* gene because tBid is activated by caspase-8 to release cytochrome *c* in the mitochondria. Thus, the interaction between pro- and anti-apoptosis proteins regulates release of cytochrome *c*

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from the mitochondria, thereby activating caspase cascades.

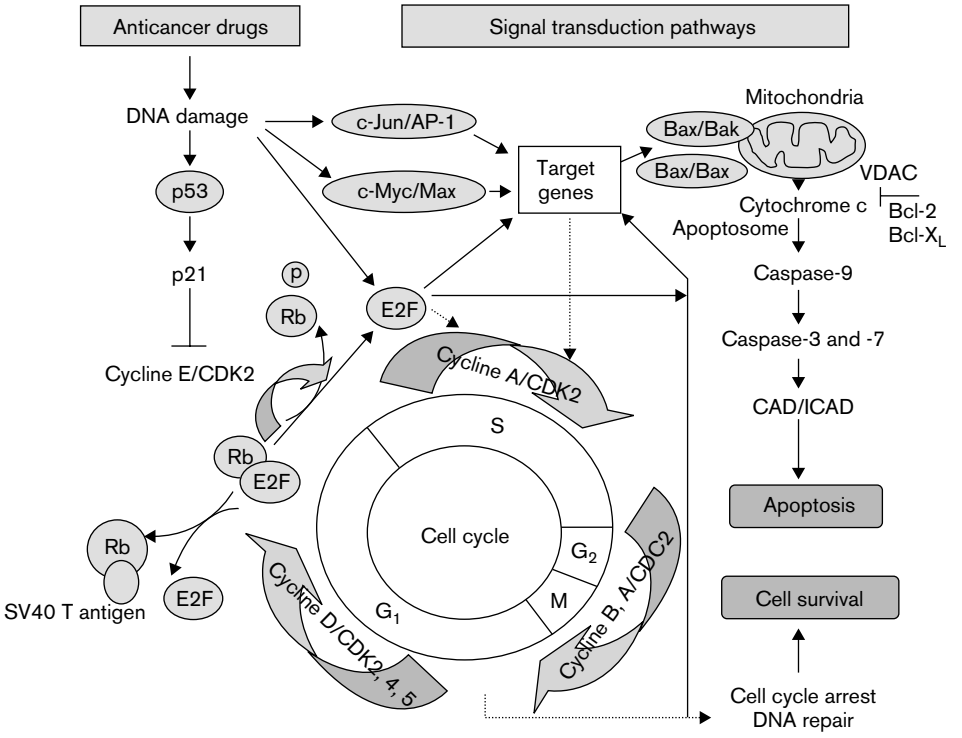
Although the molecular mechanisms underlying drug-induced apoptosis are known, the roles of transcription factors such as p53 and c-*jun*, which are active upstream in apoptotic pathways, remain unclear. The p53 tumor suppressor gene regulates the G_0/G_1 phase of the cell cycle during cell recovery or death after DNA damage [17]. Several target genes of p53 that regulate apoptosis have been identified; however, downstream genes including $p21^{Waf1}$, Fas, growth arrest and DNA damage inducible gene (GADD) 45, Bax and Bcl-2 are also activated through a p53-independent pathway [18–22]. Therefore, mutation of p53 is not necessarily correlated with resistance to apoptosis in cancer cells.

In the case of the c-*jun* gene, transcription factor AP-1, which is composed of Jun and Fos family proteins, plays biological roles in cell proliferation, differentiation and apoptosis [23,24]. It appears that the roles of transcription factors in apoptosis may differ depending on cell type and stimulus. Here, we review current knowledge regarding the roles of transcription factors in anticancer drug-induced apoptosis and discuss future directions in the study of apoptosis.

