Rb Protein Down-regulates the Stress-activated Signals through Inhibiting c-Jun N-terminal Kinase/Stress-activated Protein Kinase*

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The Rb protein is the product of the retinoblastoma susceptibility gene and loss of Rb function is detected in many types of human cancers. Rb plays important roles in the regulation of cell proliferation, differentiation, senescence, and apoptotic cell death. Here we show that Rb can physically interact with c-Jun NH2-terminal kinase/stress-activated protein kinase (JNK/SAPK), thereby inhibiting intracellular signals mediated by JNK/SAPK. Both in vitro binding and in vitro kinase studies suggest that a carboxyl-terminal domain of Rb containing amino acids 768–928 might be crucial for inhibiting JNK/SAPK. In comparison, Rb did not affect enzymatic activity of either extracellular signal-regulated kinase 1 or p38. Ectopically expressed Rb also abrogated the apoptotic cell death induced by ultraviolet radiation or the activation of MEKK1, an upstream kinase that can stimulate the JNK/SAPK cascade. JNK/SAPK inhibition highlights a novel function of Rb, which may provide a new mechanism by which Rb regulates cell death. JNK/SAPK is a major protein kinase that can be stimulated in response to a variety of cellular stresses. Our results, therefore, suggest that Rb, by inhibiting JNK/SAPK, may act as a negative regulator in stress-activated intracellular signaling cascades.

c-Jun N-terminal kinase (JNK), also known as stress-activated protein kinase (SAPK), is a serine/threonine-specific protein kinase that can be stimulated in response of cells to various environmental stresses or proinflammatory cytokines (1–5). JNK/SAPK, a member of the mammalian MAP kinase family, can be stimulated through the upstream kinases, MAPKKs and MAPKKKs. The MAPKKs for JNK/SAPK include SEK1/JNK1/MKK4 and MKK7. The MAPKKks for JNK/SAPK include MEKK1, MEKK2, MEKK3, MEK4, MLK3, ASK1, and TAK1 (4, 5). The JNK/SAPK signaling pathway is involved in the regulation of cell death and cell survival under certain conditions (4, 5). It has been reported, in particular, that JNK/SAPK mediates intracellular signals for cell death initiated by apoptotic stresses such as UV radiation (6–10).

The retinoblastoma susceptibility gene product, the retinoblastoma protein (Rb), is a negative regulator of cell growth by suppressing transition from G1 to S phase of the cell cycle (11). Rb has been shown to operate as a cell growth inhibitor and a tumor suppressor through interacting with the transcription factor E2F and other cellular proteins (11–13). For instance, the unphosphorylated or hypophosphorylated form of Rb, by binding to E2F, suppresses the expression of E2F-regulated genes that are required for DNA synthesis, leading to a cell cycle arrest (11, 12, 14, 15). Rb can also bind to other transcription factors including Elf-1, MyoD, PU.1, UBF, c-Myc, and ATF-2 as well as other regulatory proteins such as histone deacetylase, MDM2, and c-Ab1 (16–20). That Rb can interact with a variety of proteins implicates that Rb may be a multifunctional protein that can regulate not only the cell cycle but also other cellular activities. Indeed, recent studies have demonstrated that Rb can regulate apoptotic cell death (13, 21–26). Furthermore, Rb can be cleaved at the COOH terminus during apoptotic cell death by caspases, and the cleavage of Rb attenuates the anti-apoptotic function of Rb (26–28). A molecular mechanism for the anti-death effect of Rb, however, remains unclear. In the present study, we demonstrate a novel function of Rb that suppresses the JNK/SAPK signaling pathway through inhibiting JNK/SAPK activity. The inhibition of JNK/SAPK activity may be an important mechanism by which Rb negatively regulates the stress-activated cellular events including cell death.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—HEK293 cells and Saos-2 cells were routinely maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Cells were transfected with appropriate expression vectors by using LipofectAMINE (Life Technologies, Inc.). When indicated, cells were exposed to ultraviolet light (60 J/m2) after 40–48 h of transfection.

