Activin Receptor-like Kinase-7 Induces Apoptosis through Activation of MAPKs in a Smad3-dependent Mechanism in Hepatoma Cells*

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Activin receptor-like kinase (ALK7) is a type I serine/threonine kinase receptor of the transforming growth factor (TGF)-β family of proteins that has similar properties to other type I receptors when activated. To see whether ALK7 can induce apoptosis as can some of the other ALK proteins, we infected the FaO rat hepatoma cell line with adenovirus expressing a constitutively active form of the ALK7. Cells infected with active ALK7 adenovirus showed an apoptotic-positive phenotype, as opposed to those that were infected with a control protein. DNA fragmentation assays and fluorescence-activated cell sorter analysis also indicated that ALK7 infection induced apoptosis in FaO cells. We also confirmed this finding in Hep3B human hepatoma cells by transiently transfecting the constitutively active form of ALK7, ALK7(T194D). Investigation into the downstream targets and mechanisms involved in ALK7-induced apoptosis revealed that the TGF-β signaling intermediates, Smad2 and -3, were activated, as well as the MAPKs JNK and p38. In addition, caspase-3 and -9 were also activated, and cytotoxic release from the mitochondria was observed. Short interfering RNA-mediated inhibition of Smad3 markedly suppressed ALK7-induced caspase-3 activation. Treatment with protein synthesis inhibitors or the expression of the dominant-negative form of the stress-activated protein/extracellular signal-regulated kinase 1 abolished not only JNK activation but apoptosis as well. Taken together, these results suggest that ALK7 induces apoptosis through activation of the traditional TGF-β pathway components, thus resulting in new gene transcription and JNK and p38 activation that initiates cross-talk with the cellular stress death pathway and ultimately leads to apoptosis.

The transforming growth factor (TGF)-β superfamily of cytokines is responsible for regulating a wide range of cellular responses, including differentiation, cell growth, and apoptosis, in many different cell types (1–5). TGF-βs signal through a Ser/Thr kinase pathway that begins upon ligand binding to a set of two transmembrane receptors (termed type I or type II) located on the surface of the cell plasma membrane (1). The type II receptor is responsible for initial ligand binding, which then acts to recruit and activate, via phosphorylation, the type I receptor. After activation, the type I receptor phosphorylates a set of proteins (named Smads) that are specific for each kind of type I receptor. After the Smads are activated, they interact with another protein, Smad4; together, they translocate to the nucleus to modify the cellular response through transcription of other gene products. To date, the exact genes targeted by the Smad pathway have not been fully elucidated, with still less information on the mechanisms by which these genes carry out their function.

Activin receptor-like kinase (ALK)/7 is a serine/threonine kinase consistent with the characteristics of a type-I receptor. Originally identified and cloned from rat brain (6), ALK7 mRNA is present throughout the digestive and central nervous system of rats. The transmembrane receptor has a similar intracellular domain to TGF-β type I receptor and type I activin receptor (ActRIB), but a different extracellular domain. The only reported interacting ligand for ALK7 is mouse Nodal and xenopus-related Nodal (XnR1) (7). The function of ALK7 as a type I receptor was confirmed with a constitutively active mutant form that activated a TGF-β/activin response reporter (6). ALK7 has also been found to activate some components of the Smad pathway, such as Smad2 and Smad3, in fetal and adult rat pancreas (8). In the rat pheochromocytoma PC12 cell line, ALK7 not only activated both Smad2, Smad3, and the mitogen-activated protein kinases (MAPK) of extracellular signal-regulated kinase and c-Jun NH2-terminal kinase (JNK), but it inhibited cell proliferation as well (9). The human gene for ALK7 has been mapped to the genetic location of 2q24.1-q3, with most of the mRNA located in the brain, pancreas, and colon (10). Recently the human form of ALK7 has been identified along with three splice variants that are expressed in the placenta throughout various stages of pregnancy (11).

