c-Jun NH₂-terminal Kinase-mediated Activation of Interleukin-1β Converting Enzyme/CED-3-like Protease during Anticancer Drug-induced Apoptosis*

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Upon treatment with various anticancer drugs, myeloid leukemia U937 cells undergo apoptosis. In this study, we found that either etoposide (VP-16) or camptothecin (CPT) activated c-Jun N-terminal kinase (JNK), c-Jun expression, and ICE (interleukin-1β converting enzyme)/CED-3-like proteases in U937 cells. Phorbol ester-resistant U937 variant, UT16 cells, displayed a decreased susceptibility to apoptosis induced by these drugs. The drugs did not cause JNK activation, c-jun expression, nor activation of ICE/CED-3-like proteases in UT16 cells. As reported previously, benzyloxy-carbonyl-Asp-CH₂OC(O)-2,6-dichlorobenzene (Z-Asp), a preferential inhibitor of ICE/CED-3-like proteases, blocked the apoptosis of U937 cells. Interestingly, however, Z-Asp did not inhibit JNK activation in either VP-16- or CPT-treated U937 cells. The JNK antisense oligonucleotides diminished protein expression of JNK1 and inhibited drug-induced apoptosis of U937 cells, whereas sense control oligonucleotides did not. Consistent with this observation, the antisense oligonucleotide-treated cells did not respond to VP-16 or CPT with Z-Asp-sensitive proteases. These results indicate that JNK1 triggers the DNA damaging drug-induced apoptosis of U937 cells by activating Z-Asp-sensitive ICE/CED-3-like proteases.

JNK1 (c-Jun N-terminal kinase)/SAPK (stress-activated protein kinase), characterized as a member of MAPK (mitogen-activated protein kinase) family, is activated by various forms of environmental stresses, such as UV irradiation or exposure to toxic agents (1, 2). Like other members of MAPK family, JNK/SAPK requires phosphorylation at Thr-183 and Tyr-185 for its enzymatic activation (2). This phosphorylation state is controlled by, at least, a kinase and a phosphatase, such as SEK1/JNKK and MKP1, respectively (3–5). The activated JNK/SAPK, in turn, phosphorylates the c-Jun transcription factor at Ser-63 and Ser-73 within its N-terminal transactivation domain, which induces the expression of c-Jun-responsive genes, such as c-jun itself (6). These stresses are triggers for apoptosis (or programmed cell death) in certain cells and tissues. In that sense, JNK/SAPK signaling pathway, as one of stress responses, might be functionally involved in cellular survival or apoptosis. In cultured sympathetic neurons, apoptosis induced by nerve growth factor withdrawal is associated with activation of c-jun (7, 8). Recently, such c-Jun activation in PC-12 pheochromocytoma cells was found to be regulated by a JNK/SAPK but not an ERK/MAPK signaling pathway (9). This finding suggests a functional involvement of a JNK/SAPK signaling pathway in neuronal cell apoptosis, which is required for normal development of organisms.

Pathologically, failure in an apoptosis program often leads to imbalance in cell number, and some of those abnormalities might lead to tumorigenesis (10, 11). Based on this concept, control of apoptosis has emerged as an important strategy for clinical cancer therapy (11, 12). Previous works have revealed that various anticancer drugs, such as etoposide (VP-16), camptothecin (CPT), or 1-β-D-arabinofuranosylcytosine, elicit the apoptosis program in cancer cells (13–16). This also means that blockade of the apoptosis-inducing pathway could be another mechanism for multidrug resistance as well as P-glycoprotein-mediated efflux of chemotherapeutics (16, 17). Because some anticancer drugs activate JNK/SAPK (18, 19), it is interesting to determine whether the JNK/SAPK signaling pathway is functionally involved in anticancer drug-induced apoptosis of cancer cells. In addition, downstream factors controlled by the JNK/SAPK signaling pathway should be identified, too.

