The Role of c-Jun N-terminal Kinase (JNK) in Apoptosis Induced by Ultraviolet C and γ Radiation

DURATION OF JNK ACTIVATION MAY DETERMINE CELL DEATH AND PROLIFERATION*

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c-Jun N-terminal kinases (JNKs) participate in cellular responses to mitogenic stimuli, environmental stresses, and apoptotic agents. The mechanisms by which JNK integrates with other signaling pathways and regulates the diverse cellular events are unclear. We found JNK, but not p38-mitogen-activated protein kinase (MAPK) or extracellular signal-regulated kinase 2, to be persistently activated in apoptosis induced by γ radiation, UV-C, and anti-Fas treatment. Direct correlation was found between JNK activation and apoptosis induced by UV-C and γ radiation; however, JNK induction and apoptosis induced by Fas signaling were not well correlated. Overexpression of activated JNK1 caused cell death in transfected cells, and the expression of a dominant-negative mutant of MAPK kinase 1 or JNK1 (but not a dominant-negative mutant of p38-MAPK or c-Raf) prevented the UV-C- and γ radiation-induced cell death. The inductions of JNK in T-cell activation and apoptosis were distinguished by the different activation patterns, transient versus persistent, respectively. Co-treatment with a tyrosine phosphatase inhibitor (sodium orthovanadate) and T-cell activation signals (phorbol 12-myristate 13-acetate plus ionomycin) prolonged JNK induction, followed by T-cell apoptosis. Our data revealed the requirement of the JNK pathway in radiation-induced apoptosis and implicated the importance of the duration of JNK activation in determining the cell fates.

Cell death is as important as cell proliferation in regulating development and homeostasis in multicellular organisms (1, 2). Physiological cell death is usually mediated through apoptosis, which is positively or negatively regulated by various extracellular factors. Most growth factors prevent cells from undergoing apoptosis, and deprivation of growth factors usually causes the induction of cell death in growth factor-dependent cells (3). In contrast, Fas ligand and tumor necrosis factor α (TNF-α)

were shown to be potent death signal transducers (4, 5).

Besides the physiological regulators of cell death, many environmental stresses also cause apoptosis (2); however, the exact mechanism of stress-induced apoptosis remains unclear. Recent studies have identified c-Jun N-terminal kinases (JNKs), also called stress-activated protein kinases (SAPKs), to be involved in cellular responses to environmental stresses (6, 7). JNK kinases can be activated by stimuli such as UV light (8), γ radiation (9, 10), protein synthesis inhibitors (7), ceramide (11), DNA-damaging drugs (12, 13), chemopreventive drugs (14), TNF-α, and interleukin 1 (15, 16). In addition, JNK activity is also induced by mitogenic signals, including growth factors (17), oncogenic Ras (8), CD40 ligation (18, 19), and T-cell activation signals (20, 10). JNKs phosphorylate transcription factors such as c-Jun, ATF-2, and Elk-1 and strongly augment their transcriptional activity (21–23). JNK induction by TNF-α and various environmental stresses implies that JNK may regulate gene expression or other biochemical functions in cells under detrimental conditions. One potential function of JNK may be the initiation of programmed cell death. It was shown that overexpression of MEKK, the JNK kinase kinase, had a lethal effect on fibroblasts (24, 25). In addition, the tumor suppressor p53, which is essential for radiation-induced apoptosis (26), was suggested to be a substrate of JNK1 in vivo (27). These data suggest that the JNK kinase cascade may participate in apoptosis.

Previously, we showed that JNK1 was persistently activated in Jurkat cells exposed to a lethal dose of γ radiation (10). Here, we report that JNK, but not p38-MAPK or ERK2, was significantly activated in apoptotic cells treated with γ radiation, UV-C, and anti-Fas. However, JNK induction was well correlated with the radiation-induced apoptosis, but not with Fas-mediated apoptosis. Overexpression of JNK1 caused cell death in the transfected cells; in contrast, expression of dominant-negative mutants of the JNK kinase cascade blocked γ radiation and UV-C-induced cell death. JNK activation in T-cell apoptosis was characterized by a persistent kinetics in contrast to the transient induction in T-cell activation. Co-treatment of T-cell activation signals PMA plus ionomycin with a phosphatase inhibitor extended the duration of JNK1 activation and caused apoptosis in Jurkat cells. Therefore, JNK is involved in both cell proliferation and apoptosis, and the duration of JNK induction may be the crucial factor in mediating the signaling decision.