We studied the effects of infecting the FaO rat hepatoma cell line and Hep3B human hepatoma cell line with a genetically modified adenovirus expressing hemagglutinin (HA)-tagged ALK7 to determine whether or not ALK7 could induce apoptosis in these cells. The FaO cell line has proven to be a useful model system to study apoptosis, especially for TGF-β, which induces cell death in liver cells both in vitro and in vivo. Apoptosis normally occurs in cells by one of two pathways: cellular stress or death ligand (12). In both pathways, the final step involves activation of the effector caspase proteins from their inactive forms by means of large multi-protein complexes.
Once activated, the caspases act as proteases, cleaving various substrates that lead to the death of the cell. In FaO cells, the inhibition of caspases has been shown to prevent TGF-β1-induced apoptosis (13–15). The release of cytochrome c from the mitochondria and in TGF-β1-sensitive cells, where inhibition of cytochrome c release can completely abolish TGF-β1-induced apoptosis, is an important step in the cellular stress apoptotic pathway (16). Use of protein synthesis inhibitors suggests that new protein synthesis is required for TGF-β1-mediated apoptosis. Recently, microarray data of TGF-β1-treated FaO cells indicated a number of antioxidative genes that are down-regulated, as well as many reactive oxygen species that are up-regulated (17). These genes may be the intended targets of the TGF-β pathway and could have a direct impact on the two major apoptotic pathways.

Infection with ALK7 did, in fact, cause apoptosis in FaO cells. More specifically, ALK7 infection activated a number of the same proteins and mechanisms necessary for TGF-β1-induced apoptosis, including both Smad and caspase proteins; it also triggered cytochrome c release from the mitochondria. In addition, ALK7 was found to activate JNK and p38, MAPKs known to be involved in cell-cycle regulation. Coupled to the fact that the dominant-negative form of the upstream stress-activated protein kinase/extracellular signal-regulated kinase kinase (SEK1) blocked ALK7-induced apoptosis, it is likely that JNK, p38, and the caspase proteins are downstream targets of the Smad pathway transcription products. In support of the idea of cross-talk between the Smad and apoptotic pathways, protein synthesis inhibitors blocked ALK7-induced apoptosis. Thus, ALK7-mediated apoptosis seems to employ some of the same proteins used in cross-talk in TGF-β1-induced apoptosis.

MATERIALS AND METHODS

Cell Culture, Transfection, and Treatment—FaO rat hepatoma cells and Hep3B human hepatoma cells were maintained at 37 °C in Dulbecco’s modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum. The 293-derived PHOENIX E (kind gift of Lisa Choy, University of California) or GP-293 packaging cells were maintained in Dulbecco’s modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum. FaO stable cells were transfected in six-well plates using Lipofectin (Invitrogen) according to the manufacturer’s instructions. For TGF-β1 treatment, cells were incubated with 5 ng/ml TGF-β1 for 24 h in media. For treatment with protein synthesis inhibitors, cells were incubated with puromycin and cycloheximide for 24 h in media.

Plasmids and Adenoviral Infections—Recombinant adenoviruses expressing LacZ, HA-tagged active ALK5, and HA-tagged active ALK7 was used at a multiplicity of infection (m.o.i.) ranging from 0 to 250 with single viruses (as described by Fujii et al., Ref. 18). High-titered stocks of recombinant adenoviruses were grown in 293 cells and purified. Infection of recombinant adenoviruses was performed at a multiplicity of infection (m.o.i.) of less than 8 × 103 (plaque-forming units/cell). FaO cells were seeded at 0.3 × 105 cells per well in six-well plates and cultured in 2 ml of Dulbecco’s modified Eagle medium supplemented with 10% fetal bovine serum. Twenty-four hours later, the medium was replaced with fresh medium and adenovirus vectors (m.o.i. = 100–250) were added. The cells were incubated for 8 h for infection; 24 h after infection, the cells were harvested for gene expression.

DNA Fragmentation Assay—FaO cells were treated with lysis buffer (10 mM Tris-Cl, pH 7.4, 10 mM NaCl, 10 mM EDTA, 0.5% SDS, and 0.1 mg/ml proteinase K) and were incubated at 50 °C for 2 h. The lysate was extracted with phenol, phenol/chloroform (1:1), and chloroform and precipitated with 2.5 volumes of ice-cold ethanol. The DNA was resuspended in Tris-EDTA buffer supplemented with 100 μg/ml RNase A. DNA samples were electrophoretically separated on 2% agarose gel for 1 h at 120 V.

Flow Cytometry Analysis—For flow cytometry assay, FaO cells were grown in six-well plates and incubated for 24 h at 37 °C and then infected with adenoviruses carrying ALK7. After 36 h, the cells were harvested and washed twice with PBS buffer (pH 7.4). After fixing in 80% ethanol for 30 min, cells were washed twice and resuspended in phosphate-buffered saline (pH 7.4) containing 0.1% Triton X-100, 5 μg/ml propidium iodide (PI), and 50 μg/ml ribonuclease A for DNA staining. Cells were then analyzed by a FACScan cytometer (program CellQuest, BD Biosciences). Red fluorescence due to PI staining of DNA was expressed on a logarithmic scale simultaneously to the forward scatter of the particles, and 4000 events were counted on the scatter gate. The number of apoptotic nuclei was expressed as a percentage of the total number of events.

