Biochemical and Biophysical Research Communications **289,** 34–38 (2001)
doi:10.1006/bbrc.2001.5928, available online at http://www.idealibrary.com on IDEAL®

Constitutive Hyperexpression of $p21^{WAF1}$ in Human U266 Myeloma Cells Blocks the Lethal Signaling Induced by Oxidative Stress but Not by Fas

Do Kyun Kim,* Eun Sook Cho,* Su-Jae Lee,† and Hong-Duck Um*,‡,[1]

*Laboratory of Cell Biology, Yonsei Medical Research Center and ‡Brain Korea 21 Center for Medical Sciences, Yonsei University College of Medicine, CPO Box 8044, Seoul 120-752, Korea; and †Laboratory of Radiation Effect, Korea Cancer Center Hospital, Seoul 139-706, Korea

Received October 17, 2001

$p21^{WAF1/CIP1}$ is expressed in a majority of myeloma cells. To investigate the role of p21 in myeloma cell death, comparative studies using two clones of myeloma cells, Fas-sensitive RPMI8226, and Fas-resistant U266 were performed. These latter cells were also resistant to H_2O_2 up to 100 μM, whereas the former cells were not. SAPK/JNK was found to be a common mediator of RPMI8226 cell death induced by both H_2O_2 and Fas. Interestingly, the concentrations of H_2O_2 which activated SAPK/JNK in RPMI8226 cells failed to do so in U266 cells. In contrast, Fas ligation activated SAPK/JNK in both cells almost equally. U266 cells expressed p21 to levels much higher than in RPMI8226 cells. When the p21 levels were reduced using its antisense, H_2O_2 killed U266 cells by activating SAPK/JNK. However, the reduction in p21 levels neither rendered the U266 cells susceptible to Fas-mediated cell death, nor significantly influenced Fas-induced SAPK/JNK activation. Overall, our data suggest that the p21 hyperexpression in U266 cells blocks the lethal signaling that is induced by H_2O_2, but not by Fas. The mechanism whereby U266 cells resist Fas-mediated cell death is discussed.

Key Words: **p21; myeloma cells; multiple myeloma; cell death; hydrogen peroxide; oxidative stress; Fas; SAPK/JNK.**

Multiple myeloma is characterized by the uncontrolled expansion of malignant plasma cells (myeloma cells) which predominantly appear in the bone marrow. Unlike many other types of malignant cells, most myeloma cells have a very low proliferative activity. Considering that the outgrowth of neoplastic clones can be achieved not only by an enhanced rate of cell proliferation but also by a decreased rate of cell death (1), this latter mechanism may be particularly important in the expansion of slowly growing myeloma cells. Indeed, many, but not all, clones of myeloma cells were shown to withstand a lethal stimulus such as an agonistic anti-Fas antibody (anti-Fas) (2–5). However, little information exists as to whether myeloma cells are also resistant to other forms of lethal stimuli. Moreover, the mechanisms whereby myeloma cells survive lethal stimuli have been poorly described, although this information is essential in understanding the pathogenesis of multiple myeloma and for developing new therapeutic strategies.

$p21^{WAF1/CIP1}$ is an inhibitor of cyclin-dependent kinases, and therefore plays a major role in cell cycle arrest (6). Recently, evidence has accumulated that p21 can also control the susceptibility of neoplastic cells to lethal stimuli. For instance, an overexpression of p21 rendered glioblastoma cells resistant to chemotherapeutic agents such as nitrosourea and cisplatin (7). Consistent with this observation, the reduction of p21 levels using its antisense construct resulted in an increase in human lymphoma cell death that was by anti-IgM and an anticancer agent (8, 9). Although these observations support a cytoprotective role for p21, a completely opposite function of p21 has been proposed under other experimental conditions. An example of this is that the ectopic expression of p21 increased the susceptibility of hepatocarcinoma cells to ceramide (10). When these results are taken together, p21 appears to either suppress or promote cell death depending on the experimental circumstances.

Consistent with the slowly proliferating characteristics of myeloma cells, a majority of them constitutively express p21 (11). Although this observation implicates that p21 is involved in the regulation of myeloma cell death, it is difficult to predict its precise role in this context because of the variability of p21 functions. In

[1] To whom correspondence and reprint requests should be addressed at Laboratory of Cell Biology, Yonsei Medical Research Center, Yonsei University College of Medicine, CPO Box 8044, Seoul 120-752, Korea. Fax: 82-2-313-3059. E-mail: hdum@yumc.yonsei.ac.kr.