MATERIALS AND METHODS

Cells, Antibodies, and Reagents—Human Jurkat T cells (clone J.ELI) and 293T human embryonic kidney cells were cultured as described previously (28). The anti-CD28 monoclonal antibody ascites (clone 9.3; used at a 1:1,000 dilution) were provided by Dr. L. Chen (Bristol-Meyers Squibb).

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Squibb Research Institute; Seattle, WA). The anti-Fas monoclonal Ab (CH-11, immunoglobulin M) and interleukin-1β-converting enzyme (ICE) inhibitor Z-YVDA-FK were purchased from Kamiya Biomedical. Rabbit anti-JNK1 antiserum (Ab101) was described previously (10). Anti-ERK2 and anti-p38-MAPK antibodies were purchased from Santa Cruz Biotechnology. PMA and ionomycin were obtained from Sigma. Myelin basic protein (Bio-Rad) was prepared at a concentration of 5 mg/ml in kinase reaction buffer.

Plasmids—GST-Jun(1–79)-expressing plasmid was obtained from Dr. M. Karin (University of California, San Diego, CA). pCIneo-JNK1, pEECMV, pCMV-ΔMEKK1, and pUna3-MEKK1(KR) were described previously (28, 44). pcDNA3-Flag-JNK1(APF) and pCMV-Flag-p38(AGF) were described previously (8, 16). The Raf-BXB-301 plasmid was a gift from Dr. J. Bruder (GenVec, Rockville, MD).

Radiation Treatments, Cell Extract Preparation, and DNA Fragmentation Assay—Cells were γ-irradiated by using a Gammacell1000 137Cs source (10 Gy/min), and UV irradiation was performed by using a UV Stratalinker 1800 (Stratagene). To prepare whole cell extract, 10^7 cells were harvested and lysed in 200 ml of lysis buffer (20 mM HEPES, pH 7.9, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 20% glycerol, 2 μg/ml leupeptin, 5 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, and 1 mM Na3VO4). The cell lysates were vigorously vortexed several times and cleared by centrifugation at 15,000 g for 3 min. The DNA fragmentation assay was performed as described previously (10).

Transient Transfection Death Assay—This assay was performed as described (29). Briefly, 293T cells were plated 24 h before transfection at a density of 1.3 × 10^6/100-mm culture dish. Cells were co-transfected with pCMV-βGal plasmid expressing β-galactosidase and empty vectors or plasmids expressing a kinase as indicated. The calcium phosphate precipitation protocol (Specialty Media) was used for transfection. Cells were collected 48 h after transfection and lysed in a buffer containing 250 mM Tris, pH 7.4, and 5 mM EDTA. To determine the β-galactosidase activity, 2 μl of each lysate was diluted in 48 μl of distilled water and mixed with 50 μl of 2 × reaction buffer (200 mM sodium phosphate, pH 7.3, 2 mM MgCl2, 100 mM β-mercaptoethanol, and 1.33 mg/ml o-nitrophenyl β-D-galactopyranoside) in a 96-well microtiter plate. The reaction was carried out at 37 °C for 1 h and was terminated by adding 150 μl of 1 M Na2CO3. The absorbance at 420 nm was measured using a microtiter plate reader (SLT Spectra).