Transcription factors in apoptosis

Although more than 100 transcription factors have been identified, the biological roles of these factors are not fully understood. In general, it is difficult to clarify the biological roles of specific transcription factors due to difficulties in identifying target genes. In addition, several target genes can be cross-linked with each other in a single signal transduction pathway. Because in the DNA and influence, transcription factors bind to regulatory regions in gene expression, they may affect particular signal transduction pathways. Although transcription factors play critical roles in signal transduction, the biological consequences of transcription factor binding may differ between target genes. Therefore, experimental findings will hopefully clarify the complex signal transduction pathways that regulate cell proliferation, differentiation and apoptosis. In the case of apoptosis, evaluating the biological role of transcription factors is even more difficult because activation of transcription occurs upstream of the apoptotic pathways. In fact, several transcription factors activate or down-regulate expression of the target genes in response to DNA damage and the target gene plays important roles in the signal transduction pathways associated with the cell death or survival decision (Fig. 1).

Fig. 1



Model of transcription factor involvement signal transduction pathways in cell death or survival decisions following DNA damage in a cancer cell. Several transcription factors activate or down-regulate expression of the target genes in response to DNA damage and these target genes play important roles in the signal transduction pathways associated with the cell death or survival decision.

p53 gene

The p53 gene is a tumor suppressor gene that is required for anticancer drug-induced apoptosis. In contrast to glucocorticoids, anticancer drugs such as etoposide (VP-16) cannot induce apoptosis in tumors transplanted into p53 knockout mice [25]. The p53 gene also regulates the G_0/G_1 and G_2/M phases of the cell cycle following exposure of cells to DNA-damaging agents [26,27]. Because DNA damage can prevent initiation and progression of the cell cycle at multiple points, checkpoint delays may allow time for DNA repair, thereby preventing replication or segregation of a damaged genome. p53 triggers the G_1 checkpoint arrest through transcriptional activation of the cyclin-dependent kinase inhibitor $p21^{Waf1}$ [28]. p53-induced proteins that contribute to maintenance of G_2 cell cycle arrest include GADD45, $p21^{Waf1}$ and 14-3-3σ [21,29,30]. In cancer cells carrying the wild-type p53 (wp53) gene, G_0/G_1 or G_2/M arrest may occur after treatment with DNA-damaging agents so that the cell can repair DNA damage or commit to apoptosis. Cancer cells with a mutant p53 (mp53) gene lose this arrest and the cell cycle cannot be stopped after DNA damage, resulting in resistance to apoptosis. Therefore, it is conceivable that cancer cells with wp53 are more susceptible to apoptosis following DNA damage due to anticancer drugs.

p53 is also important in regulating cell proliferation. Germline mutation of p53 causes Li–Fraumeni syndrome; patients with this syndrome are predisposed to developing various malignancies [31]. Possible target genes of p53 include cell cycle-regulated genes, such as $p21^{Waf1}$ and GADD45, and apoptosis-related genes, such as Bax, Bcl-2 and Fas [18–22]. Activation of p53 induces expression of the pro-apoptosis gene, Bax, and inhibits expression of the anti-apoptosis gene, Bcl-2, resulting in apoptosis [19]. Although wp53 is more effective than mutant p53 at activating downstream target genes involved in anticancer drug-induced apoptosis, expression of $p21^{Waf1}$ and Bax can be induced by mp53 through a p53-independent pathway [32], indicating that there are both p53-dependent and -independent signal transduction pathways leading to apoptosis [33]. Findings suggest that mutation of p53 does not necessarily yield resistance to apoptosis induced by anticancer drugs. Rather, the transcriptional activation of target genes by mp53 might be more important for apoptosis. In this regard, a previous study showed that in human lymphoblastic leukemia CEM cells levels of the p53 protein, which contained mutations in amino acids 175 and 248, increased after treatment with teniposide (VM-26), a topoisomerase (Topo) II inhibitor. In the drug-resistant cells, the level of mutant p53 protein did not increase following treatment [34]. Levels of mp53 in drug-sensitive cells increased in association with activation of downstream target genes of $p21^{Waf1}$ and GADD45 following treatment with VM-26 and radiation. Although the mutant of p53 cannot bind to the p53 consensus sequence (unpublished findings), findings suggest that mp53 may play a functional role in activating the signal transduction pathways involved in anticancer drug-induced apoptosis.