Interleukin-1β converting enzyme (ICE) and related cysteine-proteases, such as CED-3, CPP32/Yama/apopain, Ich-1/Nedd2, Ich-2/ICE₃₃₂/II/XT, or Mch2, are thought to be downstream regulators of apoptosis (20, 21). Overexpression of these proteases leads to apoptosis of various cell types (22–29). Although some of these proteases seem to constitute a multiple protease cascade (21), the molecular mechanism of the cascade triggering is poorly understood. Recently, we reported that benzyloxy carbonyl-Asp-CH₂OC(O)-2,6-dichlorobenzene (Z-Asp), an aspartate-based inhibitor of ICE family proteases, inhibited apoptosis of monoblastoid leukemia U937 cells induced by various stimuli, including anticancer drugs (30). This apoptosis of U937 cells is associated with the activation of an unidentified Z-Asp-sensitive protease that is distinct from ICE (31). However, the signaling pathway responsible for this protease activation remains to be determined.
In this study, we examined the relationship between the JNK/SAPK signaling pathway and the activation of the Z-Asp-sensitive protease during apoptosis induced by anticancer drugs VP-16 and CPT. Furthermore, employing a JNK1-antisense trial, we demonstrated that JNK1 positively regulated VP-16- and CPT-induced protease activation and apoptosis of U937 cells.

**MATERIALS AND METHODS**

**Cell Lines and Cell Culture**—Human monoblastoid leukemia U937 cells were grown as described previously (32). A phorbol ester-resistant U937 variant, UT16, was established and characterized as described (33).

**Drug-induced Apoptosis of U937 Cells**—A topoisomerase II inhibitor, VP-16, and a topoisomerase I inhibitor, CPT, were kindly provided by Bristol Meyers-Squibb (Tokyo, Japan) and Yakult (Tokyo, Japan), respectively. Logarithmically growing cells were treated with 10 μg/ml either VP-16 or CPT at 2.5 × 10^6 cells/ml. Apoptosis was monitored through characteristic changes in cellular morphology, an ability to exclude trypan blue, and the appearance of chromosomal DNA fragmentation. DNA fragmentation was analyzed by electrophoresis in 2% agarose gels, as described previously (14). An ICE/CED-3-like protease inhibitor, benzoylcarbonyl-Asp-CH₂OC(O)-2,6-dichlorobenzene (Z-Asp) (30, 34), was kindly provided by Kirin Brewery (Gunma, Japan).

**Activation of ICE/CED-3-like Protease by JNK1**—A fusion vector for glutathione S-transferase (GST)/c-Jun-(1–92) was constructed by cloning the c-jun gene fragment (corresponding to the amino acid codons 1–92) into a pGEX-5X-1 vector (Pharmacia Biotech Inc.). The fusion protein was expressed in bacteria and purified with glutathione-Sepharose 4B (Pharmacia). The cellular extract was prepared as described (6). JNK1 enzyme assay was performed as described (35) with slight modifications. Briefly, 1 mg of cellular extract was incubated with protein A-Sepharose (Sigma) and 1 μg of rabbit anti-JNK1 (Santa Cruz Biotechnology, Santa Cruz, CA) for 2 h at 4°C. Immunoprecipitates were washed extensively and assayed for kinase activity at 37°C for 20 min using 1.5 μg of GST/c-Jun(1–92) as a specific substrate. Proteins in the reaction were resolved by SDS-polyacrylamide gel electrophoresis (PAGE) and subjected to autoradiography.

**ERK1 Enzyme Assay**—The cellular extract was prepared as described (33). One milligram of the extract was incubated with protein A-Sepharose and 1 μg of rabbit anti-ERK1 (Santa Cruz Biotechnology) for 2 h at 4°C. Immunoprecipitates were washed extensively and assayed for kinase activity at 37°C for 20 min in the buffer containing 50 mM HEPES (pH 7.5), 10 mM MgCl₂, 1 mM 2,3-dihydroxybutane-1,4-dithiol, 0.5 mM NaF, 0.5 mM Na₃VO₄, 2 μg/ml of ATP, and 5 μg of myelin basic protein (Sigma) as a specific substrate. Proteins in the reaction were resolved by SDS-PAGE and subjected to autoradiography.

