Stimulation of p38 Mitogen-activated Protein Kinase Is an Early Regulatory Event for the Cadmium-induced Apoptosis in Human Promonocytic Cells*

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Pulse treatment of U-937 promonocytic cells with cadmium chloride (2 h at 200 μM) provoked apoptosis and induced a rapid phosphorylation of p38 mitogen-activated protein kinase (p38MAPK) as well as a late phosphorylation of extracellular signal-regulated protein kinases (ERK1/2). However, although the p38MAPK-specific inhibitor SB203580 attenuated apoptosis, the process was not affected by the ERK-specific inhibitor PD98059. The attenuation of the cadmium-provoked apoptosis by SB203580 was a highly specific effect. In fact, the kinase inhibitor did not prevent the generation of apoptosis by heat shock and camptothecin, nor the generation of necrosis by cadmium treatment of glutathione-depleted cells, nor the cadmium-provoked activation of the stress response. The generation of apoptosis was preceded by intracellular H2O2 accumulation and was accompanied by the disruption of mitochondrial transmembrane potential, both of which were inhibited by SB203580. On the other hand, the antioxidant agent butylated hydroxyanisole-inhibited apoptosis but did not prevent p38MAPK phosphorylation. In a similar manner, p38MAPK phosphorylation was not affected by the caspase inhibitors Z-VAD and DEVD-CHO, which nevertheless prevented apoptosis. These results indicate that p38MAPK activation is an early and specific regulatory event for the cadmium-provoked apoptosis in promonocytic cells.

Cadmium and other heavy metals are frequent environmental contaminants with well known mutagenic, carcinogenic, and teratogenic effects (1). Another property of heavy metals (and of other physical and chemical agents, such as heat and inhibitors of energy metabolism) is the capacity to induce the stress response, characterized by the synthesis and accumulation of heat-shock proteins (HSPs) (2). The stimulation of HSP gene expression by stress inducers in higher eukaryotes seems to be specifically regulated by heat-shock factor 1 (HSF1). Thus, under stressful conditions, HSF1 undergoes trimerization, translocation to the nucleus, binding to the heat-shock consensus elements, and finally hyperphosphorylation to fully acquire the transactivation capacity (3). In addition, stress inducers may provoke cell death, either apoptotic or necrotic, depending on the intensity of the treatments (4, 5). The morphological characteristics of apoptosis and necrosis are well known (6–8). Thus, during apoptosis the cells undergo nuclear and cytoplasmic shrinkage, the chromatin is partitioned into multiple fragments, and the cells are broken into multiple membrane-surrounded bodies (“apoptotic bodies”), but the cell membrane retains its integrity during the process. By contrast, necrosis is characterized by cell swelling and a rapid loss of membrane integrity. However, the regulation of apoptosis and necrosis and the factors that decide the selection of one or the other mode of death are still poorly known.

One of the most relevant aspects in the regulation of both the stress response and apoptosis is the involvement of mitogen-activated protein kinases (MAPKs), a family of serine/threonine kinases that mediate intracellular signal transduction in response to different stimuli (9). The MAPK family members are themselves activated by reversible dual phosphorylation on a Thr-Xaa-Tyr conserved motif, by specific mitogen-activated protein kinase kinases. To date, three major MAPKs have been identified, namely the extracellular signal-regulated kinases (ERK1/2, p44/p42), the stress-activated protein kinase 2 (c-Jun NH2-terminal kinases and p38 MAPK). To date, three major MAPKs have been identified, namely the extracellular signal-regulated kinases (ERK1/2, p44/p42), the stress-activated protein kinase 2 (c-Jun NH2-terminal kinases, stress-activated protein kinase 1), and the p38 mitogen-activated protein kinases (p38MAPK, stress-activated protein kinase 2). ERK1/2 are mainly (although not exclusively) activated by growth factors (10, 11) and are critically involved in the regulation of mitogenesis. On the other hand, c-Jun NH2-terminal kinases and p38MAPK are mainly activated by cytotoxic insults and are often associated with apoptosis (12–18).