Wild-type p53 is more sensitive than mutated forms to anticancer drugs with DNA-damaging agents and antimetabolites such as anthracyclines, cisplatin, 5-fluorouracil (5-FU), alkylating agents and gemcitabine [35,36], whereas the mutated forms of p53 does not contribute to a decrease in sensitivity to taxanes and camptothecin [37]. Findings suggest that sensitivity to DNA-damaging agents and antimetabolites is associated with a p53-dependent pathway, whereas sensitivity to taxanes is determined by a p53-independent pathway. Thus, the most advantageous chemotherapy and the resulting clinical response may be predicted by p53 status.

Other important p53 target genes that confer drug resistance are multidrug-resistance gene MDR1 and multidrug resistance-related protein gene MRP. The proteins are involved in a drug-efflux pump, tumor progression and metastatic potential. The MRP1 promoter is suppressed by wp53, and is activated by mp53 and in cancer cells [38,39]. Similarly, transcriptional activation of the MDR1 gene is mediated by mp53 [40–42]. In contrast, wp53 suppresses transcriptional activation of MDR1 and MRP1 [38,42,43]. These findings support the notion that the p53 gene plays a crucial role in conferring susceptibility to apoptosis. Nevertheless, the therapeutic effects brought about by introducing the p53 gene into cancer cells via an adenovirus vector have not been sufficient to decrease tumor volume, indicating that p53 alone cannot trigger apoptosis [8].

Myc

Myc is a basic helix-loop-helix leucine zipper (bHLHZip) protein that plays central roles in regulating cell proliferation, differentiation and apoptosis [44]. Myc must heterodimerize with Max to have biological activity and for the function of the Myc/Max/Mad Mnt network [45]. Selective dimerization permits switching on and off of functional activity. Myc does not form homodimers; instead, it forms heterodimers with Max, Mad and Mid. Max forms homodimers. Myc/Max dimers activate gene transcription, whereas Mad/Max and Mnt/Max complexes are Myc/Max antagonists that act as repressors [46].

There are three forms of Myc—c-Myc, N-Myc and L-Myc—which are related nuclear oncoproteins that bind similar DNA sites and cooperate with activated *ras* oncogenes to transform cells. All three oncoproteins accelerate apoptosis of cancer cells after interleukin (IL)-3 withdrawal, yet overexpression c-*myc* sensitizes cancer cells to cytotoxic cells, whereas overexpression of N-*myc*

and L-*myc* yields drug-resistance [47]. Overexpression of c-*myc* also increases cisplatin-induced apoptosis, whereas down-regulation of c-*myc* increases cisplatin resistance in colon cancer cell lines [48]. The apoptosis induced by anticancer drug and IL-3 withdrawal with c-*myc* overexpression is ameliorated by Bcl-2 and Bcl-X_L [49]. In neuroblastoma cells, cytotoxic drugs efficiently induce cell death in cancer cells overexpressing N-*myc*, despite neither conditional expression of N-*myc* alone nor a low level of drug-triggered apoptosis [50]. N-*myc* influences drug-induced apoptosis through up-regulation of CD95 and CD95 ligand. Although overexpression of c-*myc* is associated with poor prognosis and drug resistance in some human cancers, introduction of the c-*myc* gene increases sensitivity of cisplatin-resistant cells to tumor necrosis factor (TNF) and cisplatin [51,52], whereas the down-regulation of c-Myc decreases sensitivity to cisplatin of colon cancer [52], small cell lung cancer [53] and melanoma cells [54]. Further, expression of c-*myc* by HepG2 human hepatoma cells increases following treatment with a camptothecin derivative, 10-hytdroxycamptothecin [5]. In contrast, treatment of human erythroleukemia K562 cells with Topo II inhibitors, including amsacrine and doxorubicin, decreases c-*myc* mRNA levels following treatment associated with drug-induced growth inhibition [56]. These findings suggest that overexpression of c-*myc per se* does not necessarily enhance apoptosis and it appears that other downstream factors in apoptotic signal transduction pathways might be more important for modulating these pathways.