**Western Blot Analysis**—The cellular extract for detection of JNK1 was prepared as described above. The cellular extracts for detection of Bel-2 family were prepared according to the method of Reynolds et al. (36). The samples (20 μg of protein) were separated by SDS-PAGE and electrophoretically transferred to nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). The membranes were soaked in 10% skim milk/phosphate-buffered saline at room temperature for 1 h and incubated with rabbit anti-JNK1, McI-1, Bax, or mouse anti-Bcl-2 (Oncogene Science, Cambridge, MA) for 1 h. The membranes were then washed with 0.1% Tween 20/phosphate-buffered saline and incubated for 1 h with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (Amersham Japan, Tokyo, Japan). The specific signals were detected on XAR-5 x-ray films (Eastman Kodak Co.) using an enhanced chemiluminescence detection system (Amersham Japan).

**RNA Extraction and Northern Blot Analysis**—Total RNA was prepared from 3 × 10⁶ cells using an acidguanidinium/phenol/chloroform method (37). Northern blot analysis was performed as described previously (32).

**ACA (Actin-cleaving Activity) Assay**—Cells were resuspended with 10 mM Tris-Cl (pH 8.1), 5 mM 2,3-dihydroxybutane-1,4-dithiol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin and disrupted by a freeze–thawing technique. The lysate was centrifuged at 100,000 × g for 20 min at 4°C to obtain the supernatant as the cytosolic fraction. Rabbit muscle actin (Sigma) was biotinylated, and ACA assay was performed as described previously (30).

**Inhibition of JNK1 by Antisense Oligonucleotides**—JNK1 sense (SON, 5'-ATC ATG AGC AGA AGC AAG CTT GAC-3') and antisense (ASON, 5'-GTC AGT CCT GCT TCT GCT CAT GAT-3') oligonucleotides were synthesized in the phosphorothioate-modified conditions and purified by high performance liquid chromatography (Greiner Japan, Tokyo, Japan). These sequences represent the amino acid codons 1 to +7 of JNK1. The oligonucleotides were dissolved in 30 mM HEPES (pH 7.0) and added into culture media. After treatment with the oligonucleotides for 72 h, cells were analyzed as described above.

**RESULTS**

**Apoptosis of U937 Cells Induced by VP-16 or CPT**—When U937 cells were continuously treated with 10 μg/ml either VP-16 or CPT for the indicated periods, a, effects of VP-16 and CPT on U937 and UT16 cells. Cellular viability was determined by trypan blue staining. Each point represents the mean ± S.D. (bars) of three experiments, each done in duplicate. Circle, U937; square, UT16; open, Z-Asp (-); closed, Z-Asp (+). DNA fragmentation induced by VP-16. Cellular DNAs were extracted and fractionated by agarose gel electrophoresis. C, expression of Bel-2-related proteins. Cells were lysed, and 20 μg of protein was assayed for expression of Bel-2, McI-1, Bax, and Bak protein by Western blot analysis.

**Fig. 1. VP-16- and CPT-induced apoptosis of U937 cells.** Cells were treated with 10 μg/ml either VP-16 or CPT for the indicated periods. A, effects of VP-16 and CPT on U937 and UT16 cells. Cellular viability was determined by trypan blue staining. Each point represents the mean ± S.D. (bars) of three experiments, each done in duplicate. Circle, U937; square, UT16; open, Z-Asp (-); closed, Z-Asp (+). DNA fragmentation induced by VP-16. Cellular DNAs were extracted and fractionated by agarose gel electrophoresis. C, expression of Bel-2-related proteins. Cells were lysed, and 20 μg of protein was assayed for expression of Bel-2, McI-1, Bax, and Bak protein by Western blot analysis.