It was recently reported that cadmium chloride induced the stress response and caused a dose-dependent activation of ERK1/2 and p38MAPK (but not of c-Jun NH2-terminal kinases) in association with mitogenesis and apoptosis, respectively, in rat brain tumor cells (19). Whereas it was demonstrated that both kinases were involved in the regulation of the stress response, it is not clear whether they were also required to induce cell death. In the present work we investigated the...
capacity of cadmium chloride to induce p38MAPK and ERK1/2 activation and to cause cell death in U-937 human promonocytic cells. It was concluded that p38MAPK activation is an early and specific requirement for the cadmium-provoked apoptosis, which precedes and probably modulates the expression of other regulatory events.

**EXPERIMENTAL PROCEDURES**

**Materials**—All components for cell culture were obtained from Life Technologies, Inc. Butylated hydroxyanisole (BHA), camptothecin, 2-buthionine-SR-sulfoximine (BSO), N-acetyl-L-cysteine (NAC), propidium iodide (PI), and Nonidet P-40 were from Sigma (St. Louis, MO). Mammalian cell culture medium (DMEM) was purchased from Life Technologies, Inc. Butylated hydroxyanisole (BHA), camptothecin, DL-β-dihexylocarbocyanine iodide were from Molecular Probes, Inc. (Eugene, OR). The MAPK inhibitors SB203580 and PD98059 and the caspase inhibitors Z-Val-Ala-Asp (OMe)-CH2F (Z-VAD) and acetyl-Asp-Glu-Val-Asp aldehyde (DEVD-CHO) were obtained from Calbiochem-Novabiochem. All antibodies against mitogen-activated protein kinase family members were rabbit anti-human phosphorylated p38MAPK pAb, rabbit anti-human phospho-p38MAPK (Thr180/Tyr182) pAb, rabbit anti-human p44/42 MAPK phospho-p44/42 MAPK pAb, and rabbit anti-human phospho-p44/42 MAPK (Thr202/Tyr204) pAb, were purchased from New England Biolabs, Inc. (Beverly, MA); mouse anti-human HSP70 monoclonal antibody (clone C92F34–5) and rabbit anti-human HSP70 monoclonal antibody was from Amersham Pharmacia Biotech. The goat anti-rabbit and anti-mouse peroxidase-conjugated antibodies were from Dako A/S (Glostrup, Denmark). The Annexin V-fluorescein isothiocyanate kit was purchased from Bender MedSystems (Vienna, Austria).

**Cells and Treatments**—U-937 promonocytic leukemia cells (20) were routinely grown in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, as described previously (21). Sixteen hours prior to treatments, cells were seeded at 2 × 10^6 cells/ml in medium containing 1.5% fetal calf serum. Cadmium chloride and BSO were dissolved in distilled water at 0.1 m and used after use. SE205580, PD98059, camptothecin, and Z-VAD were dissolved in MeSO at the concentrations of 13.2, 20, 10, and 20, mm, respectively, and DEVD-CHO in distilled water at 10 mm. All these solutions were stored at −20 °C. For cadmium treatment, cells were incubated for 2 h with 200 μM cadmium chloride. In some experiments, the cells were washed after treatment with prewarmed (37 °C) RPMI medium and allowed to recover under standard culture conditions. For heat shock, cells were placed in a bath at the desired temperature for the required time period and then allowed to recover under standard culture conditions. As controls, cells were subjected to the same manipulations as treated cells, in the absence of cadmium. For GSH depletion, cells were incubated for 18–24 h with 1 mM BSO, as earlier reported (22). Under these conditions BSO reduced the intracellular GSH level by more than 90% without affecting cell proliferation or viability.

**Determination of Apoptosis and Necrosis**—To analyze changes in chromatin structure, cells were collected by centrifugation, washed with phosphate-buffered saline (PBS), resuspended in PBS, and mounted on glass slides. After fixation in 70% (v/v) ethanol, the cells were stained for 20 min at room temperature with 4,6-diamino-2-phenylindole (1 μg/ml) and examined by fluorescence microscopy. Cells with fragmented chromatin were considered as apoptotic. Nucleosome DNA cleavage was determined by agarose gel electrophoresis, as described previously (21).

To measure the loss of DNA, cells were collected by centrifugation and incubated for 30 min in PBS containing 0.5 mg/ml RNase A. After the addition of 50 μg/ml PI and permeabilization with 0.1% (v/v) Nonidet P-40, the cells were analyzed by flow cytometry. Cells with sub-G₀ PI incorporation were considered as apoptotic (23). Within the experimental time periods used here necrotic cells did not exhibit sub-G₀ PI incorporation.