E2F family

The E2F gene family is a family of transcription factors that regulate S phase entry and cause apoptosis of some cell types when overexpressed. E2F activity is modulated by formation of a complex with the retinoblastoma protein (pRb), and related proteins p107 and p130 [57,58]. E2F-1, -2 and -3 localize in the nucleus, and preferentially bind pRb, whereas E2F-4 and -5 have no nuclear localization signal, and bind p107/p130 [59]. E2F-6 suppresses the transcriptional activity of other E2F proteins. DP-1 and -2 form heterodimers with each of the E2F proteins. Like E2F-1 and DP-1, E2F-4 induces growth arrest and caspase-dependent apoptosis in Chinese hamster cell lines, although the mechanism differs from that of E2F-1 and DP-1 [59]. IL-3 deprivation of 32D.3 myeloid cells overexpressing E2F induces apoptosis and when E2F activity is augmented by co-expression of DP-1, the effects of survival factors are abrogated [60]. E2F-1 sensitized cells to apoptosis, induced by treatment with the Topo I inhibitor camptothecin and the Topo II inhibitor VP-16 [61,62]. Co-expression of Bcl-2 and E2F-1 in myeloid cells protects them from VP-16-induced apoptosis.

When overexpressed, E2F-1 and p53 cause apoptosis independently in some types of human cancer cells. Adenovirus-mediated transfer of E2F-1 can induce apoptosis in several types of cancer cells and overexpression of E2F-1 has been shown to induce apoptosis. Human esophageal cancer cells overexpressing E2F-1 show marked growth inhibition due to apoptosis, associated with a decrease in levels of anti-apoptosis proteins including Bcl-2, Mcl-1 and Bcl-X_L [63,64]. Adenovirus-mediated transfer of the E2F-1 gene induces apoptosis of human gastric cancer cells, due to activation of caspase-3 and PARP cleavage [65]. Similarly, marked overexpression of E2F-1 induces apoptosis of human melanoma cells, and involves a decrease in levels of anti-apoptosis proteins including Mcl-1 and Bcl-X_L [66]. E2F-1 protein levels increase following treatment with DNA-damaging agents such as adriamycin and VP-16, and induction of E2F-1 in the tumor cells correlates with their sensitivity of the cells to the drugs [67]. Further, cells from E2F-1 knockout mice are more resistant to DNA damage, in comparison to cells from normal mice [68], and overexpression of wild-type E2F-1 protein enhances cytotoxicity following treatment of lung, colon and bladder cancer cells with DNA-damaging agents, resulting in enhanced apoptosis. Combined treatment with Topo II inhibitors, such as adriamycin and VP-16 and E2F-1 adenovirus therapy enhances apoptosis of human osteosarcoma cells more than treatment with anticancer drug alone does [69]. Moreover, human osteosarcoma cells expressing E2F-1 under the control of a tetracycline-responsive promoter are hypersensitive to the Topo I inhibitor camptothecin as well as to VP-16. Overexpression of E2F-1 enhances sensitivity to Topo I and II inhibitors by activating Topo II α gene promoter, and by an increase in Topo I levels and activity [62]. Findings suggest that overexpression of E2F-1 is sufficient to increase sensitivity to anticancer drugs targeting Topo I and II. Treatment with UCN-01 decreases expression of E2F-1, which is reversed by ubiquitin-dependent proteasome inhibitors and E2F-1 lacking the C-terminal region [70].

E2F proteins are thought to promote entry into the S-phase, whereas p53 can arrest cells in the G_1 phase and thereby prevent entry into the S phase. Therefore, these proteins appear to have opposite functions with respect to cell growth. Transcriptional activation of p53 is inhibited by E2F-4 and -5, but not by E2F-1, -2 and -3, indicating differential regulation of p53 by two subclasses of E2F transcription factors [71]. Sequential transfer of the p53 and E2F-1 genes efficiently induces apoptosis, and E2F overexpression activates expression of p14 (ARF), which inhibits MDM2–mediated p53 degradation to stabilize p53 levels in human esophageal cancer cells. Such findings suggest that ectopic overexpression of E2F-1 may stabilize endogenous levels of p53 through an

E2F-1/ARF/MDM2/p53 pathway [72]. Thus, members of the E2F family of transcription factors play an important role in regulating the cell cycle and apoptosis.