Transient Transfection Protection Assay—Cells were transfected as described above with duplicates performed for each transfection. After removing the transfection mixture, the cells were incubated in complete medium for 6 h for recovery and then treated with or without radiation. Cells were harvested 24 h after radiation and fixed in 1% paraformaldehyde in PBS. The fixed cells were washed with PBS once, resuspended in staining solution (PBS, pH 7.4, 1 mM MgCl2, 10 mM K4(Fe(CN)4), 10 mM K3(Fe(CN)4), 0.1% Triton X-100, and 1 mM X-gal) for 2 h, and then washed with PBS twice. The ratio of β-galactosidase-expressing cells (blue) were examined with a hemacytometer. Cell survival was determined as (% of blue cells in irradiated group/% of blue cells in nonirradiated group) × 100%.

Fluorescence Staining and Flow Cytometry Assay—An apoptosis detection kit (R & D Systems) was used to determine apoptotic cells. Cells after different treatments were stained with annexin V-fluorescein and propidium iodide by following the manufacturer’s instructions and analyzed by flow cytometry (Epics Profile, Coulter Co.). For DNA staining, 10^6 cells were collected and washed with PBS once and then fixed with 70% ethanol. Fixed cells were washed with PBS to remove residual ethanol, pelleted, and resuspended in PBS containing propidium iodide (Sigma; 50 μg/ml). The stained cells were analyzed by flow cytometry. Forward light scatter characteristics were used to exclude the cell debris from the analysis. Apoptotic cells were determined by their hypochromic, subdiploid staining profiles.

Solid-phase and Immunocomplex Kinase Assays—A solid-phase ki-
Sustained JNK Activation and Apoptosis

RESULTS

JNK1, but not p38-MAPK or ERK2, Is Persistently Activated in Apoptosis—In our previous report, we showed that JNK is activated by γ radiation in a dose-dependent manner. The ability of γ radiation to activate JNK and to induce apoptosis are correlated (10). Since different members of the MAP kinase family are usually coordinately regulated, e.g. JNKs and ERKs are activated by mitogenic signals (17, 20), and environmental stresses or proinflammatory cytokines induce JNK and p38-MAPK kinase activities simultaneously (16), we examined whether apoptotic signals specifically induced JNK activation or whether they also activated other MAP kinases. A lethal dose of γ radiation (100 Gy) induced apoptosis (as determined by DNA fragmentation and propidium iodide staining; Ref. 10 and data not shown) and persistent JNK1 activation, which coincided with the DNA fragmentation (data not shown), in Fas-mediated apoptosis in Jurkat cells (Fig. 1A). Besides JNK, γ radiation also induced the p38-MAPK activation (Fig. 1A), but the induction was weaker than the JNK1 induction. UV light is a potent agent for apoptosis (2) and JNK induction (6). However, the JNK activation induced by a low dose of UV light was reported to be rapid and transient (8). After irradiating the Jurkat cells with a lethal dose of UV-C (300 J/m²), we observed an immediate JNK1 induction within 30 min (Fig. 1B), which was followed by a sustained JNK1 kinase activation. We did not detect any p38-MAPK induction paralleled with JNK1 activation after the lethal UV radiation (Fig. 1B). In both γ and UV radiation, ERK2 activity did not change significantly in the early phase, but gradually decreased 5–6 h after radiation (Fig. 1A and B). Besides JNK activation in radiation-induced apoptosis, we detected a sustained JNK induction, which coincided with the DNA fragmentation (data not shown), in Fas-mediated apoptosis in Jurkat cells (Fig. 1C). These results revealed that apoptotic signals strongly induced JNK activation but did not significantly affect other MAP kinases.

To examine whether the radiation-induced JNK activation is a unique phenomenon in lymphocytes, we used different cell types to repeat the experiments. Both γ and UV-C radiations induced sustained JNK activation in 293T embryonic kidney cells (Fig. 1D and E). In 293T cells, γ radiation induced JNK activity within 30 min, which was different from the delayed induction in Jurkat cells (Fig. 1A). γ radiation, but not UV-C,
induced weak p38-MAPK activation, as observed in Jurkat cells (Fig. 1, D and E). ERK2 activity did not change significantly by the radiation. In addition, we detected γ radiation-induced JNK activation in myeloid leukemia cells, HL-60 and BV173, which were susceptible to γ radiation-induced apoptosis (data not shown). However, we failed to detect JNK activation in γ-irradiated murine primary fibroblasts, which did not show apparent cell death after radiation (data not shown). This suggests that γ radiation does not induce JNK kinase activity in all cell types; however, in the cells that are susceptible to γ radiation-induced apoptosis, the same doses of γ radiation can always induce JNK activity.