0006-291X/01 $35.00

the present study, we performed functional studies to directly address the question as to the role of p21 in myeloma cell death. Our data suggests that p21 protects myeloma cells from certain, but not all, forms of lethal stimuli by inhibiting the activation of stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK). It appears, therefore, that the up-regulation of p21 in myeloma cells is beneficial to their expansion.

MATERIALS AND METHODS

Materials. All the antibodies used in this study were raised against human antigens. Anti-Fas was purchased from Upstate Biotechnology (Lake Placid, NY). Anti-p21 and anti-SAPK were obtained from PharMingen/Transduction Laboratories (San Diego, CA). The recombinant c-Jun protein was purchased from New England Biolabs (Beverly, MA).

Cell culture, DNA transfection, and treatments. RPMI8226 and U266 cells were cultured in a RPMI 1640 medium supplemented with 10% heat-inactivated FBS and gentamicin (50 μg/ml). To treat cells with anti-Fas and H_2O_2, the cell density was adjusted to 3×10^5/ml. The untreated control and treated cells were maintained at 37°C in 5% CO_2 and high humidity for the periods of time indicated. For DNA transfection, the dominant negative mutant of MKK4/SEK1 (12) and the p21 coding region (13) were cloned into the pcDNA vector in the sense and antisense orientations, respectively. The expression plasmids were delivered into the specified cell types by electroporation. The transfected cells were selected by using 1 mg/ml of G418 sulfate, after which they received the indicated treatments.

Analysis of cellular viability. The incubated cells received propidium iodide (5 μg/ml) followed by flow cytometry analysis to simultaneously monitor the uptake of propidium iodide (FL-2 channel) and cell size (forward light scatter). The cells that displayed both a normal size and a low permeability to propidium iodide were understood to be viable cells, as previously defined (14). All other populations were understood to be dead.

Western blot analysis. Cell lysates were prepared as described previously (15). Equal amounts of proteins (70 μg) were separated by 15% SDS–PAGE. The proteins were then electrotransferred to Immobilon membranes (Millipore, Bedford, MA), which were subsequently blotted using anti-p21 and visualized by chemiluminescence (ECL; Amersham, Arlington Heights, IL).

In vitro kinase assay. The cells were lysed in a Hepes buffer (50 mM, pH 7.4) containing 100 mM NaCl, 10% glycerol, 1% NP-40, 1 mM EDTA, 20 mM β-glycerophosphate, 1 mM NaF, 1 mM *p*-nitrophenyl phosphate, 1 mM sodium orthovanadate, and the protease inhibitors (2 μg/ml aprotinin, 2 μg/ml leupeptin, and 100 μg/ml PMSF). The lysates were clarified by centrifugation at 13,000*g* for 15 min. Immunoprecipitation was performed by using 400 μg of the lysate proteins and the anti-SAPK. The precipitates were resolved in 20 μl of a kinase buffer containing 20 mM Hepes (pH 7.4), 10 mM $MgCl_2$, 20 mM β-glycerophosphate, 10 mM NaF, 1 mM DTT, 0.5 mM sodium orthovanadate, 50 μM ATP, and 10 μCi [γ-^{32}P]ATP. The kinase reactions were initiated by adding 2 μg of c-Jun protein to the solution. After the specified incubation times, the reaction was stopped by adding a boiled sample buffer, and the proteins were then separated by 12% SDS–PAGE. The gels were dried, and a PhosphoImager using Tina 2.0 software visualized the radioactive bands.

RESULTS AND DISCUSSION

Differential susceptibility of RPMI8226 and U266 cells to H_2O_2. To define a mechanism whereby myeloma cells survive lethal stimuli, we performed a comparative study using two clones of myeloma cells, RPMI8226 and U266. Despite similar levels of Fas expression, only the RPMI8226 cells, and not the U266 cells, died in response to anti-Fas treatment (3–5). To explore whether these two myeloma cell types can respond to other lethal stimuli in a similar manner, they were exposed to various concentrations of H_2O_2. The cellular viabilities were compared 24 h after the treatment by flow cytometric analysis. More than 50% of the RPMI8226 cells lost their viability in response to concentrations of 50 μM H_2O_2 (Fig. 1). The RPMI8226 cell death further increased in a dose-dependent manner, as the concentration of H_2O_2 was elevated up to 250 μM. In contrast, the U266 cells did not significantly lose their viability even when exposed to 100 μM H_2O_2. Higher concentrations (250 μM) of H_2O_2 were necessary for an efficient induction of U266 cell death. Therefore, U266 cells can not only withstand Fas-mediated cell death, but also are less susceptible to H_2O_2 than are RPMI8226 cells.