The criteria used to determine necrosis was the loss of membrane integrity, as measured by permeability to trypan blue or by massive influx of PI in nonpermeabilized cells. In the later case, cells were washed with PBS, PBS was replaced with PI-containing buffer (100 μM of a buffer consisting of 10 mM HEPES (pH 7.4), 150 mM NaCl, 5 mM KC1, 1 mM MgCl₂, and 1.8 mM CaCl₂, containing 20 μg of PI and 3 μl of fluorescein isothiocyanate-conjugated human annexin V. The cells were then analyzed by flow cytometry using appropriate color filters to determine the PI-derived reddish orange fluorescence (emission peak 590 nm) and the fluorescein isothiocyanate-derived greenish fluorescence (emission peak 530 nm). Apoptotic cells were characterized by annexin V binding but with null or low PI influx, whereas necrotic cells were characterized by annexin V binding and a great increase in red fluorescence because of the massive influx of PI (24).

**Measurement of Hydrogen Peroxide and Mitochondrial Transmembrane Potential**—The intracellular H₂O₂ content was determined by flow cytometry after loading the cells with dichlorodihydrofluorescein diacetate, as described earlier (25).

To evaluate mitochondrial transmembrane potential (ΔΨₘ), cells were washed with PBS and then incubated for 20 min at room temperature with PBS containing 0.1 mM 3,3′-dihexyloxy carbocyanine iodide (3). After washing twice with fetal calf serum, the cells were resuspended in PBS, and the fluorescence was measured by flow cytometry.

**Immunoblot Assays**—Whole cell extracts were fractionated by SDS-polyacrylamide gel electrophoresis in 10% polyacrylamide minigels and transferred to Immobilon-P transfer membranes (Millipore Corp., Bedford, MA). After blocking with 3% nonfat milk in TTBS buffer (0.1% (v/v) Tween 20, 25 mM Tris, 150 mM NaCl, pH 7.5), the membranes were incubated overnight with the primary antibody, then extensively washed with TTBS, and incubated for 1 h with the secondary antibody. After extensive washing with TTBS, the immune complexes were detected by chemiluminescence using the Western blotting kit from Pierce.

**Gel Retardation Assays**—Nuclear extracts from 10³ cells were prepared as earlier described (26) and stored at −70 °C. For HSF1 binding assay, 3 mg of nuclei were prepared in 1 ml binding buffer (60 mM KCl, 1 mM MgCl₂, 12% glycerol, 1 mM 1,4-dithiothreitol, and 20 mM HEPES, pH 7.9) containing 5 ng of the labeled probe, 8 μg of total nuclear proteins, 2 μg of poly(dI:dC), and 2 μg of salmon sperm DNA. Fifty-fold excess heat shock element, unrelated (AP-1-recognizing) unlabeled probes, or anti-HSF1 antibody were used to check the specificity of the binding reaction. The samples were electrophoresed in 4% polyacrylamide gels that were dried and later autoradiographed.

**RESULTS**

**Apoptosis Induction**—We have previously shown that pulse treatment with high cadmium concentrations (100 μM and above) induced the stress response and caused death in U-937 promonocytic cells (28, 29). To determine the mode of death, the expression of apoptotic and necrotic markers was measured in cells pulse-treated for 2 h with 200 μM cadmium chloride followed by recovery. It was observed that the treatment caused chromatin fragmentation (Fig. 1A), accumulation of cells with sub-G₁ DNA content (Fig. 1B), and nucleosome-sized DNA fragmentation (Fig. 1C), all of which are characteristics of apoptosis. Apoptotic cells were already detected at 1 h of recovery after treatment, reaching approximately 60% at 6 h. In contrast, no significant necrosis was detected during this period of recovery, as revealed by trypan blue staining (Fig. 1A) or free PI uptake (see Fig. 6B). Necrosis was not detected even at a cadmium chloride concentration of 1 mM (result not shown). Nevertheless, after prolonged recovery periods (12 h and thereafter) some trypan blue-stained cells started to be detected (result not shown), which suggests secondary necrosis, derived from apoptosis. Hence, 6 h was the maximum recovery period adopted for further experiments.