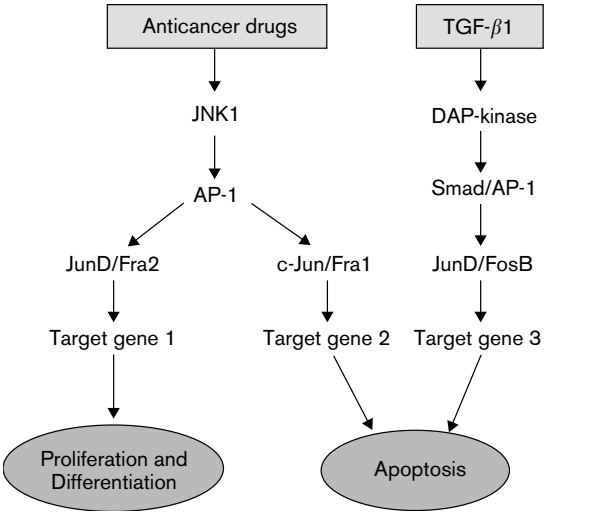
AP-1 (Jun/Fos)

AP-1 is composed of a mixture of homo- and heterodimers of Jun and Fos proteins. Jun and Fos family members vary significantly in their relative abundance and in their interactions with other proteins. c-Jun has been implicated in the regulation of many important biological processes, including cell cycle progression, transformation, differentiation and apoptosis [9,20,73]. Activation of c-Jun is mediated by c-Jun N-terminal kinase (JNK), which regulates its activity through phosphorylation of Ser63 and Ser73 [74]. Several anticancer drugs induce transcription of the c-*jun* and c-*fos* genes, which is associated with an increase in AP-1 binding activity [75–77]. The AP-1-mediated signaling pathway for apoptosis is involved in the activation of JNK, mitogen-activated protein kinase [78], extracellular signal-regulated kinase [79] and p38, which regulates transcription factors. Ceramide is an important lipid mediator in the signal transduction pathway activated by AP-1/JNK in TNF-induced AP-1 activation. Treatment with ceramide activates NF-κB-dependent reporter gene expression in a time-dependent manner in Jurkat T cells but not JcaM1 cells [80]. Activation of AP-1 is necessary for ceramide-induced apoptosis in human leukemia HL-60 cells and inhibition of c-*jun* expression by antisense oligonucleotides blocks ceramide-induced apoptosis [81].

Vinblastine and other microtubule inhibitors (except paclitaxel) activate AP-1 in human KB-3 carcinoma cells [82]. Vinblastine has a selective effect on AP-1 proteins: it increases levels of c-Jun, Jun B, Jun D and Fra-1, but not c-Fos, Fos B and Fra-2. Although increased AP-1 binding activity is mediated by Jun D/Fra-2 heterodimers in untreated cells, in treated cells AP-1 binding activity involves c-Jun/Fra-1 heterodimers [83]. Findings suggest that increased AP-1 binding activity through c-Jun/Fra-1 heterodimers plays a role in transactivating target genes in association with apoptosis. Such findings are in agreement with those of our previous study showing that VM-26-induced AP-1 binding activity is mediated by c-Jun/Fra-1 heterodimers in human CEM drug-sensitive cells and that decreased AP-1 binding activity in drug-resistant cells may be explained by attenuation of the signal transduction pathways that transactivate target genes leading to apoptosis [84]. It was recently reported that Smad proteins and AP-1 complex are involved in transforming growth factor (TGF)-β1 signaling for apoptosis, which is mediated by activation of the Jun D/Fos B85. Fos B enhances Smad3- and Smad4-dependent transcription, and a dominant-negative form of Fos B inhibits TGF-β1-dependent apoptosis. Thus, AP-1 activity in a specific cell depends on the relative amounts of specific Jun/Fos proteins as well as other interacting proteins. The diversity of AP-1 components has complicated our understanding of AP-1 function and has resulted in a paucity of information about the precise roles of individual AP-1 members in distinct cellular processes (Fig. 2).

