p21-activated kinase 1 (Pak1) has been shown recently to induce hyperplasia in the mammary epithelium, a phenotype also manifested by overexpression of cyclin D1, a known indicator of the proliferative stage. Here we investigated the role of the Pak1 pathway in the expression of cyclin D1 using tissue culture models and transgenic mice expressing activated Pak1 in mammary glands. We found that hyperplastic mammary glands from catalytically active Pak1 transgenic mice exhibit a 5- to 7-fold increased expression of cyclin D1 as compared with stage-matched wild-type mice. In addition, Pak1 levels were elevated in human breast tumors and also correlated well with increased cyclin D1 expression. Increased expression of Pak1 in breast cancer cells stimulated cyclin D1 promoter activity, elevated levels of cyclin D1 mRNA, protein, and nuclear accumulation of cyclin D1. Conversely, Pak1 inhibition by an auto-inhibitory peptide (amino acids 83–149) or Pak1 knockdown by short interference RNA markedly reduced the expression of cyclin D1, suggesting a requirement of a functional Pak1 pathway for optimal expression of cyclin D1. Results from deletion and mutant analysis indicate that Pak1 regulates cyclin D1 transcription by means of an NF-kB-dependent pathway. Together, these findings suggest a model wherein Pak1 regulation of cyclin D1 expression might involve an NF-kB-dependent pathway and that hyperplasia in the mammary glands of Pak1-TG mice may be associated, at least in part, with the up-regulation of cyclin D1, and that Pak1 is up-regulated in human breast tumors.

The small GTPases, including Cdc42 and Rac1, have been implicated in the regulation of mammalian cell morphology and motility (1). More specifically, Rac1 induces cortical actin polymerization, which is seen as membrane ruffling and lamellipodia, and Cdc42 induces the formation of peripheral actin polymerization, which is seen as membrane ruffling and lamellipodia (2–4). The small GTPases regulate cytoskeletal structures by means of a family of microspikes and filopodia (2–4). The small GTPases, including Cdc42 and Rac1, have been shown to be up-regulated in ovarian tumors (16) and breast (17) cancers. Furthermore, Pak1 protein has been shown to be up-regulated in ovarian tumors (16) and breast cancer (15, 18, and this study).

More recently, Pak1 has been shown to directly phosphorylate estrogen receptor-α (ER) at Ser-305 and to promote its transactivation functions (19). Additionally, expression of kinase-active T423E Pak1 transgene in mammary glands induces hyperplasia in the mammary epithelium (19), a phenotype manifested by several other oncogenes including cyclin D1 (20).
of the cyclin D1 promoter (21). Also, NF-κB interaction with the NF-κB binding sites in the cyclin D1 promoter is required for cyclin D1 expression, leading to cell cycle progression. The small GTPase Rac1 signaling has been found to activate cyclin D1 transcription by means of an NF-κB-dependent pathway in murine NIH3T3 cells (22, 23). In addition, up-regulation of cyclin D1 by ERα signaling is accompanied by an increased proliferative response in breast cancer cells (24, 25), as ERα-stimulated proliferation could be effectively blocked by antisense cyclin D1 or by microinjection of anti-cyclin D1 antibodies (25, 26) and reversed by cyclin D1 overexpression (27). Up-regulation of cyclin D1 expression has also been found in hyperplastic mammary glands and proliferative human breast disease (28, 29). Together, these observations suggest that cyclin D1 may constitute an important downstream target of diverse upstream signals, with a role in mammary gland development and tumorigenesis. Despite the widespread role of cyclin D1 in the biology of breast cancer, its involvement in Pak1 signaling, a common point of convergence of growth factor signaling in breast cancer cells, remains unknown. Here we investigated the role of Pak1 signaling pathways in the expression of cyclin D1 using breast cancer cell culture models and transgenic mice expressing activated Pak1 in mammary glands.

MATERIALS AND METHODS

Cell Cultures and Reagents—MCF-7 cells, MDA-MB-231 human breast cancer cells, HeLa cells, and endometrial Ishikawa cells were maintained in Dulbecco’s modified Eagle’s medium F12 (1:1) supplemented with 10% fetal calf serum. HC11 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and epidermal growth factor (10 ng/ml). Antibodies were purchased from the following companies: anti-Pak1 from Cell Signaling, anti-cyclin D1 from Santa Cruz Biotechnology, anti-vinculin from Sigma, and anti-HA from Roche Applied Science.

Metabolic Labeling—MCF-7 cells constitutively expressing active Pak1 were grown to 50% confluency and were metabolically labeled with 20 μCi/ml of [35S]methionine for 24 h in a methionine-free medium.
containing 2% dialyzed fetal bovine serum in the absence or presence of doxycycline (Dox) (30). Conditioned media with equal trichloroacetic acid precipitable counts were immunoprecipitated with the desired or control antibody, resolved on PAGE gels, and analyzed using autoradiography.