Co-immunoprecipitation—Nuclear extracts were obtained from cultured HEK293 cells as described previously (29) and immunoprecipitated with either rabbit anti-Rb polyclonal antibody (Santa Cruz) or pre-immune rabbit IgG for a negative control. Immunoprecipitates were subjected to electrophoresis on 12% SDS-polyacrylamide gel, immobilized with mouse anti-JNK1/SAPK monoclonal antibody (Pharmingen), and visualized using an enhanced chemiluminescence system (Amersham Pharmacia Biotech).

Immunocomplex Kinase Assays—Cultured cells were harvested, and lysed with a lysis buffer (10). Solubilized fractions were immunoprecipitated with appropriate antibodies, and the resultant immunopellets were assayed for indicated protein kinases, as described previously (10, 30). The phosphorylated proteins were separated by SDS-polyacrylamide gel electrophoresis on a 10% polyacrylamide gel, and quantified
by autoradiography and densitometry. Either GST-c-Jun(1–135) or GST-ATF2(1–109) was used as a substrate for JNK, GST-ATF2(1–109) for p38, and myosin basic protein for ERK. GST-SEK1(K129R) was used as a substrate for MEKK1 assay. Mouse monoclonal anti-Flag M2 (Kodak), anti-HA (Roche Molecular Biochemicals), or anti-JNK1 antibody was used as indicated.

Protein Kinase Assay for GST-SEK1—HEK293 cells were transfected with pEBG vector expressing GST-SEK1 (3). Transfected cells were lysed, and solubilized with 1% Triton X-100. Solubilized fraction was applied to glutathione-agarose, and GST-SEK1 was eluted from the resin and assayed for SEK1 activity using hexahistidine-tagged p38 as a substrate protein, as described previously (31).

In Vitro Binding Assay—SAPK\(_b\) and ERK1 were in vitro translated with a rabbit reticulocyte lysate system (Promega) using \[^{35}S\]methionine. In vitro translation product was incubated for 2 h at 4 °C with the indicated GST fusion proteins immobilized on glutathione-Sepharose beads in the binding buffer, and the beads were rinsed extensively with the washing buffer, as described (30). The \[^{35}S\]labeled proteins were eluted from the beads, separated by electrophoresis on 10% SDS-polyacrylamide gel, and visualized by autoradiography.

Apoptotic Cell Death—Saos-2 cells were transfected with pEGFP (CLONTECH) and various cDNA constructs by LipofectAMINE (Life Technologies, Inc.). When indicated, transfected cells were exposed to UV (60 J/m\(^2\)) after a 40-h incubation. Then, cells were fixed with 3% paraformaldehyde, permeabilized with 0.2% Triton X-100, and stained with 4,6-diamidino-2-phenylindole. 4,6-Diamidino-2-phenylindole-stained nuclei were observed with a Zeiss Axiovert microscope. GFP-expressing cells were scored for apoptotic nuclei. The percentage of GFP-producing cells that were apoptotic was determined from four independent dishes.

RESULTS AND DISCUSSION

Rb Protein Suppresses the JNK/SAPK Signaling Pathway—To investigate whether Rb protein might modulate a function of the JNK/SAPK pathway, we examined the action of Rb on JNK activity in cultured cells. HEK293 cells were transiently transfected with plasmids expressing GST-SEK1 and Rb, as indicated. After a 48-h incubation, transfected cells were exposed to UV light (60 J/m\(^2\)), incubated further for 1 h, and lysed. GST-SEK1 was purified from cell lysates by using glutathione-agarose resin, and assayed for phosphorylation of hexahistidine-tagged p38 (His-p38). Rb does not affect MEKK1 activity. HEK293 cells were transfected with plasmids expressing MEKK1-flag and Rb, as indicated. After transfected cells were exposed to UV light (60 J/m\(^2\)), enzymatic activity of MEKK1-flag expressed in transfected cells was determined by immunocomplex kinase assay. In panels A-B, fold increase in phosphorylation of each substrate protein is indicated. IB, immunoblotting of transfected cells with anti-Flag or anti-GST antibody, as indicated.