RESULTS

Constitutive Active ALK7 in FaO Cells Results in Apoptosis—To investigate the possible role ALK7 plays in apoptosis, a constitutive active mutant form bearing an HA-tag was inserted into an adenovirus expression system and used for infection into cultures of FaO cells. Two other adenoviruses constructed expressing either HA-tagged ALK5 or LacZ were infected into separate sets of FaO cells. To confirm the expression of the virally infected proteins, the cells were harvested for their protein and detected using an antibody against HA. Both
the HA-tagged proteins were successfully expressed (Fig. 1a), whereas the LacZ protein could not be detected. After sufficient time had elapsed, the phenotype of the cells was observed and compared with the known apoptotic-positive control phenotype exhibited by cells treated with TGF-β/H92521. Both the active ALK5- and ALK7-infected cells showed positive apoptotic phenotypes, whereas the cells infected with LacZ did not (Fig. 1, b–d). The ALK7 also induced apoptosis in the untransformed hepatocyte cell line AML12 (data not shown).

To further analyze whether ALK7 activation could cause apoptosis, cells were infected with increasing amounts of the active mutant form along with cells that were treated with TGF-β1, serum-starved, unaltered, or infected with LacZ and run in a DNA-laddering assay. When cells undergo apoptosis, a series of small DNA fragments separated by a hundred or so base pairs are generated, creating a characteristic “ladder” appearance (19). DNA in cells treated with ALK7 resembled the DNA ladder seen in the TGF-β1 apoptotic-positive control, whereas the other DNA did not (Fig. 1c). As a final test to see whether ALK7 induces apoptosis in FaO cells, a fluorescence-activated cell sorter (FACS) analysis was conducted of cells that had been infected with LacZ or the active forms of either ALK5 or ALK7. The resulting percentage of cells that appeared in the population of cells in sub-G1 phase for ALK7-infected cells was similar to the apoptotic-positive control ALK5-infected cells and significantly higher than LacZ (Fig. 1d).

We next examined the effect of ALK7 on apoptosis in Hep3B human hepatoma cells. We transiently transfected the constitutively active form of ALK7 (ALK7-TD) and kinase-inactive form of ALK7 (ALK7-KR), which acts as a dominant-negative receptor. Comparable expression levels of the ALK7 mutant proteins were obtained when immunoblotting was performed using the anti-HA antibody (Fig. 2a). By 48 h after transfection, the phenotype of the cells was observed and compared with the known apoptotic-positive control phenotype exhibited by cells treated with TGF-β1. Hep3B cells transfected with ALK7-TD showed positive apoptotic phenotypes, whereas the cells transfected with ALK7-KR did not (Fig. 2, a–c). Taken together, these results indicate that activated ALK7 induces apoptosis in hepatoma cells.
ALK7-induced Apoptosis in FaO Cells Activates JNK and Requires New Protein Synthesis—To better understand the mechanisms and pathways involved in ALK7-induced apoptosis, we examined the possible activation of MAPKs known to be important in other cytokine-mediated cell death. The MAPKs play a crucial role in relaying extracellular signals regarding cell-cycle regulation and cell proliferation (20). To study the possible role of MAPK involvement in ALK7-induced apoptosis, increasing amounts of active ALK7 were introduced into FaO cells, and the activity of three MAPKs were measured. The amount of the active forms of these three MAPKs (JNK, p38, and extracellular signal-regulated kinase) was measured using antibodies specific to the phosphorylated forms of each kinase. Only active, phosphorylated JNK (p-JNK) showed increased levels of expression with increasing amounts of virus (Fig. 3).