Cell Extracts, Immunoprecipitation, Immunoblotting Assays—For preparation of cell extracts, cells were washed 3× with phosphate-buffered saline and lysed in buffer (50 mM Tris-HCl, pH 7.5, 120 mM NaCl, 1% Triton X-100, 100 mM NaF, 200 mM NaVO₅, 1 mM phenylmethanesulfonyl fluoride, and protease inhibitor mixture (GIBCO) for 15 min on ice. The lysates were centrifuged in an Eppendorf centrifuge at 4 °C for 15 min. Cell lysates containing equal amounts of protein were immunoprecipitated, resolved on a 10% SDS-PAGE, transferred to nitrocellulose, and probed with the appropriate antibodies using an ECL method.

Northern Blotting—Total cytoplasmic RNA (20 µg) was analyzed by Northern blot analysis. Northern blots were probed with 32P-labeled cyclin D1 and Pak1 cDNA probe. Actin mRNA was used to assess the integrity of the RNA and to control for the RNA loading.

Cyclin D1 Promoter-Reporter Assays—Subconfluent cells cultured in 6-well plates were transiently cotransfected with cyclin D1-luciferase reporter constructs and Pak1 cDNA probe. Actin mRNA was used to assess the integrity of the RNA and to control for the RNA loading.

RESULTS AND DISCUSSION

Pak1 Regulates Cyclin D1 Expression in a Transgenic Model—In an attempt to define the role of Pak1 in the mammary gland, we recently demonstrated that overexpression of a kinase-active T423E Pak1 transgene in the murine mammary gland leads to a widespread hyperplasia (19). Because cyclin D1 overexpression in the mammary tissues has been reported to cause hyperplasia (28, 29) and the finding that Rac, an upstream effector of Pak1, regulates cyclin D1 transcription activity (23), we hypothesized that Pak1 might also regulate...
cyclin D1 expression. Therefore, we explored the possibility of involvement of cyclin D1 in the action of Pak1 by examining the status of cyclin D1 expression in mammary glands from T423E transgenic and stage-matched wild-type mice. Immunohistochemical analysis revealed a background level of cyclin D1 staining in wild-type mammary glands. In contrast, mammary epithelium from T423E Pak1 transgenic mice exhibited an intense cyclin D1 nuclear staining (Fig. 1A). Quantitation of signals in three independent transgenic lines indicated an 5- to 7-fold-increased expression of cyclin D1 in T423E-Pak1 transgenic mammary glands (Fig. 1B). These results suggest that hyperplasia in mammary glands of Pak1-TG mice may be associated, at least in part, with the potential up-regulation of cyclin D1.

Overexpression of Pak1 in Breast Tumors—To assess the significance of Pak1 in breast cancer, we next determined the status of Pak1 expression in breast tumor specimens. We performed immunohistochemical staining of Pak1 and cyclin D1 in tumor arrays containing 60 breast cancer sections. Overexpression of Pak1 was observed in 34 tumors (55%) and cyclin D1 in 42 tumors (70%). Subcellular localization of Pak1 was variable. Of the 34 Pak1-positive tumors, an intense Pak1 cytoplasmic staining was observed in 19 tumors (56%) (Fig. 2A), nuclear staining in 5 tumors (15%) (Fig. 2B), and both nuclear and cytoplasmic Pak1 immunoreactivity in 10 breast tumor specimens (29%) (Fig. 2C). Pak1 expression also correlated well with the overall cyclin D1 expression in about 40% of tumors. Representative examples of co-expression of Pak1 and cyclin D1 in the same tumor specimen are shown in Fig. 2 (I–L).

Pak1 Stimulates Cyclin D1 Promoter Activity—To investigate the potential Pak1 regulation of cyclin D1 expression, we next examined the effects of wild-type (WT) Pak1 or catalytically active T423E Pak1 on the transcriptional regulation of cyclin D1 using the cyclin D1-promoter luciferase reporter system (−1745 cyclin D1 luciferase) (23). Cotransfection of WT-Pak1 or T423E Pak1 in murine normal mammary epithelial HC11 cells stimulated transcription from the cyclin D1 promoter as compared with the transcription driven by control vector (Fig. 3). The stimulatory effect of Pak1 signaling on the cyclin D1 promoter was a wide-spread phenomenon, as WT-Pak1 or T423E Pak1 efficiently stimulated cyclin D1 promoter activity in invasive breast cancer MDA-MB-231, cervical cancer HeLa, and in well differentiated endometrial Ishikawa cells (Fig. 3). Together, these results implied a role of the Pak1 pathway in the expression of cyclin D1.