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complex kinase assay (35). As shown in Fig. 2
CPT, JNK1 activity in U937 cells was monitored by immuno-
Cells—
313
RNAs loaded and stained with ethidium bromide (EtBr)
Fig. 2. JNK1 activation during apoptosis of U937 but not of
UT16 cells. Cells were treated as in Fig. 1. A, effects of VP-16 and CPT on JNK1 activity. Cellular extract was prepared, and JNK1 activity was monitored by phosphorylation of bacterially expressed GST/c-Jun-(1–92) as a specific substrate of the kinase (upper panels). The amounts of JNK1 protein were also monitored by Western blot analysis (lower panels). B, effects of VP-16 and CPT on c-jun expression. Total RNA was resolved on 1% agarose gel and transferred to a nylon membrane. The blot was hybridized with c-jun probe (upper panel). Also shown are the RNAs loaded and stained with ethidium bromide (EtBr). C, effects of VP-16 and CPT on ERK1/MAPK activity in U937 cells. Cellular extract was prepared, and ERK1 activity was monitored by phosphorylation of myelin basic protein as a specific substrate of the kinase. Also shown is a positive control derived from TPA (10 ng/ml)-treated cells.

increase). Neither Z-Asp nor its solvent, dimethyl sulfoxide, alone had much effect on the cell growth (data not shown).

 UT16 cells, TPA-resistant variants of U937 cells (33), exhibited decreased susceptibility to apoptosis induced by these agents. In the presence of VP-16, for instance, viability of UT16 cells at 9 h was 43% (6.7-fold resistant compared with the viability of U937 cells; Fig. 1A). Interestingly, dying UT16 cells exhibited little DNA fragmentation but rather a smear pattern of DNA, which was probably due to random cleavages of base pairs during necrosis-like process (Fig. 1B). Dying U937 cells exhibited swelling morphology rather than formation of apoptotic bodies (data not shown). Although Z-Asp enhanced viability of VP-16- and CPT-treated U937 cells (Fig. 1A), the extent of survival was less than in the parental U937 cells; Z-Asp equalized the susceptibility of the two cell lines to the drug-induced apoptosis.

Upon treatment with VP-16 or CPT, there were no major differences between the cell lines in cellular amounts of apoptosis-inhibitory (Bcl-2, Mcl-1) and -accelerating (Bax, Bak) proteins (40–46); the loss on Mcl-1 was striking, but it was also observed in UT16 cells (Fig. 1C).

JNK1 Activation during Apoptosis of U937 but Not of UT16 Cells—During the course of apoptosis induced by VP-16 or CPT, JNK1 activity in U937 cells was monitored by immuno-complex kinase assay (35). As shown in Fig. 2A, both agents caused transient activation of JNK1 in U937 cells (indicated by phosphorylation of GST/c-Jun-(1–92) recombinant protein as a JNK1-specific substrate). This JNK1 activation preceded the appearance of the laddering pattern of chromosomal DNA. For example, when U937 cells were treated with CPT, JNK1 activation was detected at 1 h after treatment, whereas DNA fragmentation had not been detected at that time (data not shown). Maximum activation of this kinase occurred at 3 h after treatment with each agent. Western blot analysis revealed that these JNK1 activations were not due to enhanced expression of JNK1 protein. However, in UT16 cells, which exhibited delayed cell death with little DNA laddering (Fig. 1, A and B), very low JNK1 activity was detected even in the absence of any drugs. Interestingly, neither VP-16 nor CPT significantly enhanced the activity in UT16 cells. The amounts of JNK1 protein were comparable between the cell lines, although dying U937 cells exhibited less (suggesting the specific down-regulation of JNK1) (Fig. 2A, lower panels).