Extent of JNK Activation and Apoptosis Induced by Radiation Are Correlated—Because UV-induced JNK activation was suggested to play roles in the protection from UV-induced damages and in the tumor promotion effect of UV light (8), we studied the correlation between JNK induction and apoptosis induced by different doses of UV light. Both a low dose (60 J/m²) and a high dose (300 J/m²) of UV-C induced a rapid JNK1 induction (Fig. 2A). However, the low dose of UV-C induced a weaker and less sustained JNK1 induction in comparison with the persistent JNK1 activity induced by the high dose UV-C. DNA fragmentation assays showed that the UV-induced JNK activation preceded the occurrence of DNA fragmentation, and the extent of DNA degradation was correlated with the intensity of JNK1 activation (Fig. 2B).

Jurkat cells transfected with a JNK1 expression vector (pCIneo-JNK1) were selected by incubation with G418 for stable transfectants overexpressing JNK1. Several independent clones were isolated; however, no detectable elevation of JNK1 protein expression was detected in the transfectants in comparison with parental cells (data not shown). This may be because Jurkat T cells with higher JNK levels were in an unfavorable condition and were eliminated in the selection process. However, the responsiveness of these Jurkat clones to γ radiation were different. The basal JNK activities in these clones remained at the same low levels, but γ radiation-induced JNK activities were significantly higher in clones 8A and 8G than in other clones (Fig. 3A). All of the Jurkat clones, except clone 8D, showed detectable levels of apoptosis 12 h after γ radiation treatment, as determined by propidium iodide staining (Fig. 3B). With the higher JNK induction, clones 8A and 8G were also more susceptible to the γ radiation-induced apoptosis than the other clones (Fig. 3B). The γ radiation-induced JNK activation and apoptosis in parental Jurkat cells were comparable to that of clone 8C (data not shown). The correlation between JNK activation and apoptosis induced by γ radiation suggests the involvement of the JNK kinase cascade in γ radiation-induced apoptosis.

When we examined the JNK activation and apoptosis induced by anti-Fas in those T-cell clones, we found that JNK induction by γ radiation and Fas ligation were not correlated in the individual clones (Fig. 3A). This suggested that JNK induction by γ radiation and Fas signaling were mediated by different pathways. Five of seven Jurkat clones (8B, 8D, 8G, 8H, and 12G) had comparable levels of JNK activation and apoptosis induced by anti-Fas (Fig. 3, A and C). However, clone 8A, which was highly susceptible to anti-Fas treatment, showed a weak JNK induction, and clone 8C, which was relatively resistant to Fas-mediated apoptosis, had a strong JNK activation. The dissociation of JNK activation and apoptosis caused by Fas ligation implicates the existence of JNK-independent pathways in Fas-mediated apoptosis.

Inhibitor of ICE Blocks Anti-Fas-induced JNK Activation—ICE family proteases are the effector molecules for apoptotic cell death (31). We preincubated Jurkat cells with ICE protease inhibitor Z-YVAD-FK and then studied JNK activation and apoptosis induced by different agents. We were able to detect JNK induction by UV-C and γ radiation in the presence of ICE inhibitor (Fig. 4A), even though the DNA fragmentation was completely inhibited (Fig. 4B). This result indicated that the activation of JNK was either upstream of or independent of the activation of ICE-like proteases in UV-C- and γ radiation-induced apoptosis. In contrast, along with the inhibition of
DNA fragmentation, the ICE inhibitor abolished the JNK induction by anti-Fas treatment (Fig. 4, A and B), which indicated that JNK induction in Fas-mediated apoptosis occurred after the ICE protease activation. Since activation of ICE-like proteases was considered as a late event in apoptosis, JNK activation may not be involved in the initiation of Fas-mediated apoptosis.