Effect of Kinase-Active T423E Pak1 on Cyclin D1 Expression—To further implicate a regulatory role for Pak1 signaling in the regulation of cyclin D1 expression, we next used a previously characterized MCF-7 breast cancer cell clone expressing HA-tagged-T423E Pak1 under the control of an inducible tetracycline promoter (15). As expected, induced expression of HA-tagged T423E Pak1 in MCF-7 cells stimulated transcription from the cyclin D1 promoter-luciferase reporter (Fig. 4A). The increased expression of kinase-active Pak1 in MCF-7 cells was also accompanied by elevated steady-state levels of cyclin D1 mRNA (Fig. 4B) and cyclin D1 protein (Fig. 4C), as well as in the levels of newly synthesized metabolic-labeled 35S-cyclin D1 level (Fig. 4D). To gain clues about the functional conse-
quence of Pak1-mediated increased expression of cyclin D1, we
determined the subcellular distribution of cyclin D1 in MCF-7
cells expressing kinase-active Pak1 by quantitative confocal
scanning microscopy. After 24 h of Dox treatment, both Pak1
and cyclin D1 protein expression were greatly induced, with
the majority of cyclin D1 accumulating in the nucleus. (Fig.
5A). Quantitation of cellular localization of cyclin D1 upon
Pak1 signaling is presented in Fig. 5B. The observed nuclear
accumulation of cyclin D1 seems to be due to increased cyclin
D1 expression as well as to increased levels of phosphorylated
cyclin D1 in MCF-7 cells with a hyperactivated Pak1 pathway
(Fig. 5C). Taken together, these results suggest that Pak1
signaling regulates the expression of cyclin D1 and that Pak1
may be an important mediator of upstream signals leading to
cyclin D1 expression.

**Pak1 Status Modulates Cyclin D1 Expression**—To validate
the effect of Pak1 on cyclin D1 expression, we next used previ-
ously characterized MCF-7 clones 10 and 17, which ectopically
express an auto-inhibitory peptide fragment of Pak1 (amino
acids 83–149) that does not interact with GTPases and thus
acts as a dominant-negative Pak1 (7, 32). We observed that
inhibition of the Pak1 pathway in MCF-7 cells substantially
suppressed cyclin D1 transcription but not transcription from a
control luciferase reporter (Fig. 6A). The expression of Pak1
inhibitory domain 83–149 in stable clones is shown in Fig. 6A,
*inset.* Consistent with this finding, we also observed a marked
reduction in the baseline level of cyclin D1 protein expression
in clones 10 and 17 (Fig. 6B). These findings suggested that
the Pak1 pathway might have an important role in the biology
of cyclin D1 in breast cancer cells. To further confirm the signif-
icance of Pak1 signaling in the optimal expression of cyclin D1,
we next selectively knocked down the endogenous Pak1 expres-
sion in MCF-7 breast cancer cells by using the siRNA method-
ology (33). We demonstrated previously the efficacy of Pak1-
specific siRNA in down-regulating endogenous Pak1 using
Western blotting and confocal microscopy and the ability of
Pak1-specific siRNA to suppress Pak1-driven pathways (19).
Interestingly, reducing Pak1 expression by Pak1-siRNA but
not by control siRNA was accompanied by a significant reduc-
tion of cyclin D1 expression (Fig. 6C), suggesting a potential
role of functional Pak1 pathway for optimal expression of cyclin
D1. To study further whether the inhibitory effect of Pak1
(83–149) on cyclin D1 expression can be relieved by an activat-
ing point mutation (Pak1 L107F), transfection studies were
performed using plasmids containing Pak1 (83–149) and Pak1
(83–149) L107F on cyclin D1 promoter. Interestingly, activat-
ing point mutant Pak1 (83–149) L107F partially but sig-
nificantly relieved the repression induced by Pak1 (83–149) (Fig.
6D), validating the requirement of a functional Pak1 pathway
for optimal cyclin D1 expression.

**Pak1 Regulates Cyclin D1 Transcription via an NF-κB-Depen-
dent Pathway**—Data from the literature suggest that cyclin
D1 transcription could be up-regulated through multiple sig-
alling pathways, including Stat5a and NF-κB transcription
could be up-regulated through multiple sig-

![Fig. 6. Pak1 status modulates cyclin D1 expression.](image)