**Activation of Mitogen-activated Protein Kinases**—Earlier observations indicated that cadmium caused a dose-dependent activation of ERK1/2 and p38MAPK, as measured by their increased phosphorylation, in rat brain tumor cells (19). Hence, we decided to measure the phosphorylation/activity of those kinases in cadmium-treated U-937 cells. As shown in Fig. 2, cadmium provoked a rapid increase in p38MAPK phosphorylation, which was already detected after 30 min of treatment, hence preceding the expression of apoptotic markers and followed during the whole treatment and recovery periods. In addition, cadmium provoked a late increase in ERK1/2 phosphorylation, which started at 1–3 h of recovery, coincident with
The rapid increase in p38MAPK phosphorylation and the delayed increase in ERK1/2 phosphorylation might indicate that these kinase activities are involved in the triggering and the execution of apoptosis, respectively. To investigate this, we analyzed the effect of the p38MAPK-specific inhibitor SB203580 and the ERK-specific inhibitor PD98059 on the cadmium-provoked apoptosis.

Effect of Kinase Inhibitors on Apoptosis—To rule out the possibility of a general, nonspecific interference of SB203580 with the apoptotic machinery, new experiments were carried out using other apoptotic agents, namely p38MAPK phosphorylation/activation by inhibiting mitogen-activated protein kinase/extracellular signal-regulated kinase activity by competing with ATP for binding (31), but it was later demonstrated that the drug also prevents p38MAPK phosphorylation/activation (32). We observed that treatment of U-937 cells with 30 μM PD98059 totally abolished ERK phosphorylation, whereas treatment with 5–20 μM SB203580 (because higher concentrations were toxic) only partially inhibited p38MAPK phosphorylation (Fig. 3A). However, although PD98059 failed to alter (either inhibit or potentiate) the expression of apoptotic markers, SB203580 attenuated apoptosis by approximately 50% (Fig. 3, B and C). These results suggest that p38MAPK activation is required, at least in part, for the cadmium-provoked apoptosis, whereas ERK activation is irrelevant for the process.

The determinations in A were repeated three times with similar results. All other conditions were as in Figs. 1 and 2.
the stress inducer heat shock (2 h at 42.5 °C followed by recovery) (28, 29) and the antitumor drug camptothecin (as a continuous treatment at 0.4 μM). Whereas heat shock did not significantly induce p38MAPK phosphorylation, this kinase was transiently phosphorylated by camptothecin (Fig. 4A). Nevertheless, SB203580 failed to attenuate the generation of apoptosis by both agents, as demonstrated by chromatin fragmentation (Fig. 4B) and the accumulation of cells with sub-G1 DNA content (result not shown).

**Stress Response and Necrosis Induction**—To exclude the possibility of a general, nonspecific cadmium inactivation by SB203580 (e.g. by direct drug-metal interaction) we analyzed the action of this kinase inhibitor on other effects of cadmium, namely the stress response and necrosis induction. The stress response was measured by HSF1 binding at 2 h of treatment and by HSP70 expression at different times of recovery. It was observed that SB203580 did not affect the cadmium-provoked accumulation of HSP70 (Fig. 5A) nor the stimulation of HSF1 binding (Fig. 5B), suggesting that the p38MAPK is not relevant for the induction of the stress response by cadmium in these cells. Moreover, SB203580 also failed to inhibit the increase in HSP70 caused by heat shock, whereas as expected (33), the increase was abolished or greatly reduced by the antioxidant agents NAC and BHA (Fig. 5C). This excludes the possibility that SB203580 may act as a radical oxygen scavenger in U-937 cells.

It was reported that depletion of intracellular GSH may potentiate the lethality of cytotoxic drugs, occasionally switching the mode of death from apoptosis to necrosis (34). We observed that a 18–24-h incubation with the GSH depletor BSO (1 mM) was innocuous (Fig. 6, A and B) and did not affect p38MAPK and ERK1/2 phosphorylation (results not shown). However, preincubation with BSO potentiated the cadmium toxicity, causing necrosis instead of apoptosis, as revealed by free uptake of trypan blue (Fig. 6A) and PI (Fig. 6B). Under these conditions p38MAPK was phosphorylated with the same efficacy, and ERK1/2 with greater efficacy, than in cells treated with cadmium alone (Fig. 6C). However, neither SB203580 nor PD98059 were able to prevent the necrotic response (Fig. 6, A and B). Taken together, the present results indicate that the inhibition by SB203580 of cadmium-provoked apoptosis is a highly specific effect.