FIG. 1. Rb inhibits JNK/SAPK in intact cells. A, Rb inhibits the UV-induced stimulation of JNK/SAPK in HEK293 cells. HEK293 cells were untransfected or transfected with cDNA constructs for SAPK\(_b\)-flag or JNK1-HA, and Rb, as indicated. After 48 h of transfection, cells were exposed to UV light (60 J/m\(^2\)), and incubated further for 1 h. Enzymatic activities of ectopically expressed JNK in transfected cells were measured by immunocomplex kinase assay. B, Rb does not affect either ERK or p38 MAP kinase activity in HEK293 cells. HEK293 cells were transiently transfected with cDNA constructs for ERK1-HA or p38-flag, and Rb, as indicated. After a 48-h incubation, transfecants were exposed to either UV light (60 J/m\(^2\)) or p38 stimulation or 12-O-tetradecanoylphorbol-13-acetate (100 nM, 15 min) for ERK1 stimulation. Protein kinase activities in transfected cells were determined by immunocomplex kinase assay. C, ectopically expressed Rb inhibits the JNK/SAPK stimulation in Saos-2 cells. Saos-2 cells were transiently transfected with SAPK\(_b\)-flag alone or SAPK\(_b\)-flag plus Rb, as indicated. Transfected cells were exposed to UV irradiation, and assayed for SAPK\(_b\) activity, as in panel A. In panels A-C, fold increase in phosphorylation of each substrate protein is indicated. IB, immunoblotting of transfected cells with anti-Flag or anti-HA as indicated.

FIG. 2. Rb cannot inhibit JNK/SAPK upstream kinases. A, Rb does not affect SEK1 activity. HEK293 cells were transiently transfected with plasmids expressing GST-SEK1 and Rb, as indicated. After 48 h of transfection, transfected cells were exposed to UV light (60 J/m\(^2\)), incubated further for 1 h, and lysed. GST-SEK1 was purified from cell lysates by using glutathione-agarose resin, and assayed for phosphorylation of hexahistidine-tagged p38 (His-p38). Rb does not affect MEKK1 activity. HEK293 cells were transfected with plasmids expressing MEKK1-flag and Rb, as indicated. After transfected cells were exposed to UV light (60 J/m\(^2\)), enzymatic activity of MEKK1-flag expressed in transfected cells was determined by immunocomplex kinase assay. In panels A-B, fold increase in phosphorylation of each substrate protein is indicated. IB, immunoblotting of transfected cells with anti-Flag or anti-GST antibody, as indicated.
Immunopellets were subjected to electrophoresis on 12% SDS-polyclonal anti-Rb IgG, or mouse monoclonal anti-JNK1 IgG, respectively. Were immunoprecipitated by using pre-immune rabbit IgG, rabbit polyassociates with JNK in intact cells. Nuclear extracts from HEK293 cells, and performed immunoprecipitation; in vitro. In vitro GST-Rb fusion proteins were visualized by autoradiography. The input 35S-labeled proteins were immobilized onto glutathione-Sepharose beads. Bound proteins were eluted, and separated by SDS-polyacrylamide gel electrophoresis. 35S-Labeled SAPK activity in transfected Saos-2 cells. These findings indicate that a carboxyl-terminal domain of Rb containing amino acids 768–928 may be important for binding to JNK/SAPK. GST-Rb(379–928) did not interact with 35S-labeled SAPKβ. These data indicate that an inhibitory function of Rb was specific to the JNK/SAPK path- way among the mammalian MAP kinase cascades. The ability of Rb to repress JNK/SAPK activity was stimulated after UV irradiation (Fig. 2A). Ectopic expression of Rb did not affect the UV-induced SEK1 activation in transfected cells. Rb also had little effect on an enzymatic activity of MEKK1 for phosphorylating GST-SEK1 protein (Fig. 2B). These findings suggest that Rb may block the JNK/ SAPK signaling cascade by acting on JNK/SAPK.