Overexpression of Activated JNK1 Induces Cell Death—A transient transfection death assay was used to study the effect of JNK1 on cell death. 293T cells were cotransfected with β-galactosidase expression plasmid (pCMV-βGal) plus either empty vectors or different combinations of effector plasmids for JNK1, dominant-negative JNK1 mutant (JNK1(APF)), and activated MEKK1 (ΔMEKK1). Transfected cells were harvested 48 h after transfection and then assayed for β-galactosidase activity. A decrease in β-galactosidase activity in comparison with the control group was used as an indicator of cell death. A combination of transfected ΔMEKK1 and JNK1 induced significant levels of cell death in 293T cells, whereas vector alone or ΔMEKK plus JNK1(APF) did not have any cytotoxic effects (Fig. 5A). There was a direct correlation between the cytotoxicity observed in the death assay and JNK kinase activity in the transfected cells (Fig. 5B). These data indicate that activation of JNK may initiate apoptosis.

Dominant-negative Mutants of JNK and MEKK1 Block Cell Death Induced by Radiation—Transient transfection assays were also used to examine the protective effects of dominant-negative mutants MEKK1(KR) and JNK1(APF) of the JNK kinase pathway on radiation-induced cell death. 293T cells were transfected with pCMV-βGal, with or without mutant kinases expressing plasmids, and each transfection was duplicated for treatments with or without radiation. The cells were harvested 24 h after radiation and stained by X-gal staining to examine the β-galactosidase-expressing cells (blue). The survival rate of transfected cells after radiation treatment was determined as the percentage of blue cells in the irradiated group divided by the percentage of blue cells in the nonirradiated group times 100%. Data presented are the mean and S.D. (bars) of three experiments. B, X-gal colormetric staining of vector- or JNK1(APF)-transfected cells treated with or without γ radiation.
cells after UV radiation (Fig. 7, A and B). A combination of MEKK1(KR) and JNK1(APF) did not have synergistic effects on protection of UV-C-induced cell death. A dominant-negative mutant of c-Raf or p38-MAPK, Raf-BXB301 or p38(AGF), respectively, did not show any effect on cell survival (Fig. 7, A and B). These results demonstrate that interfering with JNK signaling can prevent radiation-induced cell death, indicating that the JNK kinase cascade is required for cell death signaling. In contrast, the Raf-ERK and p38-MAPK pathways are not involved in cell death signaling.

Phosphatase Inhibitor Prolongs JNK1 Activation Induced by T-cell Activation Signals and Causes Apoptosis—Since JNK activity is also induced by various mitogenic stimuli, it is intriguing to see the same kinase participating in two completely opposing pathways—cell proliferation and apoptosis. We treated the Jurkat cells with anti-CD28 plus PMA or PMA plus ionomycin and examined the JNK induction. JNK1 activation by anti-CD28 plus PMA displayed a rapid and transient induction pattern (Fig. 8A, top panel). Similar kinetics was observed in the JNK1 activation induced by PMA plus ionomycin (Fig. 8B, top panel). Both T-cell stimulation signals induced a moderate ERK2 activation (Fig. 8, A and B, bottom panels) but had no significant effect on p38-MAPK activity (Fig. 8, A and B, middle panels). These data indicate that the duration of JNK induction is regulated differentially in T-cell activation and apoptosis.

JNK kinase activities are down-regulated by dual specificity phosphatases (32, 33). The different kinetics of JNK induction in T-cell activation and apoptosis may be due to the preferential activation of the phosphatases in T-cell activation but not in T-cell apoptosis. We treated Jurkat cells with sodium orthovanadate (a tyrosine phosphatase inhibitor) in the presence or absence of PMA plus ionomycin for 1 h. Sodium orthovanadate alone caused a transient JNK1 induction, which occurred immediately after the treatment and sharply increased at the 2-h time point and then vanished after the 4-h time point (Fig. 9A). This transient induction may be due to the temporary inhibition of cellular phosphatase activities. A combination of
the phosphatase inhibitor and PMA plus ionomycin caused a rapid JNK1 induction within 1 h, as in the T-cell activation signal alone. However, the JNK1 activation induced by PMA and ionomycin was significantly prolonged by co-incubation with sodium orthovanadate (Fig. 9A). JNK activity remained higher than the basal level even 6 h after the co-treatment.