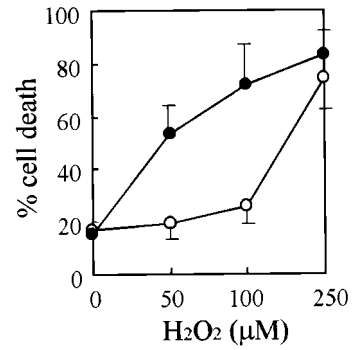

FIG. 1. Differential susceptibility of RPMI8226 and U266 cells to H_2O_2. RPMI8226 (closed circle) and U266 cells (open circle) were exposed to the indicated concentrations of H_2O_2 for 24 h. The cellular viability was analyzed by flow cytometry. The values are the means of three separate experiments with the error bar representing the standard deviations.

SAPK is a mediator of myeloma cell death induced by H_2O_2 and anti-Fas. SAPK has emerged as a key mediator of cell death that is induced under diverse experimental conditions (12, 16–18). To investigate the role of SAPK in myeloma cell death, RPMI8226 cells were exposed to lethal concentrations of H_2O_2 (50–250 μM) and anti-Fas (100 ng/ml). At the end of various incubation times up to 30 min, the SAPK activity was analyzed by an *in vitro* kinase assay using c-Jun as the substrate. The treatment with either H_2O_2 (Figs. 2A and 2B) or anti-Fas (Fig. 2C) resulted in increased SAPK activities. A previous report also demonstrated the ability of anti-Fas to activate SAPK in RPMI8226 cells (19). Therefore, it appears that SAPK is commonly involved in the myeloma cell death induced by both H_2O_2 and anti-Fas. To confirm this hypothesis, RPMI8226 cells were stably transfected with either an empty pcDNA vector or the vector containing the dom-

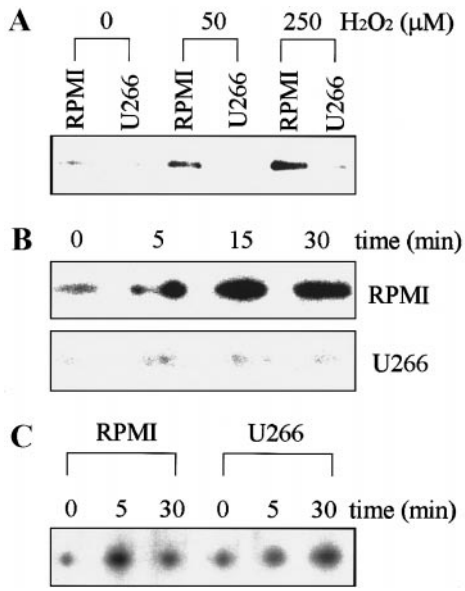

FIG. 2. Comparison of SAPK activation in RPMI8226 and U266 cells. RPMI8226 and U266 cells were exposed to the indicated concentrations of H_2O_2 for 30 min (A), or to 250 μM H_2O_2 (B) and 100 ng/ml anti-Fas (C) for the indicated time periods. SAPK activities in the treated and untreated cells were compared by an *in vitro* kinase assay using c-Jun as the substrate.

inant negative mutant of MKK4, which is a mitogen activated protein kinase kinase that can activate SAPK (20). The introduction of the MKK4 mutant resulted in a reduction in the SAPK activation that was induced by either H_2O_2 or anti-Fas (Fig. 3A). The suppression of H_2O_2-induced SAPK activation by the MKK4 mutant has also been reported using other cell types (16). To determine whether the suppression of SAPK activation influenced cellular responses to lethal stimuli, the transfected cells were treated with various concentrations of H_2O_2 and anti-Fas for 24 h, and then compared for their viability. As shown in Fig. 3B, the cell death induced by both stimuli was attenuated by the expression of the MKK4 mutant. All these results suggested that SAPK can act as a common mediator of myeloma cell death that is induced by both H_2O_2 and anti-Fas. However, it should be noted that the protective effect of the MKK4 mutant against H_2O_2-induced cell death was evident only when relatively low concentrations (50–100 μM) of H_2O_2 were employed. Higher concentrations (250 μM) of H_2O_2 killed the transfectants regardless of the MKK4 mutant expression almost equally. Given that the MKK4 mutant suppressed the SAPK activation induced by 250 μM H_2O_2 (Fig. 3A), this relatively high concentration of H_2O_2 seemed to kill RPMI8226 cells in a manner that was independent of the SAPK activity, possibly because of its cytotoxicity.