Dominant-negative SEK1 Blocks ALK7-induced Apoptosis by Preventing Caspase Activation and Cytochrome c Release—The MAPK JNK can be activated by upstream kinases, including SEK1 (21). To determine whether ALK7-induced apoptosis activates JNK through SEK1, the dominant-negative form of SEK1 (dnSEK1) was created and introduced into FaO cells (22) that were infected with increasing amounts of ALK7. JNK activation is almost completely abolished in cells expressing dnSEK1 when compared with the cells with the control vector (Fig. 4a). To see whether the loss of activated JNK could halt apoptosis, LacZ-infected or ALK7-infected cells carrying the dnSEK1-expressing vector or the control vector were subjected to FACS analysis. As expected, the dnSEK1-containing cells had a similar number of cells in sub-G1 phase when compared with the LacZ-containing cells, even in the presence of ALK7 (Fig. 4b). This result confirms that, through the inhibition of JNK activation, dnSEK1 prevents apoptosis. It also suggests that SEK1 acts upstream of JNK in the ALK-7 apoptotic pathway.

Caspase proteins are known to be important in most apoptotic pathways. To see whether caspase activation plays a role in ALK7-induced apoptosis, increasing amounts of active ALK7 protein were infected in FaO cells and caspase activation was measured. Using antibodies that were specific to the active, inactive, or both forms of caspase-3, -7, -8, and -9, it was
discovered that only caspase-3 and -9 are activated by ALK-7. (Fig. 5a) To determine whether caspase-9 activation is upstream or downstream of SEK1, FaO cells expressing dnSEK1 or vector control were infected with either LacZ or ALK7 adenovirus. ALK7 activation of caspase-9 is almost completely abolished in cells that also express dnSEK1 (Fig. 5b), indicating that caspase-9 activation occurs downstream of SEK1. To confirm that caspase activation is necessary for apoptosis, the polycaspase inhibitor Z-VAD-fmk was added to FaO cells infected with ALK7 or LacZ and compared with cells treated with Me2SO in a FACS analysis. The number of cells in sub-G1 phase in the cells treated with inhibitor resembles the number seen in the control cells, which was significantly lower than the number seen in ALK7-infected cells without inhibitor (Fig. 5c).

ALK7 Activates Smad2 and Smad3 Which, in Turn, Can Activate JNK and p38 MAPKs—ALK7 has been shown to activate both the receptor Smad2 and Smad3 protein in a variety of cell types (8, 9), which is consistent with the function of ALK-7 as a type I receptor. To investigate whether ALK7 can activate Smads in both FaO and Hep3B cells, increasing amounts of active ALK7 were infected into cells and analyzed for Smad activation using antibodies specific to the phosphorylated forms of Smad2 or Smad3. Both Smad2 and Smad3 were activated in the presence of a significant expression of ALK7 (Fig. 7a) and Hep3B cells (Fig. 7c). To see whether over-expression of Smad proteins is sufficient to stimulate activation of JNK and p38 MAPKs in the presence of ALK7, FaO cells were infected with active ALK7 and transfected with FLAG-tagged Smad2, Smad3, Smad4, or a control vector and analyzed for JNK activation. Smad expression was confirmed with an antibody specific for Methods). Like ALK5 and TGF-β1, ALK7 also induced cytochrome c release when compared with the control (Fig. 6a). To see whether cytochrome c release occurs downstream of SEK1, FaO cells were infected with LacZ, ALK5, or ALK7, or treated with TGF-β1 and transfected with dnSEK1. Cytochrome c release was reduced in all instances when compared with cells not treated with dnSEK1. (Fig. 6b).

ALK7 Induces Apoptosis

Fig. 4. Dominant-negative SEK1 blocks ALK7-induced apoptosis and JNK activation. FaO cells expressing either dnSEK1 or the control vectors (pcDNA3) were described previously (22). These cells were infected with increasing amounts of adenovirus vectors carrying constitutively active ALK7. a, after sufficient time had passed for infection, the cells were harvested for protein, which was then subjected to Western blot analysis. Antibodies specific to p-JNK were used to measure JNK activation, and antibodies specific to β-actin were used to confirm sample normalization. b, FACS analysis showing the percentage of cells counted in sub-G1 phase when FaO cells were infected with LacZ or ALK7 (m.o.i. = 250) in cells expressing either dnSEK1 or pcDNA3 vectors.
the FLAG-tag. Though the three Smads tested all activated JNK to some degree, only the receptor-activated Smads, Smad2 and Smad3, showed significant activation in FaO cells (Fig. 7b). By contrast, overexpression of the co-Smad4 protein did not result in similar levels of activation, but was comparable with the control vectors in overall activation. We also examined activation of JNK and p38 MAPKs in the presence of ALK7 in Hep3B cells (Fig. 7d). ALK7 induced activation of JNK and p38, as well as caspase-3 activation.