In the early phase of apoptosis, the cells maintain an intact plasma membrane that enables them to exclude propidium iodide but expose phosphatidylserine to the outer membrane; this then enables them to bind annexin V (34). We found a significant percentage of cells stained by annexin V-fluorescein isothiocyanate but excluded propidium iodide in the cells (Fig. 9B). In addition, using propidium iodide to stain the permeabilized cells, we detected an increase of apoptotic cells, which have the subdiploid staining profile, in Jurkat cells treated with the phosphatase inhibitor and T-cell activation agents but not in the cells treated with each of the individual agents (Fig. 9C). Taken together, phosphatase activities may be responsible for the rapid down-regulation of JNK activity in T-cell activation. Inhibition of tyrosine phosphatase activities during T-cell activation converted the mitogenic signals into an apoptotic signal, and this effect may be mediated through the sustained induction of JNK activity.

**DISCUSSION**

Many regulatory molecules, such as the Bcl-2 family members (35) and ICE family proteases (31), have been shown to be involved in the control of apoptosis, but the exact regulatory mechanisms for apoptosis remain unknown. We found that JNK was activated in apoptosis induced by γ radiation, UV-C, Fas signaling, and DNA-damaging drugs (13). JNK activation and apoptosis induced by γ radiation were correlated. In peripheral lymphocytes, which were very sensitive to γ radiation, JNK activity and apoptosis can be induced by 5 Gy of γ radiation (10). In contrast, no significant JNK activation and cell death were observed in murine fibroblasts treated with γ radiation (15–100 Gy; data not shown). In myeloid leukemia cells, HL-60 and BV173, JNK activation and apoptosis can be induced with less than 20 Gy (data not shown). In Jurkat T cells and 293T cells, which are more resistant to γ radiation, apparent cell death and JNK activation only occurred after receiving more than 40–50 Gy (10 and data not shown). To exclude the possibility that a high dose of γ radiation induced cell death (e.g., necrosis) other than apoptosis, by JNK induction, the cell morphology change, DNA fragmentation, phosphidium iodide staining, and blocking by an ICE inhibitor were used to classify that the cell death induced by the radiation treatments was in fact apoptosis (data not shown; Ref 10 and Fig. 4). In summary, although JNK induction did not always occur in cells exposed to γ radiation, in the cells that were susceptible to γ radiation-induced apoptosis, the same dose of γ radiation always induced JNK activation.

Our data showed that different mechanisms may have existed for the radiation-induced and Fas-mediated JNK induction. In subcloned Jurkat cells, JNK activation was well correlated with γ radiation-induced, but not Fas-mediated, apoptosis. UV-C- and γ radiation-induced JNK activation occurred in the presence of ICE inhibitor, although the DNA fragmentation was completely inhibited. In contrast, preincubation of the ICE inhibitor blocked both JNK induction and apoptosis induced by anti-Fas. This data indicate that JNK induction by UV-C and γ radiation were independent of ICE-like proteases or were upstream of these proteases; however, Fas-mediated JNK induction was a downstream effect of ICE-like protease activation. Because the progression of Fas-mediated apoptosis was very quick (the DNA fragmentation occurred in 2 h, which coincided with the JNK activation), and the ICE-like proteases were considered end effectors of apoptosis, the JNK kinase pathway may not be important for the initiation of Fas-mediated apoptosis.