**Rb Interacts with JNK/SAPK—** In order to test whether Rb directly interacts with JNK/SAPK, we carried out *in vivo* interaction analysis (Fig. 3A). We prepared nuclear extracts from HEK293 cells, and performed immunoprecipitation with anti-Rb antibody. Immunoblot analysis of protein complexes immunoprecipitated with anti-Rb antibody demonstrated that Rb was coimmunoprecipitated with JNK. We further tested if Rb directly binds to JNK/SAPK in *in vitro* binding assay using recombinant GST-Rb proteins that were immobilized onto glutathione-Sepharose beads (Fig. 3B). Either GST-Rb(379–928) or GST-Rb(768–928) physically associated with *in vitro* translated [35S]methionine-labeled SAPKβ, whereas GST-Rb(379–767), GST-Rb(776–830), or GST alone did not interact with [35S]-labeled SAPKβ. These data indicate that Rb can directly inhibit a catalytic activity of JNK/SAPK, and that the COOH-terminal domain of Rb is critical in the regulation of JNK by Rb.

**Inhibition of JNK by Rb**—There are at least two possible mechanisms by which Rb inhibits JNK/SAPK activity through interacting with the enzyme. One possible mechanism is that Rb may directly suppress a catalytic activity of JNK/ SAPK. Another possibility is that Rb may interfere to the JNK/SAPK phosphorylation reaction catalyzed by a JNK/ SAPK kinase such as SEK1. We, therefore, tested these possibilities in the following experiments.

In order to test a possibility that Rb may directly inhibit JNK/SAPK activity, we examined the *in vitro* effects of GST-Rb fusion proteins on JNK/SAPK activity (Fig. 4A). Enzymatic activity of JNK/SAPK was markedly inhibited *in vitro* by both Rb(379–928) and Rb(768–928), whereas the effect of either Rb(379–767) or Rb(776–830) on the enzymatic activity was minimal. Thus, these data were in excellent agreement with the *in vitro* binding data shown in Fig. 2. We also confirmed the inhibitory function of Rb(781–928) in transfected cells (Fig. 4B). Enzymatic activity of ectopically expressed SAPKβ-flag was stimulated after cells were exposed to UV irradiation, and SAPK stimulation was blocked by Rb(761–928). Taken together, our findings suggest that Rb can directly inhibit an enzymatic activity of JNK/SAPK, and that the COOH-terminal domain of Rb is critical in the regulation of JNK by Rb.

We then investigated another possibility that Rb may inhibit the JNK/SAPK phosphorylation reaction catalyzed by SEK1. In order to test this possibility, we examined the effect of GST-Rb(768–928) on the *in vitro* phosphorylation of SAPKβ by SEK1 (Fig. 5). We used SAPKβ(K55R), a kinase-inactive SAPKβ mutant that has no autophosphorylation activity, as a substrate for SEK1 in the *in vitro* kinase assay. We found that the SEK1 phosphorylation by SEK1 was inhibited by GST-Rb(768–928). Interestingly, GST-Rb(768–928) did not affect the phosphorylation of myosin basic protein by SEK1, suggesting that Rb(768–928) does not affect a catalytic activity of SEK1. On the basis of our data that Rb (768–928) blocks the SAPK phosphorylation *in vitro*, therefore, it may be proposed that Rb, through interacting with JNK/SAPK, may interfere to the phosphorylation of JNK/SAPK by SEK1.