H_2O_2 fails to activate SAPK in U266 cells. Having determined the role of SAPK in myeloma cell death, dysregulation of SAPK activation was expected to confer myeloma cells with a resistance to those lethal stimuli. To explore whether such a mechanism operates in U266 cells, the ability of H_2O_2 to activate SAPK in RPMI8226 and in U266 cells was compared. While H_2O_2 in concentrations as low as 50 μM efficiently activated SAPK in RPMI8226, even concentrations as high as 250 μM H_2O_2 failed to do so in U266 cells (Figs. 2A and 2B). These results suggested that H_2O_2 does not efficiently kill U266 cells because of its failure to activate SAPK. In this regard, the U266 cell death that is induced by 250 μM H_2O_2 (Fig. 1) might reflect the SAPK-independent toxicity of H_2O_2 in relatively high concentrations, as already mentioned above.

Hyperexpression of p21 in U266 cells. The above results raised the possibility that U266 cells express a protein that can block H_2O_2-induced SAPK activation. Based on the ability of p21 to suppress UV-induced SAPK activation (21), the levels of p21 in RPMI8226 and U266 cells were compared using a Western blot analysis. While p21 was detected in RPMI8226 cells, its level was dramatically elevated in the U266 cells (Fig. 4A). Therefore, p21 seemed to be a factor that blocks the H_2O_2-induced SAPK activation in U266 cells.

p21 blocks the SAPK activation and cell death induced by H_2O_2. To investigate this possibility, U266 cells were stably transfected with a pcDNA vector containing the p21 antisense construct (U266/p21AS).

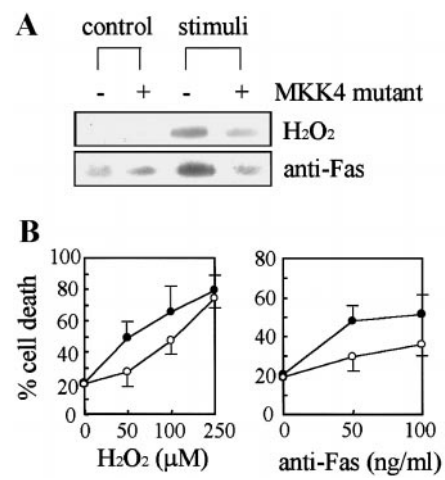

FIG. 3. SAPK is a common mediator of RPMI8226 cell death that is induced by both H_2O_2 and Fas. RPMI8226 cells were stably transfected with either the empty control pcDNA vector or the vector containing the dominant negative mutant of MKK4. (A) These transfectants were exposed to 250 μM H_2O_2 for 30 min or to 100 ng/ml anti-Fas for 5 min. The SAPK activities in the treated and untreated control cells were analyzed by an *in vitro* kinase assay. (B) The transfectants that received either the control vector (closed circle) or the MKK4 mutant (open circle) were treated with the indicated concentrations of H_2O_2 and anti-Fas for 24 h, and compared for their viability.