**Down-regulation of Endogenous Smad3 Markedly Decreases ALK7-induced Caspase-3 Cleavage**—In the previous study (13), we demonstrated that overexpression of Smad4 did not enhance the level of apoptosis induced by TGF-β1, and that Smad2 overexpression slightly enhanced TGF-β1-induced apoptosis. However, Smad3 overexpression significantly enhanced apoptosis induced by TGF-β1, suggesting that Smad3 activation may mediate apoptosis in hepatoma cells. To confirm the critical role of Smad3 in the ALK7-induced caspase-3 cleavage, we performed loss of function studies using Smad3-specific siRNA to evaluate the specific role of Smad3 on ALK7-induced apoptosis. We tried to reduce endogenous Smad3 expression through RNA-mediated interference using Smad3-specific siRNA. Transfection of the Smad3 siRNA (100–200 nM) resulted in a >70–90% decrease in Smad3 protein levels (Fig. 8). When transfected with Smad3-specific siRNA, caspase-3 cleavage by ALK7 was markedly reduced (Fig. 8).

**DISCUSSION**

In this study, we have demonstrated the first reported data that ALK7 can induce apoptosis. By using a modified form of adenovirus expressing a constitutively active ALK7 protein, we have demonstrated that ALK7 creates the same apoptotic-positive phenotype that treatment with TGF-β causes in FaO cells.
cells. We have also shown that ALK7 infection generates the breakup of DNA into smaller segments or ladders, which is consistent with cells that are undergoing apoptosis and not another form of cell death.

Aside from confirming apoptosis in FaO cells, we have shown some of the possible pathway components and mechanisms that could be responsible for ALK7-induced apoptosis. These include the activation of the Smad, caspase, JNK, and p38 MAPKs, as well as the release of cytochrome c from the mitochondria. We have also shown that new protein synthesis is required for both JNK activation and cell-cycle arrest. Finally, we have demonstrated that dominant-negative SEK1 blocks ALK7-induced apoptosis, suggesting that SEK1 is a crucial intermediate protein.

The data are highly similar to data reported for TGF-β/H9252 signaling or active ALK5 pathways in general. This is not surprising, as ALK7 has a very similar intracellular domain to ALK5 and is known to activate some of the same receptor Smads and reporter constructs (9). This suggests that ALK7 may stimulate transcription of the same genes that are activated by Smads in the ALK5 or TGF-β signaling or apoptotic pathways.

Some similarities to TGF-β/H9252-induced apoptosis and ALK7-induced apoptosis in FaO cells are present in this study, including the release of cytochrome c and JNK activation. JNK activation, coincidentally, has been implicated as a possible downstream target of the Rho family of proteins, which have been shown to be involved in cross-talk during TGF-β signaling. This is in agreement with our results, which suggests that cross-talk between the Smad and other pathways, such as the cellular stress death pathways, must be occurring. Evidence of this cross-talk is found in our data with the activation of caspase-9, which is activated upon interaction with Apaf1, a component of the apoptosome. However, Apaf1 requires cytochrome c release to self-aggregate and interact with the inactive form of caspase-9 (12), thus strongly supporting the theory.
that ALK7-induced apoptosis occurs via cross-talk with the cellular stress pathway. Importantly, caspase-8 was not activated, suggesting that the death ligand response does not play a role in ALK7-induced apoptosis.

Experiments involving a tetracycline-inducible active form of ALK7 have been shown to create morphological changes and arrest proliferation in the rat pheochromocytoma PC12 cell line (9). In addition, ALK7 activated Smad2 and Smad3 as well as stimulated transcription from Smad-binding elements in these cells, including genes often activated by TGF-β stimulated transcription from Smad-binding elements in these cells, often activated by TGF-β. An analysis of the microarray data of gene transcription upon TGF-β treatment of FaO cells found up-regulation of many pro-apoptotic genes, such as CTGF, which promotes fibroblast proliferation, and down-regulation of many antioxidant genes, such as GLCLC, which helps synthesize glutathione (17). Based on the high similarity of the downstream targets studied so far, it’s likely that ALK7 activation stimulates these same kind of pro- and anti-apoptotic genes in FaO cells. Further work is also required to identify the ligand responsible for signaling apoptosis in these cells, though Nodal is a possible candidate (7).

Experiments that find ALK7 induction of apoptosis in human cells will also provide some valuable information, notably in brain development, where it is thought that high levels of ALK7 mRNA are located.