**Rb Prevents the JNK-involved Cell Death**—There are increasing lines of evidence that JNK/SAPK can mediate intracellular signals for cell death initiated by cellular stresses such as UV radiation (6–10). We, therefore, tested whether ectopically expressed Rb protein could suppress the JNK-involved cell death induced by UV irradiation. Ectopic expression of Rb had little effect on UV-induced cell death (Fig. 6A). This finding is consistent with previous data that Rb can suppress the expression of pro-apoptotic genes, such as bax and caspase-3 (6). We, therefore, concluded that ectopic expression of Rb could suppress the JNK-involved cell death.

**Fig. 3. Rb directly interacts with JNK/SAPK.** A. Rb physically associates with JNK in intact cells. Nuclear extracts from HEK293 cells were immunoprecipitated by using pre-immune rabbit IgG, rabbit polyclonal anti-Rb IgG, or mouse monoclonal anti-JNK1 IgG, respectively. Immunoprecipitates were subjected to electrophoresis on 12% SDS-polyacrylamide gel, and immunoblotted with anti-JNK1 antibody. IP, immunoprecipitation; IB, immunoblotting. B, binding of JNK/SAPK to GST-Rb fusion proteins *in vitro*. *In vitro* translated [35S]-labeled SAPKβ or ERK1 was applied to the indicated GST-Rb fusion proteins immobilized onto glutathione-Sepharose beads. Bound proteins were eluted, and separated by SDS-polyacrylamide gel electrophoresis. [35S]-Labeled proteins were visualized by autoradiography. The input [35S]-labeled protein (33%) is shown. In the diagram of Rb fragments, A, B, and C represent the A-, B-, and C-pocket domains of Rb, respectively.
cell death in Saos-2 cells. UV light, a strong activator of the JNK pathway, caused apoptotic cell death in Saos-2 cells (Fig. 6A). UV-induced cell death was decreased in cells overexpressing a dominant-negative mutant of SEK1, SEK1(K129R), indicating the involvement of JNK pathway in UV-induced apoptosis. Ectopic expression of the full-length Rb or Rb(761–928) in Saos-2 cells converted transfected cells to a UV-resistant phenotype (Fig. 6A). In comparison, UV-induced apoptosis was not affected by Rb(1–886). Rb(1–886) contains the intact Rb A- and B-pocket domains, but has a disrupted C-pocket domain. We also examined an effect of Rb on DMEKK1-induced cell death. DMEKK1, a constitutively active form of MEKK1, has been shown to induce apoptosis (8), and JNK activity was markedly stimulated in DMEKK1-transfected Saos-2 cells (data not shown). Overexpression of DMEKK1 in Saos-2 cells induced cell death, and DMEKK1-induced cell death was abrogated by SEK1(K129R) (Fig. 6B). These data suggest that DMEKK1 induces cell death through the MEKK1-SEK1-JNK/SAPK signaling cascade. DMEKK1-induced cell death was prevented by the full-length Rb or Rb(761–928), but not by Rb(1–886) (Fig. 6B). Rb does not affect the catalytic activities of the JNK/SAPK upstream kinases, MEKK1 and SEK1 (Fig. 2). Taken together, these findings suggest that Rb repress JNK activation and this inhibitory function of Rb may be an important mechanism by which it prevents JNK-mediated cell death.

Our results in this study suggest a novel function of Rb: Rb silences the function of JNK/SAPK. In this way, Rb may repress the stress-activated signals that are mediated by the JNK pathway. Interestingly, Rb suppresses apoptosis by a mechanism that is poorly understood (21–25). Furthermore, Rb can be cleaved by a caspase during apoptosis, and the cleavage of Rb appears to lose its anti-cell death function (26–28). The cleavage occurs at the COOH-terminal region between Asp^{886} and Gly^{887}, generating a Rb protein that lacks 42 amino acids.