In nerve growth factor (NGF)-differentiated PC-12 neuronal cells, JNK and p38-MAPK activities were persistently activated during apoptosis induced by NGF withdrawal (36). Repression of JNK or p38-MAPK activation by transfection of dominant-negative mutants of upstream kinases (MEKK1 and MKK3 for JNK and p38-MAPK, respectively) blocks cell death induced by NGF depletion. These findings along with our data indicate that the JNK kinase cascade plays an important role in the regulation of apoptosis induced by various agents. In apoptosis induced by NGF withdrawal (36), ERK activity is significantly reduced in PC-12 cells; in contrast, constitutive activation of ERK prevents apoptosis. It was suggested that ERK and the JNK/p38 cascade may have opposing effects on nerve cells, and the dynamic balance between ERK and the JNK-p38 kinase pathways is important in determining whether the cells will undergo proliferation or apoptosis. We detected a sustained JNK activation during apoptosis induced by γ radiation and UV-C. The ERK2 activity was either unchanged or decreased slightly 6 h after the radiation treatments. Since the reductions of ERK2 activity in radiation treatments appeared hours after DNA fragmentation, they are unlikely to be involved in the initiation of apoptosis. Therefore, the regulation of ERK and JNK kinase cascades in radiation-induced apoptosis was distinct from that in apoptosis induced by NGF withdrawal. This difference may be due to the nature of different cell types or different apoptotic stimuli. In addition, we observed a strong JNK activation, along with ERK2 induction, in Jurkat cells stimulated with T-cell activation signals.

Since the JNK kinase cascade is also involved in and required for mitogenic signaling (17, 20, 23), ERK and JNK may not always have opposing effects, and these two MAP kinase cascades may have distinct functions in different cell types, or in different stages of the same cell type. The observations of: (i) JNK activation in apoptosis induced by different agents; (ii) induction of cell death by overexpression of JNK; and (iii) blockage of cell death by a dominant-negative JNK mutant indicate the essential role of JNK in apoptosis. The activation
Sustained JNK Activation and Apoptosis

of JNK kinases may be the executing step of cell death in response to the accumulating genomic and/or structural damages after treatment with apoptotic agents. Similarly, in neuronal cells, deprivation of NGF may render the cells in an unfavorable condition that causes activation of JNK and initiates the cell death program. Constitutive activation of ERK may actually mimic the survival signal of NGF that in turn prevents the cell death process.

The different patterns of JNK induction between T-cell activation and apoptosis imply that the duration of JNK induction may be the determining factor in the cell-signaling decision. Transient activation of JNK may induce the genes required for T-cell proliferation, and persistent activation of JNK may cause aberrant gene expression and subsequent T-cell apoptosis (Fig. 10). The prolonged JNK1 induction and apoptosis in Jurkat cells co-treated with T-cell activation signals and sodium orthovanadate suggest that phosphatase activity is important for controlling the duration of JNK induction and prevention of apoptosis. The different kinetics of JNK1 induction in T-cell activation and apoptosis may be due to the preferential induction of dual specificity phosphatases by T-cell activation signals but not by apoptotic signals. This hypothesis is supported by the failure of DNA-damaging agents to induce the dual specificity phosphatase CL100/MKP1 (37), which is inducible by mitogenic signals (38) and is a JNK phosphatase (32, 33). In summary, co-treatment of sodium orthovanadate with PMA plus ionomycin prolonged JNK induction and converted the T-cell activation signal into an apoptotic signal. This result strongly suggests, but does not prove, that the duration of JNK induction may determine cell proliferation versus apoptosis (Fig. 10).

Different durations of ERK activation also have distinct effects on cell fate (39). Transient induction of ERK leads to proliferation of PC12 cells, whereas prolonged ERK activation causes the differentiation of PC12 cells to sympathetic neurons (40–42). Therefore, a single kinase-signaling pathway may have distinct functions if the induction patterns are different. However, the kinetics of JNK activation may not be the only determining factor for the fate of cells; hence, we do not exclude the possibility that the wrong timing of JNK activation in the presence or absence of other cellular factors may affect the outcomes. For example, constitutive JNK activation is associated with human T-lymphotropic virus type 1–mediated tumorigenesis after certain unknown genetic changes in infected cells (43). Understanding the interaction between JNK and other apoptotic regulators will provide important information on the mechanisms of cell death as well as cell proliferation and transformation.

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