The activated JNK1 phosphorylates c-Jun at Ser-63 and Ser-73 within its N-terminal activation domain, which in turn induces c-jun expression (6, 47). So next, we used Northern blot analysis to investigate c-jun expression during apoptosis of U937 cells. As shown in Fig. 2B, treatment with either VP-16 or CPT led to transient expression of c-jun in U937 cells. Consistent with the JNK1 activation described above, maximum induction of c-jun occurred 3 h after treatment with each agent. In UT16 cells, however, c-jun expression was not detected over the course of the experiment even after prolonged exposure of the blot to an x-ray film. This finding suggests that the slight JNK1 activity detected in UT16 control (Fig. 2A) did not lead to sustained c-jun expression in those cells. Expression of c-jun is also induced by the ERK/MAPK pathway, which is activated during phorbolester-induced monocytic differentiation of U937 cells (33). In U937 cells, however, ERK1 was not activated by either VP-16 or CPT (Fig. 2C). On the other hand, phorbolester (12-O-tetradecanoylphorbol-13-acetate; TPA) did not activate JNK1 in either cell line (data not shown). Taken together, these data suggest that c-jun induction by VP-16 or CPT is correlated with the drug-induced apoptosis of U937 cells and is mediated by the JNK/SAPK rather than the ERK/MAPK pathway.

JNK1 Activation Precedes ICE/CED-3-like Protease Activation—To determine whether the failure in JNK1 activation in UT16 cells could be associated with abnormality in ICE/CED-3 proteases, we compared Z-Asp-sensitive actin-cleaving activity (31) of U937 and UT16 cells in vitro (Fig. 3A). After treating U937 cells with either VP-16 or CPT, there was a great deal of activity (determined by appearance of specifically degraded actin fragment at 15 kDa) within 3 h while the control displayed no trace of activity. This activity was maintained for at least 6 h (time period, 3–9 h after the drug treatment) and completely blocked by Z-Asp (Fig. 3B). In contrast, UT16 cells exhibited a trace of actin cleavage even without drugs (Fig. 3A). This basal activity was also blocked by Z-Asp (Fig. 3B), suggesting that the protease activity in both cells seems to be identical, and the latter cells show basal activity for unknown reasons. In UT16 cells, however, the increase in the actin cleavage mediated by VP-16 or CPT was only marginal (Fig. 3A). These data indicate that failure in elevation of Z-Asp-sensitive actin-cleaving activity is correlated with decreased susceptibility to VP-16- and CPT-induced apoptosis and the loss of JNK1 activation in UT16 cells.

To evaluate the linkage between JNK1 activation and the Z-Asp-sensitive protease activation, we monitored JNK1 activity in anticancer drug-treated U937 cells with or without Z-Asp. Interestingly, while Z-Asp protected U937 cells from VP-16- and CPT-induced apoptosis (Fig. 1A), it did not inhibit
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JNK1 activation at all in these cells (Fig. 4, upper panel). Z-Asp alone had little effect on JNK1 activation or its protein expression (Fig. 4, lower panel). This result suggests that JNK1, as an upstream regulator, might control the activity of Z-Asp-sensitive proteases in a single cascading pathway. Alternatively, JNK1 and this unidentified protease might participate in independent pathways.

**JNK1 Antisense Oligonucleotide Inhibits ICE/CED-3-like Protease Activation and Apoptosis of U937 Cells**—To further investigate the direct effect of JNK1 on the drug-induced apoptosis and Z-Asp-sensitive proteases, we designed JNK1 antisense oligonucleotide experiments. Upon treatment with JNK1 antisense oligonucleotide (ASON) for up to 72 h, cellular amounts of JNK1 protein decreased in U937 cells (Fig. 5A). Control sense oligonucleotide (SON) marginally affected the JNK1 contents. Under these conditions, susceptibility to VP-16- and CPT-induced apoptosis was examined. As shown in Fig. 5, B and C, ASON significantly blocked the apoptosis of U937 cells but SON did not. The inhibition of apoptosis was associated with the loss of ICE/CED-3-like protease activation in ASON-treated cells; lysates from dying control and SON-treated cells exhibited enhanced actin-cleaving activity, whereas those from ASON-treated cells did not (Fig. 5D). The signal intensity was correlated with the appearance of apoptotic cells. Taken together, these data indicate that JNK1 enhances the activity of Z-Asp-sensitive ICE/CED-3-like protease(s) in a single cascading pathway and positively participates in VP-16- and CPT-induced apoptosis of U937 cells.