**Caspase Activity, Oxidative Stress, and Mitochondrial Transmembrane Potential**—Finally, we wanted to investigate the possible relationship between p38MAPK activation and other events that regulate apoptosis. This included caspase activities, intracellular oxidation and changes in mitochondrial transmembrane potential (Δψm). It is known that apoptosis requires the action of ICE-like proteases (caspases) (for review see Ref. 35). Moreover, caspase activities seem also to mediate p38MAPK activation by some inducers (14). To approach the problem, we analyzed the action of the caspase-3-specific inhibitor DEVD-CHO and the nonspecific caspase inhibitor Z-VAD on apoptosis and p38MAPK activation in cadmium-treated cells. As expected, both inhibitors attenuated apoptosis (Fig. 7A). However, they did not affect p38MAPK phosphorylation (Fig. 7B). This suggests that p38MAPK plays its regulatory role on apoptosis upstream or independently of the caspase cascade.

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Earlier reports indicated that cytotoxic insults rapidly cause intracellular oxidation measured by H$_2$O$_2$ accumulation, which at least in some apoptotic models is a trigger for cell death (25, 36, 37). In addition, apoptosis involves and is regulated by a disruption of Δϕm, (for reviews see Refs. 38 and 39). Hence, we wanted to investigate the possible relationship between these phenomena and p38MAPK activation. We found that cadmium caused a rapid increase in H$_2$O$_2$ accumulation, which was already detected after 30 min of treatment (Fig. 8A), at the same time as the increase in p38MAPK phosphorylation. The treatment also caused a late disruption of Δϕm, which was first detected at 3 h of recovery (Fig. 9), hence paralleling the expression of apoptotic markers. The administration of the antioxidant agent BHA reduced cell death (Fig. 8B), proving the importance of intracellular oxidation for the cadmium-provoked apoptosis, but it did not inhibit p38MAPK phosphorylation (Fig. 8C). More potent antioxidants such as NAC and other thiol-containing agents could not be used because of the direct reactivity of –SH groups with Cd$^{2+}$ ions. Interestingly, the administration of SB203580 greatly reduced H$_2$O$_2$ accumulation (Fig. 8A) as well as Δϕm disruption (Fig. 9). Taken together, these results indicate that p38MAPK activation is an early regulatory event in cadmium-provoked apoptosis.
Fig. 9. Modulation of mitochondrial transmembrane potential (Δψm). The figure shows the degree of Δψm disruption at different times of recovery from cadmium treatment (2 h at 200 μM) in the absence (Cd) or in the presence (Cd/SB) of 10 μM SB203580, as revealed by the decrease in fluorescence upon 3,3′-dihexyloxacarboxylic iodide loading. All other conditions were as in Figs. 1–3.

DISCUSSION

Although apoptotic stimuli usually activated p38MAPK and this activation was required for apoptosis in some models (40–43, among others), it seemed to be irrelevant in others. This was proved by the inability of the p38MAPK-specific inhibitor SB203580 to prevent apoptosis in Fas- and UV-treated Jurkat T cells (14), in UV-treated U-937 cells (16), in H2O2-treated HeLa cells (17), and in nitric oxide-treated RAW macrophages (18), among other examples. Likewise, a p38MAPK dominant-negative mutant failed to prevent the induction of apoptosis in UV- and γ-radiation in 293T human embryonic kidney cells (13). It was reported that heavy metals also induce p38MAPK activation in different cell types (19, 44, 45), but the actual relevance of such activation for apoptosis was not known. Our present results indicate that treatment of U-937 promonocytic cells with apoptotic inducers, such as cadmium, rapidly causes p38MAPK activation, as judged by its increased phosphorylation, and proves that this activation is required for apoptosis, as revealed by the capacity of SB203580 to reduce both p38MAPK phosphorylation and apoptosis. Moreover, the regulation by p38MAPK of the cadmium-provoked apoptosis seemed to be a highly specific effect, which cannot be explained by a general interference of the kinase inhibitor with either the apoptotic machinery or the total cadmium activity. This conclusion is based on the following: (a) SB203580 did not prevent the generation of apoptosis by other agents, such as heat shock and camptothecin, although the later one also caused p38MAPK activation; (b) SB203580 did not prevent the generation of necrosis induced by the cadmium treatment of BSO-preincubated cells, although this treatment also increased p38MAPK phosphorylation; and (c) SB203580 did not prevent the cadmium-provoked activation of the stress response. This later result was somewhat surprising, because SB203580 was reported to prevent HSF1 activation and HSP70 induction in rat brain tumor cells treated with high apoptosis-inducing cadmium concentrations (19). Such a discrepancy suggests the existence of marked differences in the regulation of the stress response in different cell types.