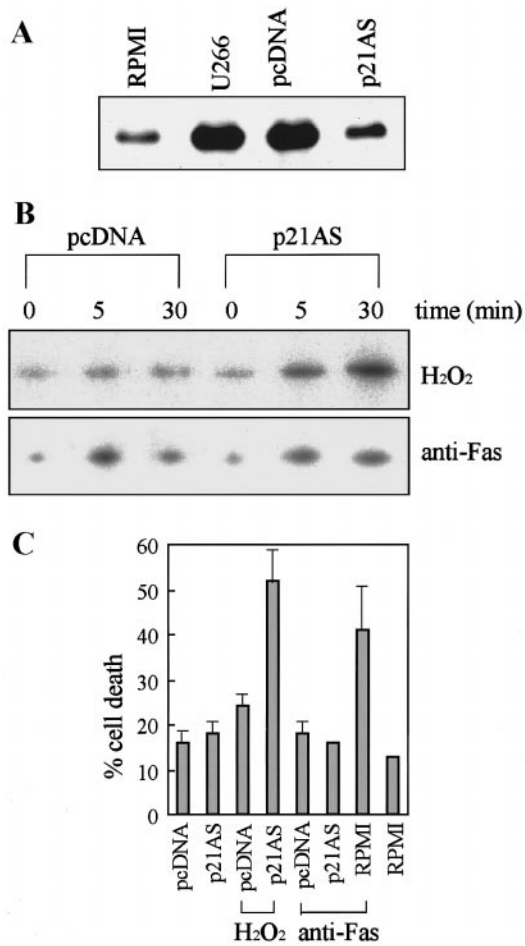

FIG. 4. Role of p21 in SAPK activation and cell death induced by H_2O_2 and Fas. (A) U266 cells were stably transfected with the empty pcDNA vector or the vector containing the p21 antisense. (A) Levels of p21 in the transfectants and untransfected U266 and RPMI8226 cells were compared using a Western blot analysis. (B) The transfectants were exposed to either 100 μM H_2O_2 or 100 ng/ml anti-Fas for the indicated time periods, and the SAPK activities were analyzed. (C) The treatments were extended to 24 h, and cellular viability was compared. For a comparison, RPMI8226 cells also received the same concentration of anti-Fas.

Levels of p21 in the cells that received the empty control vector (U266/pcDNA) were almost equal to those in untransfected U266 cells, but were dramatically reduced in the U266/p21AS cells (Fig. 4A). Similarly to the untransfected U266 cells, 100 μM H_2O_2 did not activate SAPK in the U266/pcDNA cells (Fig. 3B). However, the same concentration of H_2O_2 efficiently activated SAPK in the U266/p21AS cells. Therefore, the H_2O_2-induced lethal pathway appeared to be intact in the U266 cells, but this was blocked by p21. To confirm this possibility, the transfectants were exposed to 100 μM H_2O_2 for 24 h and their viabilities were compared. While nearly 75% of the U266/pcDNA cells were viable, more than 50% of the U266/p21AS cells lost their viability (Fig. 4C). These results are consistent with the report that a p21 overexpression reduces the H_2O_2-induced death of human U937 leukemia cells (22). This suggests that the enhanced levels of p21 protected U266 cells against oxidative stress. Our overall data suggests that the p21 hyperexpression in U266 cells suppresses H_2O_2-induced cell death by interfering with the ability of H_2O_2 to activate SAPK. To our knowledge, this is the first report that establishes p21 to be a survival factor for myeloma cells.

Anti-Fas activates SAPK in U266 cells. Whether the p21-dependent suppression of SAPK activation also underlies the resistance of U266 cells to anti-Fas was investigated next. However, anti-Fas (100 ng/ml) which efficiently killed RPMI8226 but not U266 (3, 5, and Fig. 4C), activated SAPK in both RPMI8226 and U266 cells to an almost equal extent (Fig. 2C). This suggested that dysregulation of SAPK activation is not the mechanism whereby U266 cells resist Fas-mediated cell death.

p21 does not influence the SAPK activation and cell death mediated by Fas. The observations also suggested that the p21 hyperexpression in U266 cells does not influence Fas-mediated SAPK activation. Indeed, the ability of anti-Fas to activate SAPK in U266 cells was not significantly altered by the expression of the p21 antisense (Fig. 4B). Taking this and the above results together, the SAPK-suppressing effect of p21 appears to be stimulus-specific for a single cell type. This conclusion is consistent with the view that p21 blocks upstream events involved in activating SAPK (22).

The expression of the p21 antisense also failed to render U266 cells susceptible to anti-Fas (100 ng/ml) (Fig. 4C). Therefore, p21 does not appear to be involved in the resistance of U266 cells to Fas-mediated cell death. It has been consistently reported that the induction of p21 by γ-irradiation does not significantly alter the susceptibility of human fibroblasts to anti-Fas (23).

Having determined that the p21 hyperexpression in U266 cells is protective against H_2O_2 but not against anti-Fas, U266 cells may have an additional mechanism that enables them to survive anti-Fas. Given that anti-Fas efficiently activated SAPK in the U266 cells, an alternative mechanism is thought to inhibit a downstream lethal event that is triggered by Fas-activated SAPK. This mechanism does not seem to operate against H_2O_2-activated SAPK, because the restoration of H_2O_2-induced SAPK activation in U266 cells using p21 antisense was sufficient for an increased cellular susceptibility to H_2O_2. This view implicates that the H_2O_2- and Fas-activated SAPK kill myeloma cells by different mechanisms. Indeed, the reports from many laboratories have suggested that the lethal action of SAPK can be mediated by a variety of cellular components, such as c-Jun (24), p53 and Bax (25), mitochondria (26), and FADD (27), depending on the experimental conditions.