**DISCUSSION**

Upon treatment with TPA, U937 cells differentiate into a monocyte/macrophage-like lineage (48, 49). In this process, TPA causes sequential activation of the protein kinase C/Raf-1/MEK (MAPK/ERK kinase)/ERK pathway, which in turn leads to AP-1 activation (47, 50). During an extended culture period, the differentiated leukemia cells often undergo apoptosis (51). Recent studies indicate that AP-1 activation is involved not only in differentiation but also in apoptosis. For example, AP-1 is required for ceramide-induced apoptosis of human leukemia HL-60 cells (52). In fact, AP-1 is activated by a variety of stimuli, such as UV light or tumor necrosis factor-α, which also induce apoptosis. AP-1 also regulates cellular proliferation in many other cases (50). At present, however, AP-1-mediated course of events that lead to these cellular processes is not well known.

Based on these observations, it is likely that differentiation and apoptosis programs overlap to some extent. TPA resistance with a failure in differentiation is often associated with multidrug resistance (MDR) to anticancer drugs (53–55). In typical MDR cells, several changes, such as up-regulation of P-glycoprotein, have been observed (17). However, TPA-resistant UT16 cells, which exhibited decreased susceptibility to anticancer drug-induced apoptosis, did not express P-glycoprotein (monitored at both levels of protein and mRNA by Western blot analysis and reverse transcription-polymerase chain reaction, respectively; data not shown). Also, the cellular amounts of topoisomerase II, one of the targets of VP-16 (56), were comparable between U937 and UT16 cells. In fact, DNA-protein covalent cross-links mediated by VP-16 (56) occurred equally in both cell lines. These observations indicate that the decreased susceptibility to apoptosis of UT16 cells is due to an abnormality in a cellular response subsequent to initial DNA damage caused by anticancer drugs. Alternatively, there might be another response initiated by damage to cellular components other than DNA. In other words, it could be said that cells do not die from disarray caused by DNA damage itself but rather by a triggered apoptosis program.

JNK/SAPK activation is one of the responses to genotoxic stresses, including some anticancer drugs (5, 18, 19, 57). The present data demonstrated that JNK1 activation is well correlated with the occurrence of drug-induced apoptosis. Although the cellular amounts of JNK1 protein were comparable between U937 and UT16 cells, the latter did not respond to VP-16 or CPT with JNK1 activation. These data indicate that UT16 cells might have some abnormality upstream of JNK1. Originally, UT16 cells have been established from TPA-treated U937 cells (33). Thus, TPA might have some effect on the signaling pathway upstream of JNK1 although TPA itself did not activate JNK1 in U937 cells (data not shown). Since TPA inhibits VP16-induced apoptosis of U937 cells, it is possible that TPA negatively regulates a factor that is required for JNK1 activation in U937 cells. As UT16 cells have been established by prolonged exposure of U937 cells to TPA (33), such a JNK1 activator seems to have been irreversibly repressed and neither VP16 nor CPT could activate JNK1 in the cells. Recently, it has been reported that genotoxic stress-induced activation of c-Abl tyrosine kinase leads to JNK/SAPK activation (18). Since c-Abl-deficient cells fail to activate JNK/SAPK, c-

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2 H. Seimiya, M. Toho, and T. Tsuruo, unpublished results.
3 H. Seimiya, T. Mashima, and T. Tsuruo, unpublished results.
Abl would be one of the upstream regulators of JNK/SAPK. The relationship between c-Abl and JNK1 in UT16 cells is under investigation. Alternatively, UT16 cells might have a dominant negative mutation in JNK1 that was not distinguished by Western blot analysis.