FIG. 4. Rb directly inhibits JNK/SAPK. A, JNK is inhibited in vitro by GST-Rb fusion proteins. HEK293 cells were exposed to UV irradiation, lysed, and immunoprecipitated by using anti-JNK1/SAPKγ antibody. Enzymatic activity of immunocomplex JNK1 was assayed in vitro in the presence of GST-Rb fusion proteins as indicated. B, the C-pocket domain of Rb blocks the UV-stimulated JNK/SAPK activity in HEK293 cells. Cultured cells were transfected with plasmid vectors expressing the indicated proteins, exposed to UV irradiation, lysed, and assayed for JNK/SAPK activity, as for Fig. 1. Cell lysates were also immunoblotted with anti-Flag antibody.

FIG. 5. The C-pocket domain of Rb inhibits the JNK/SAPK phosphorylation catalyzed by SEK1. HEK293 cells were transfected with pEG-SEK1 expressing GST-SEK1, and exposed to UV light after a 48-h transfection. Then, GST-SEK1 was prepared as soluble form from the transfected cells, as for Fig. 2. In vitro phosphorylation of either GST-SAPKβ(K55R) or myosin basic protein (MBP) by GST-SEK1 was carried out in the absence or presence of GST-Rb(768–928).

FIG. 6. Rb represses UV- or MEKK1-induced cell death in Saos-2 cells. A, Rb suppresses UV-induced apoptotic cell death. Saos-2 cells were transfected with plasmids expressing wild-type or mutants of Rb or SEK1(K129R) along with pEGFP. Forty hours after transfection, cells were exposed to UV light (60 J/m²) and incubated further for 12 h. Cells were fixed, permeabilized, and stained with 4,6-diamidino-2-phenylindole. GFP-positive cells were scored for apoptotic nuclei with a Zeiss Axiovert microscope. B, Rb suppresses DMEKK1-induced apoptotic cell death. Saos-2 cells were transfected with pEGFP and expression vectors producing indicated proteins. Apoptotic cell death in transfected cells was determined as in panel A, except that cells were fixed 40 h after transfection.
(26–28). The caspase-cleaved Rb can still interact with the Rb-binding proteins such as E2F or cyclin D as efficiently as a full-length Rb (13, 27, 28), whereas the cleaved form of Rb could not inhibit JNK (data not shown). This may imply that JNK might be a critical target of Rb in order for Rb to suppress stress-induced cell death. Thus, the JNK/SAPK inhibition may be a new function of Rb that negatively regulates stress-activated signaling cascades.