In summary, ALK7 is a type I serine/threonine kinase of the TGF-β signaling cytokines that causes apoptosis when transiently expressed in hepatoma cells. The pathway by which ALK7 carries out apoptosis is similar to other ALKs, in that it begins with Smad signaling, which results in the transcription of various gene products. These newly synthesized proteins are necessary to complete the final stages of apoptosis, which involve cross-talk with other cell pathways that eventually culminate in cytochrome c release and caspase-9 activation.

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REFERENCES

1. Massagué, J., and Wotton, D. (2000) EMBO J. 19, 1745–1754
2. Attisano, L., and Wrana, J. L. (2000) Curr. Opin. Cell Biol. 12, 235–243
3. Hsing, A. Y., Kodomatsu, K., Bonham, M. J., and Danielpour, D. (1996) Cancer Res. 56, 5146–5149
4. Choi, K. S., Lim, I. K., Brady, J. N., and Kim, S. J. (1998) Hepatolog y 27, 415–421
5. Choi, K. S., Eom, Y. W., Kang, Y., Ha, M. J., Rhee, H., Yoon, J. W., and Kim, S. J. (1999) J. Biol. Chem. 274, 31775–31783
6. Tsujihara, R., Sawchenko, P. E., Nishikawa, S., and Vale, W. W. (1996) Mol. Cell Neurosci. 7, 467–478
7. Reissmann, E., Jornvall, H., Blokzijl, A., Andersson, O., Chang, C., Minchioti, G., Persico, M. G., Ibáñez, C. F., and Brivanlou, A. H. (2001) Genes Dev. 15, 2010–2022
8. Watanabe, R., Yamada, Y., Ibara, Y., Someya, Y., Kubota, A., Kagimoto, S., Kurone, A., Iwakura, T., Shen, Z. P., Inada, A., Adachi, T., Ban, N., Miyawaki, K., Sunaga, Y., Teuda, K., and Seino, Y. (1999) Biochem. Biophys. Res. Commun. 254, 707–712
9. Jornvall, H., Blokzijl, A., ten Dijke, P., and Banez, C. F. (2001) J. Biol. Chem. 276, 5140–5146
10. Bondestam, J., Hustrulid, M. A., Moren, A., Ustinov, J., Kaivo-Oja, N., Kallio, J., Horelli-Kuutinen, N., Aaltonen, J., Fujii, M., Moustakas, A., ten Dijke, P., Otonkoski, T., and Ritvos, O. (2001) Cytogenet. Cell Genet. 95, 157–162
11. Roberts, H. J., Hu, S., Quo, Q., Leung, P. C., Cannigia, I., Gruslin, A., Tsang, B., and Peng, C. (2002) Biol. Reprod. 68, 1719–1726
12. Green, D. R. (1998) Cell 18, 685–698
13. Kim, B. C., Mamura, M., Choi, K. S., Calabretta, B., and Kim, S. J. (2002) Mol. Cell. Biol. 22, 1569–1578
14. Cain, K., Inayat-Hussain, S. H., Coutet, C., and Cohen, G. M. (1996) Biochem. J. 314, 27–32
15. Inayat-Hussain, S. H., Coutet, C., Cohen, G. M., and Cain, K. (1997) Hepatology 25, 1516–1526
16. Herrera, B., Alvarez, A. M., Sanchez, A., Fernandez, M., Roncero, C., Benito, M., and Fahregat, I. (2001) FASEB J. 15, 741–751
17. Coyle, B., Freetby, C., Gant, T. W., Roberts, R. A., and Cain, K. (2003) J. Biol. Chem. 278, 5920–5928
18. Fuji, M., Takeda, K., Imamura, T., Aoki, H., Sampath, T. K., Enomoto, S., Miyawaki, K., Sunaga, Y., Teuda, K., and Seino, Y. (1999) Biochem. J. 341, 3981–3983
19. Duvall, E., and Wyllie, A. H. (1986) Immunol. Today 7, 115
20. Gallo, K. A., and Johnson, G. L. (2002) Nat. Rev. Mol. Cell. Biol. 9, 663–672
21. Kishimoto, H., Nakagawa, K., Watanabe, T., Kitagawa, D., Nomura, H., Sato, J., Ichijo, H., and Miyazono, K. (1999) Mol. Biol. Cell 10, 3801–3813
22. Park, H. J., Kim, B. C., Kim, S. J., and Choi, K. S. (2003) Hepatology 35, 1360–1371