In summary, we have verified that the up-regulation of p21 can give myeloma cells a survival advantage by blocking SAPK activation. Therefore, p21 seems to be a key factor that is required for the expansion of slowly growing myeloma cells. It should also be stressed that p21 may not be solely responsible for the survival/outgrowth of the myeloma cells, because we have observed that the cytoprotective function of p21 is stimulus-specific.

ACKNOWLEDGMENT

This work was supported by a Nuclear R&D Program (2001) from the Ministry of Science and Technology in Korea.

REFERENCES

1. Korsmeyer, S. J. (1992) Bcl-2: An antidote to programmed cell death. *Cancer Surv.* **15,** 105–118.
2. Westendorf, J. J., Lammert, J. M., and Jelinek, D. F. (1995) Expression and function of Fas (APO-1/CD95) in patient myeloma cells and myeloma cell lines. *Blood* **85,** 3566–3576.
3. Shima, Y., Nishimoto, N., Ogata, A., Fujii, Y., Yoshizaki, K., and Kishimoto, T. (1995) Myeloma cells express Fas antigen/APO-1 (CD95) but only some are sensitive to anti-Fas antibody resulting in apoptosis. *Blood* **85,** 757–764.
4. Shima, Y., Nishimoto, N., Yoshizaki, K., and Kishimoto, T. (1996) Fas antigen/APO-1 (CD95) expression on myeloma cells. *Leuk. Lymphoma* **23,** 521–531.
5. Kim, D. K., Cho, E. S., Yoon, J.-H., and Um, H.-D. (2000) FLIP is constitutively hyperexpressed in Fas-resistant U266 myeloma cells, but is not induced by IL-6 in Fas-sensitive RPMI8226 cells. *Mol. Cells* **10,** 552–556.
6. El-Diery, W. S., Harper, J. W., O'Connor, P. M., Velculescu, V. E., Canman, C. E., Jackman, J., Pietenpol, J. A., Burrell, M., Hill, D. E., Wang, Y., Wilman, K. G., Mercer, W. E., Kastan, M. B., Hohn, K. W., Elledge, S. J., Kinzler, K. W., and Volgelstein, B. (1994) WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis. *Cancer Res.* **54,** 1169–1174.
7. Ruan, S., Okcu, M. F., Ren, J. P., Chiao, P., Andreef, M., Levin, V., and Zhang, W. (1998) Overexpressed WAS1/CIP1 renders glioblastoma cells resistant to chemotherapy against 1,3-bis(2-chloroethyl)-1-nitrosourea and cisplatin. *Cancer Res.* **58,** 1538–1543.
8. Marches, R., Hsueh, R., and Uhr, J. W. (1999) Cancer dormancy and cell signaling: Induction of $p21^{waf1}$ initiated by membrane IgM engagement increases survival of B lymphoma cells. *Proc. Natl. Acad. Sci. USA* **96,** 8711–8715.
9. Wang, Z., Van Tuyle, G., Conrad, D., Fisher, P. B., Dent, P., and Grant, S. (1999). Dysregulation of the cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1/MDA6}$ increases the susceptibility of human leukemia cells (U937) to 1-β-D-arabinofuranosylcysteine-mediated mitochondrial dysfunction and apoptosis. *Cancer Res.* **59,** 1259–1267.
10. Kang, K. H., Kim, W. H., and Choi, K. H. (1999) p21 promotes ceramide-induced apoptosis and antagonizes the antideath effect of Bcl-2 in human hepatocarcinoma cells. *Exp. Cell Res.* **253,** 403–412.
11. Urashima, M., Teoh, G., Chauhan, D., Hoshi, Y., Ogata, A., Treon, S. P., Schlossman, R. L., and Anderson, K. C. (1997) Interleukin-6 overcomes p21WAF1 upregulation and G1 growth arrest induced by dexamethasone and interferon-γ in multiple myeloma cells. *Blood* **90,** 279–289.
12. Kim, D. K., Cho, E. S., Seong, J. K., and Um, H.-D. (2001) Adaptive concentrations of hydrogen peroxide suppress cell death by blocking the activation of SAPK/JNK pathway. *J. Cell Sci.*, in press.