A recent report regarding putative target genes of AP-1 showed vinblastine down-regulates p53 and $p21^{WAF1}$, whereas it up-regulates TNF and Bak, indicating that vinblastine-inducible AP-1 promotes apoptotic cell death after mitotic arrest [82]. Expression of GADD153, which is also a putative target gene of AP-1, is induced by oxidative stress and anticancer drugs [86–89]. An increase in expression of GADD153 in human leukemia cells occurs following treatment with VP-16 in a dose- and time-dependent manner associated with apoptotic cell death [90]. Further, introduction of the GADD153 gene into human MKN45 gastric cancer cells increases sensitivity to VP-16, cisplatin, 5-FU and docetaxel, indicating that increased AP-1 binding activity and GADD153 expression promote apoptosis induced by anticancer drugs [91]. Other putative targets genes of AP-1 include vascular endothelial growth factor, FasL and cyclooxygenase-2, all of which are involved in tumor proliferation and apoptosis. The Fas–FasL pathway in apoptosis can be induced by AP-1 activation of the FasL promoter [92]. The AP-1–NK signaling pathway leading to apoptosis can be blocked by NF-κB, Bcl-2 and Bcl-X_L, indicating that apoptosis is regulated through interactions of these proteins [93,94].

Fig. 2



Activation of different target genes by various heterodimer forms of AP-1 in cell survival and apoptosis. Transcriptional activation of target gene by the c-Jun/Fra-1 form of AP-1 may be involved in a universal signal transduction pathway promoting apoptosis triggered by anticancer drugs.

There have been several controversial reports concerning the involvement of increased AP-1 binding activity in signal transduction pathways leading to apoptosis. Initial reports indicate that several anticancer drugs induce transcription of c-*jun* and c-*fos* mRNAs in association with apoptosis, suggesting that transactivation of c-Jun/c-Fos is involved in signal transduction pathways in cell death [75–77]. The increase in levels of Jun and Fos family members following treatment with anticancer drugs is connected to increased AP-1 binding activity associated with apoptosis. Moreover, attenuation of AP-1 binding activity is observed in drug-resistant cells [84]. However, a different report suggested that induction of c-*jun* was not involved directly in drug-induced apoptosis in human leukemia cells [95]. The authors reported that staurosporine reduces c-*jun* expression and induces apoptosis in response to ara-C; that human leukemia cells transfected with *bcl-2* are resistant to apoptosis and treatment of such cells with ara-C and mitoxantrone induces c-*jun* expression; and that paclitaxel-induced apoptosis is not associated with increased c-*jun* expression. However, such findings do not necessarily negate the direct involvement of AP-1 in drug-induced apoptosis. Although it appears that increased c-*jun* expression is not a universal phenomenon leading to apoptosis, certain extracellular stimuli or certain conditions may increase AP-1 binding activity leading to transactivation of target genes and apoptosis.

During the past several years, the molecular mechanisms by which anticancer drugs induce apoptosis have been clarified with respect to the role of mitochondrial dysfunction. The central gate of signal transduction pathways in apoptosis is release of cytochrome *c* from the mitochondria, which is mediated by a voltage-dependent anion channel in the inner mitochondrial membrane or by mitochondrial permeability transition. Release of cytochrome *c* is stimulated by pro-apoptosis proteins, such Bax and Bak homodimers and heterodimers, and by the interaction of Bid with Bax and Bak, which is classified under the rubric of the death receptor-dependent and -independent pathways induced by anticancer drugs. Mitochondrial release of cytochrome *c* induced by these pro-apoptosis proteins is inhibited by anti-apoptosis proteins such as Bcl-2 and Bcl-X_L. The released cytochrome *c* oligomerizes with procaspase-9 and Apaf-1 in the presence of ATP, and then activates effector caspase-3 and -7, leading to apoptosis. Involvement of AP-1 in apoptosis has been reported at several points along the signal transduction pathways; the involvement of AP-1 is connected to activation of pro-apoptosis proteins and inhibition of anti-apoptosis proteins. The inhibition of AP-1 signaling by dominant-negative mutant c-Jun (TAM 67), which lacks the N-terminal transactivation domain, decreases drug sensitivity and radiation sensitivity associated with apoptosis [82].