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REFERENCES

1. Derijard, B., Hibi, M., Wu, I. H., Barrett, T., Su, B., Deng, T., Karin, M., and Davis, R. J. (1994) Cell 76, 1025–1037
2. Kyriakis, J. M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E. A., Ahmad, M. F., Aurruch, J., and Woodgett, J. R. (1994) Nature 369, 156–160
3. Sanchez, I., Hughes, R. T., Mayer, B. J., Yee, K., Woodgett, J. R., Avruch, J., Kyriakis, J. M., and Zon, L. I. (1994) Nature 372, 794–798
4. Minden, A., and Karin, M. (1997) Biochem. Biophys. Acta 1333, F85–104
5. Ip, Y. T., and Davis, R. J. (1998) Curr. Opin. Cell Biol. 10, 205–219
6. Xia, Z., Dickens, M., Raingeaud, J., Davis, R. J., and Greenberg, M. E. (1995) Science 270, 1326–1331
7. Chen, Y. R., Wang, X., Templeton, D., Davis, R. J., and Tan, T.-H. (1996) J. Biol. Chem. 271, 31929–31936
8. Johnson, N. L., Gardner, A. M., Diener, K. M., Lange-Carter, C. A., Gleavy, J., Jarpe, M. B., Minden, A., Karin, M., Zon, L. I., and Johnson, G. L. (1996) J. Biol. Chem. 271, 3229–3237
9. Verheij, M., Bose, R., Lin, X. H., Yao, B., Jarvis, W. D., Grant, S., Birrer, M. J., Szabo, E., Zon, L. I., Kyriakis, J. M., Haimovitz-Friedman, A., Fuks, Z., and Kolesnick, R. N. (1996) Nature 380, 75–79
10. Park, J., Kim, I., Oh, Y. J., Lee, K., Han, P.-L., and Choi, E.-J. (1997) J. Biol. Chem. 272, 16725–16728
11. Weinberg, R. A. (1995) Cell 81, 323–330
12. Taya, Y. (1997) Trends Biochem. Sci. 22, 14–17
13. Tan, X., and Wang, J. Y. (1998) Trends Cell Biol. 8, 116–120
14. Kouzourides, T. (1995) Trends Cell Biol. 5, 443–450
15. Zarkowska, T., and Mittnacht, S. (1997) J. Biol. Chem. 272, 12738–12746
16. Goga, A., Liu, X., Hambuch, T. M., Senechal, K., Major, E., Berk, A. J., Witte, O. N., and Sawyers, C. L. (1995) Oncogene 11, 791–799
17. Xiao, Z. X., Chen, J., Levine, A. J., Modjtahedi, N., Xing, J., Sellers, W. R., and Livingston, D. M. (1995) Nature 375, 694–698
18. Brehm, A., Miska, E. A., McAnce, D. J., Reid, J. L., Bannister, A. J., and Kouzourides, T. (1998) Nature 391, 597–601
19. Magnaghi-Jaulin, L., Grousset, R., Nagulineva, I., Robin, P., Lorain, S., Le Villain, J. P., Trolan, F., Trouche, D., and Harel-Bellan, A. (1998) Nature 391, 601–605
20. Welch, P. J., and Wang, J. Y. (1993) Cell 75, 779–790
21. Clarke, A. R., Maundag, E. R., van Roon, M., van der Lught, N. M., van der Valk, M., Hooper, M. L., Berns, A., and te Riele, H. (1992) Nature 359, 328–330
22. Jacks, T., Fazeli, A., Schmitt, E. M., Bronson, R. T., Goodell, M. A., and Weinberg, R. A. (1992) Nature 359, 285–300
23. Lee, E. Y., Chang, C. Y., Hu, N., Wang, Y. C., Lai, C. C., Herrup, K., Lee, W. H., and Bradley, A. (1992) Nature 359, 288–294
24. Almasan, A., Yin, Y., Kelly, R. E., Lee, E. Y., Bradley, A., Li, W., Bertino, J. R., and Wahl, G. M. (1992) Proc. Natl. Acad. Sci. U. S. A. 92, 5436–5440
25. Haas-Kogan, D. A., Kogan, S. C., Levi, D., Dazin, P., T'Ang, A., Fung, Y. K., and Izrael, M. A. (1995) EMBO J. 14, 461–472
26. Tan, X., Martin, S. J., Green, D. R., and Wang, J. Y. J. (1997) J. Biol. Chem. 272, 9615–9616
27. Janicke, R. U., Walker, P. A., Lin, X. Y., and Porter, A. G. (1996) EMBO J. 15, 6969–6978
28. Chen, W. D., Otterson, G. A., Lipkowitz, S., Kluef, S. N., Cossen, A. B., and Kaye, F. J. (1997) Oncogene 14, 1243–1248
29. Schreiber, E., Matthias, P., Muller, M. M., and Schaffner, W. (1989) Nucleic Acids Res. 17, 6419
30. Shim, J., Lee, H., Park, J., Kim, H., and Choi, E. J. (1996) Nature 381, 804–806
31. Kim, H., Shim, J., Han, P. L., and Choi, E. J. (1997) Biochemistry 36, 13677–13681
32. Derijard, B., Raingeaud, J., Barrett, T., Wu, I. H., Han, J., Ulevitch, R. J., and Davis, R. J. (1995) Science 267, 682–685
33. Lin, A., Minden, A., Martinetto, H., Clare, P. X., Lange-Carter, C., Mercurio, F., Johnson, G. L., and Karin, M. (1995) Science 268, 286–290