13. Kim, J.-Y, Choi, J.-A, Kim, T.-H, Yoo, Y.-D, Kim, J.-I, Lee, Y. J., Yoo, S.-Y, Cho, C.-K, Lee, Y.-S, and Lee, S-J. (2001) Involvement of p38 mitogen-activated protein kinase in the cell growth inhibition by sodium arsenite. *J. Cell. Physiol.*, in press.
14. Mangan, D., Welch, G. R., and Wahl, S. M. (1991) Lipopolysaccharide, tumor necrosis factorα, and IL-1β prevent programmed cell death (apoptosis) in human peripheral blood monocytes. *J. Immunol.* **146,** 1541–1546.
15. Lee, B. R., and Um, H.-D. (1999) Hydrogen peroxide suppresses U937 cell death by two different mechanisms depending on its concentration. *Exp. Cell Res.* **248,** 430–438.
16. Verheij, M., Bose, R., Lin, X. H., Tao, B., Jarvis, W. D., Grant, S., Birrer, M. J., Azabo, E., Zon, L. I., Kyriakis, J. M., Haimovitz-Friedman, A., Fuks, Z., and Kolesnick, R. N. (1996) Requirement for ceramide-initiated SAPK/JNK signaling in stress-induced apoptosis. *Nature* **380,** 75–79.
17. Yang, X., Khosravi-Far, R., Chang, H. Y., and Baltimore, D. (1997) Daxx, a novel Fas-binding protein that activates JNK and apoptosis. *Cell* **89,** 1067–1076.
18. Cross, T. G., Scheel-Toellner, D., Henriquez, N. V., Deacon, E., Salmon, M., and Lord, J. M. (2000) Serine/threonine protein kinases and apoptosis. *Exp. Cell Res.* **256,** 34–41.
19. Chauhan, D., Kharbanda, S., Ogata, A., Urashima, M., Teoh, G., Robertson, M., Kufe, D. W., and Anderson, K. V. (1997) Interleukin-6 inhibits Fas-induced apoptosis and stress-activated protein kinase in multiple myeloma cells. *Blood* **89,** 227–234.
20. Sanchez, I., Hughes, R. T., Mayer, B. J., Yee, K., Woodgett, J. R., Avruch, J., Kyriakis, J. M., and Zon, L. I. (1994) Role of SAPK/ERK kinase-1 in the stress-activated pathway regulating transcription factor c-Jun. *Nature* **372,** 794–798.
21. Shim, J., Lee, H., Park, J., Kim, H., and Choi, E. (1996) A non-enzymatic p21 protein inhibitor of stress-activated protein kinases. *Nature* **381,** 804–807.
22. Asada, M., Yamada, T., Ichijo, H., Delia, D., Miyazono, K., Fukumuro, K., and Mizutani, S. (1999) Apoptosis inhibitory activity of cytoplasmic $p21^{Cip1/WAF1}$ in monocytic differentiation. *EMBO J.* **18,** 1223–1234.
23. Tepper, C. G., Seldin, M. F., and Mudryj, M. (2000) Fas-mediated apoptosis of proliferating, transiently growth-arrested, and senescent normal human fibroblasts. *Exp. Cell Res.* **260,** 9–19.
24. Leppa, S., and Bohmann, D. (1999) Diverse functions of JNK signaling and c-Jun in stress response and apoptosis. *Oncogene* **18,** 6158–6162.
25. Aloyz, R. S., Bamji, S. X., Pozniak, C. D. Toma, J. G., Atwal, J. Kaplan, D. R., and Miller, F. D. (1998) P53 is essential for developmental neuron death as regulated by the TrkA and p75 neurotrophin receptors. *J. Cell Biol.* **143,** 1691–1703.
26. Tournier, C., Hess, P., Yang, D. D., Xu, J., Turner, T. K., Nimnual, A., Bar-Sagi, D., Jones, S. N., Flavell, R. A., and Davis, R. J. (2000) Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. *Science* **288,** 870–874.
27. Chen, Y., and Lai, M.-Z. (2001) c-Jun NH_2-terminal kinase activation leads to a FADD-dependent but Fas ligand-independent cell death in Jurkat T cells. *J. Biol. Chem.* **276,** 8350–8367.