Expression of dominant-negative c-Jun in melanoma cells increases Fas expression and increases sensitivity to FasL-induced apoptosis [96]. One study found that the transfection of glioblastoma cells with a non-phosphorylating dominant-negative c-Jun inhibited activation of AP-1, increasing the cytotoxicity of DNA-damaging agents. These findings suggest that activation of c-Jun protects tumor cells from apoptosis due to DNA damage [74]. Depending on the target gene, induction of c-Jun/AP-1 may play various functional roles in regulating anticancer drug-induced apoptosis. Alternatively, the transactivation network in regulating apoptosis induced by anticancer drugs may be influenced by cell type and stimulus.

Future directions for transcription factor-targeted chemotherapies

Transcription factors are activated by a variety of stimuli and in different cancer cells in association with apoptosis; the functional roles of transcription factors in apoptosis are not universal. It appears that apoptotic stimuli, including anticancer drugs, TNF and Fas, activated signal transduction pathways via specific transcription factors that are cell and stimulus type dependent. In cancer cells susceptible to apoptosis, the signaling process causes release of cytochrome *c* from mitochondria, which then activates a caspase cascade. Therefore, release of cytochrome *c* acts as the central gate for drug-induced apoptosis. It would be of interest to clarify how transcription factors interact with apoptosis-related proteins to regulate apoptosis. In fact, it has been reported that several transcription factors are associated with apoptotic cell death and that expression of dominant-negative forms inhibits cell death. However, the precise mechanisms regulating apoptosis-related genes are not fully understood. Furthermore, it has proven difficult to understand the complex regulation of transcription factor networks in cell proliferation, differentiation and apoptosis in cancer cells. Once apoptotic signals are activated in cancerous cells in response to anticancer drugs, the signal transduction network for cytochrome *c* release can be induced, leading to apoptosis. Activation of transcription factors targeting downstream genes, some of which are apoptosis-related genes, can play a critical role in promoting apoptosis following treatment with anticancer drugs.

Many anticancer drugs have been shown to target transcription factors that influence cell death or survival. To develop specific chemotherapies, it will be necessary to identify which transcription factors promote apoptosis. Although transcriptional activation is an upstream event in the signal transduction pathways leading to apoptosis, the Bcl-2 family genes are important targets for regulating anticancer drug-induced apoptosis. For example, STI-571 is a tyrosine kinase inhibitor of Bcr/Abl and is a

transcription factor that promotes progression of leukemia. This compound has high hematological and cytogenetic response rates in patients with chronic myelogenous leukemia and in those experiencing blast crises [97]. The therapeutic efficacy of STI-571 is due to inhibition of the ATP binding site in the kinase domain of Bcr/Abl. Treatment of leukemia cells with STI-571 suppresses expression of Bcl-X [98], through interactions with pro-apoptosis proteins, resulting in apoptosis. Findings indicate that although STI-571 targets the ATP binding site of Bcr/Abl, the critical downstream target for promoting apoptosis was also identified. Therefore, It is likely that identification of downstream target proteins or transcription factors involved in apoptosis will be necessary for development of an effective transcription factor-targeted chemotherapy for cancer.

During the past decade, many of the molecular mechanisms by which anticancer drugs induce cell death have been clarified; therefore, several molecular targets involved specifically in apoptosis have been the focus of research related to enhancing chemotherapeutic effects in patients with cancer. Transcriptional activation by various factors is necessary to induce apoptosis; however, activated transcription factors are located upstream of signal transduction pathways. The downstream target genes regulated by transcription factors in apoptotic pathways will be an important focus of future research in the area of anticancer drug-induced cell death. It is now clear that transcriptional activation is too complex to be explained by one universal theory. It will be important to understand the roles of these transcription factors for the identification of target genes involved in apoptosis of cancer cells. Thus, the current strategy of targeting apoptosis-related transcription factors may eventually clarify how transcriptional activation or suppression is connected to activation of apoptosis-related proteins in the signal transduction pathways leading to apoptosis. cDNA microarray analyses may provide information regarding signal transduction networks in individual cancer patients.

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