The rapid phosphorylation of p38MAPK suggested that this kinase plays an early role in the regulation of the cadmium-provoked apoptosis. This conclusion was also proved by examining other regulatory events, such as caspase activity, Δψm disruption, and intracellular oxidation. It is known that apoptosis requires the activation of a cascade of ICE-like proteases, which, at least in myeloid cells, ends with the activation of the effector caspase-3 (46, 47). Moreover, caspases may also regulate p38MAPK activation by some apoptotic inducers, as in Fas-treated Jurkat T cells (14). However, our experiments showing that the activation of p38MAPK by cadmium was insensitive to the pan-caspase inhibitor Z-VAD or the caspase-3-specific inhibitor DEVD-CHO suggest that the regulation by p38MAPK of the cadmium-provoked apoptosis is either upstream or independent of the caspase cascade. The attenuation by SB203580 of the fall in Δψm suggests that the regulatory role of p38MAPK is also upstream of Δψm disruption, a phenomenon that in some cases is an early event while in others (as in cadmium-treated U-937 cells) occurs simultaneously with the execution cell death (48–50). Finally, the interaction between p38MAPK activation and intracellular oxidation was more intriguing, because both phenomena took place rapidly upon cadmium administration and could not be separated based on kinetic data. Earlier reports indicate that the toxicity of cadmium is the result at least in part of intracellular oxidation (51, 52). This was confirmed in our experiments showing an increase in H2O2 production by cadmium and a reduction in apoptosis by the antioxidant agent BHA. Moreover, it was found that SB203580 attenuated H2O2 accumulation, whereas BHA did not affect p38MAPK phosphorylation. The possibility that SB203580 acts as a radical oxygen scavenger may be excluded, because this agent did not prevent the heat-provoked induction of HSP70, as done by typical antioxidants (33 and Fig. 5C). Hence, we conclude that p38MAPK activity probably plays a role in the triggering of intracellular oxidation by cadmium. An attractive hypothesis is that p38MAPK is required for apoptosis only when apoptosis is regulated via intracellular oxidation, a subject that is at present under investigation in our laboratory. Interestingly, a recent report indicates that SB203580 inhibits NADPH oxidase in human neutrophils, suggesting that p38MAPK kinase regulates the oxidative burst in these cells (53).

In addition to their inducibility by mitogenic stimuli, ERKs may also be activated by heavy metals and other cytotoxic insults (17–19, 44, 45). In most cellular models, ERK activation seems to inversely correlate with apoptosis. For instance, suppression of ERK1/2 activation by PD98059 enhanced apoptosis in H2O2-treated HeLa cells (17) and in nitric oxide-treated RAW macrophages (18), and conversely, the activation of the ERK pathway inhibited apoptosis in neuronal PC-12 cells (11). However, in other models PD98059 inhibited apoptosis, e.g., in nerve growth factor-deprived pheochromocytoma cells (40) and in H2O2-treated CG4 oligodendrocytic cells (54), suggesting a pro-apoptotic role for ERK1/2 activity. Watabe et al. (55) showed that bufalin caused a late ERK activation in U-937 cells, which could explain the generation of apoptosis by this agent. In our experiments cadmium caused a similar late ERK1/2 activation, as indicated by their increased phosphorylation, which paralleled the execution of apoptosis. Moreover, preincubation with BSO followed by cadmium treatment caused necrosis instead of apoptosis and overincreased ERK1/2 phosphorylation, suggesting that the extent of kinase activation might also be important in determining the mode of death. However, PD98059 failed to prevent apoptosis in cadmium-treated cells and necrosis in BSO plus cadmium-treated cells, indicating that ERK activity is irrelevant for death induction (either apoptotic or necrotic) by cadmium. Studies using other apoptotic agents and different procedures to modulate ERK1/2 activity are in progress to better define the possible role of this kinase in death induction in promonocytic cells.

REFERENCES