ICE/CED-3-like proteases, such as ICE and CPP32, are functionally involved in apoptosis of many cell types (20, 21). Consistent with these observations, Z-Asp inhibits apoptosis in U937 cells induced by various stimuli (30). This finding suggests a common participation by the Z-Asp-sensitive protease in apoptosis of U937 cells. The present data indicate that JNK1 activates the Z-Asp-sensitive protease and facilitates apoptosis of U937 cells. Recently, we have identified the Z-Asp-sensitive protease that is activated during apoptosis of U937 cells. This protease does not have a proline-directed serine/threonine residue, which is preferentially phosphorylated by ERK/JNK family (58). Therefore, it is unlikely that JNK1 directly phosphorylates and activates the protease. There must be additional transducers that connect two events, JNK1 activation and the protease activation. These transducers are our major concern to be elucidated in the future. Meanwhile, the present data suggest a threshold effect in the signaling cascade of apoptosis. In Fig. 5, while 30% of JNK1 protein remains to exist in the ASON-treated cells, apoptosis and the protease activation were more efficiently inhibited. Similarly, although UT16 cells have basal activity of JNK1 and Z-Asp-sensitive protease even without any drugs, the cells grow normally compared with parental U937 cells (33). These observations support the idea that apoptosis signaling has a threshold effect.

ICE/CED-3-like proteases are regulated not only by post-translational processing but also by transcriptional control. For example, interleukin regulatory factor-1 induces translational processing but also by transcriptional control. For example, interleukin regulatory factor-1 induces transcription during DNA damage-induced apoptosis of mitogen-activated T-lymphocytes (59). AP-1, one transcription factor, is activated by TPA, U937 cells undergo differentiation rather than apoptosis (49). It should also be noted that c-Jun, one of AP-1 components, is not necessarily required for all types of apoptosis (60, 61). Alternatively, it is possible that other known substrates for JNK1, such as ATF-2 (57), might also be involved in the protease activation and apoptosis in a more specific manner. Meanwhile, p53, which regulates genotoxic stress-induced growth arrest and apoptosis, is another substrate for JNK1 (62). However, U937 cells are p53-negative and, at least in these cells, interaction between JNK1 and p53 could not be associated with the protease activation or with apoptosis.

Recently, Verheij et al. (63) demonstrated that JNK/SAPK signaling is required for ceramide-mediated apoptosis of U937 cells, and Johnson et al. (64) found a JNK/SAPK-independent mechanism for MEK kinase-induced apoptosis of Swiss 3T3 cells. This discrepancy suggests that JNK/SAPK as well as ERK/MAPK could display different functions in different cell types.

Here we demonstrate that in U937 cells JNK1 activation causes apoptosis by activating Z-Asp-sensitive ICE/CED-3-like protease. If this also occurs in clinical events, defects in such apoptosis signaling might be associated with therapeutic difficulties in that cancers often exhibit broader patterns of resistance to chemotherapy and radiation than those defined by the classical MDR phenotype. In that sense, the JNK/SAPK pathway especially in solid tumors would be worth studying.

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Fig. 5. Effect of JNK1 antisense oligonucleotide (ASON) on apoptosis of U937 cells. A, effect of ASON on cellular amounts of JNK1 protein. U937 cells were treated with JNK1 sense (SON) or antisense (ASON) oligonucleotides for 72 h. Cellular extract was prepared, and the amounts of JNK1 protein were examined by Western blot analysis. Lane 1, HEPES buffer (100%); lane 2, 50 μM ASON (48%); lane 3, 100 μM ASON (30%); lane 4, 100 μM SON (75%); value in parentheses indicates relative amount of JNK1 protein determined by densitometry. B, effect of ASON on drug-induced apoptosis of U937 cells. Cells were treated with 100 μM oligonucleotides as in A followed by exposure to 10 μg/ml either VP-16 or CPT for 4 h. Cells were scored for the incidence of apoptotic changes by a photomicroscopy (more than 300 cells per sample were counted). Each column represents the mean ± S.D. (bars) of three experiments. C, effect of ASON on VP-16-induced DNA fragmentation. DNAs from the cells treated as in B were assayed for DNA fragmentation. AS, ASON; S, SON; D, effect of ASON on actin-cleaving activity of U937 cells. The cytosolic fraction was prepared from cells treated as in B, and actin cleaving activity was measured. Lanes 1–3, HEPES buffer; lanes 4–6, ASON; lanes 7, SON; lanes 1 and 4, no treatment; lanes 2 and 5, VP-16; lanes 3 and 6–7, CPT.
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