A. Stable clones expressing Pak1 inhibitor (83–149) and pcDNA control cells were
transfected with 1.0 ng of cyclin D1-luciferase reporter (*left panel*) and
with a control cytomegalovirus-luciferase reporter (*right panel*). After
24 h, the cells were lysed, and luciferase activity was measured (n = 3).
The activity was normalized with β-galactosidase activity. B. Western
blot analysis of cyclin D1 in stable clones expressing Pak1 inhibitor
(83–149). C, reduction in cyclin D1 protein expression by Pak1-siRNA.
MCF-7 cells were cotransfected with 10 ng Pak1, control, or glyceral-
dehyde-3-phosphate dehydrogenase (GAPDH) siRNA for 48 h and an-
alyzed for the status of cyclin D1 and Pak1 protein expression (n = 2).
D, HC11 cells were transfected with 0.5 ng of cyclin D1-luciferase
reporter and with either 1.5 μg of glutathione S-transferase (GST)-
vector, 1.5 μg of GST-Pak1 (83–149), or with 1.5 μg of GST-Pak1
(83–149) L107F. After 24 h, the cells were lysed, and luciferase activity
was measured (n = 3). The activity was normalized with β-galactosid-
ase activity.

The activity was normalized with β-galactosidase activity.

- Fig. 6. Pak1 status modulates cyclin D1 expression. A. Stable
  clones expressing Pak1 inhibitor (83–149) and pcDNA control cells were
  transfected with 1.0 ng of cyclin D1-luciferase reporter (*left panel*) and
  with a control cytomegalovirus-luciferase reporter (*right panel*). After
  24 h, the cells were lysed, and luciferase activity was measured (n = 3).
  The activity was normalized with β-galactosidase activity. B. Western
  blot analysis of cyclin D1 in stable clones expressing Pak1 inhibitor
  (83–149). C, reduction in cyclin D1 protein expression by Pak1-siRNA.
  MCF-7 cells were cotransfected with 10 ng Pak1, control, or glyceral-
  dehyde-3-phosphate dehydrogenase (GAPDH) siRNA for 48 h and an-
  alyzed for the status of cyclin D1 and Pak1 protein expression (n = 2).
  D, HC11 cells were transfected with 0.5 ng of cyclin D1-luciferase
  reporter and with either 1.5 μg of glutathione S-transferase (GST)-
  vector, 1.5 μg of GST-Pak1 (83–149), or with 1.5 μg of GST-Pak1
  (83–149) L107F. After 24 h, the cells were lysed, and luciferase activity
  was measured (n = 3). The activity was normalized with β-galactosid-
  ase activity.

To investigate the mechanism of this regulation, cotransfection
studies were performed using reporter plasmids containing the
human cyclin D1 promoter-deletion constructs and T423E-
Pak1. The results showed that Pak1 strongly activated cyclin
D1 gene expression and that this regulation mapped to regions
between −1745 to −630 and −261 to −66 within the promoter
(Fig. 7C). Sequence analysis of the human cyclin D1 promoter
identified three potential NF-κB binding sites at positions
−858, −749, and −39 (37), so we extended our study to exam-
ine which promoter regions were critical for Pak1 regulation of
cyclin D1. Accordingly, deletion of −858 and −749 sites in
−630 cyclin D1-luciferase resulted in a significant reduction in
promoter activity. Also, site-directed mutagenesis of one NF-κB
binding site at −39 (23) in the −66 cyclin D1 IκBMT luciferase
abolished cyclin D1 promoter activity (Fig. 7D), strongly sug-
Fig. 7. Pak1 regulates cyclin D1 transcription via an NF-kB-dependent pathway. A, HC11 cells were cotransfected with 0.5 μg of cyclin D1-luciferase reporter, 0.5 μg of dominant-negative Stat5a, 0.5 μg of dominant-negative Stat5a, and 0.5 μg of dominant-negative NF-κB. After 24 h, the cells were lysed, and luciferase activity was measured (*n* = 3). The activity was normalized with β-galactosidase activity. B, HC11 cells were cotransfected with 0.5 μg of cyclin D1-luciferase reporter, 0.5 μg of WT Pak1, 0.5 μg of dominant-negative Stat5a, and 0.5 μg of dominant-negative Stat5a, and 0.5 μg of dominant-negative IκBMT. After 24 h, the cells were lysed, and luciferase activity was measured (*n* = 3). The activity was normalized with β-galactosidase activity. C, MCF-7 cells were cotransfected with 1.0 μg of reporter plasmids containing various deletions of the human cyclin D1 promoter and 0.5 μg of T423E Pak1. After 24 h, the cells were lysed, and luciferase activity was measured (*n* = 3). The activity was normalized with β-galactosidase activity. D, MCF-7 cells were cotransfected with 1.0 μg of reporter plasmids containing various deletions of the human cyclin D1 promoter and 0.5 μg of T423E Pak1. After 24 h, the cells were lysed, and luciferase activity was measured (*n* = 3). The activity was normalized with β-galactosidase activity.

In summary, the results presented here demonstrate that (i) Pak1 regulates the expression of cyclin D1 in diverse cell types; (ii) Pak1 signaling is an important regulator of cyclin D1 expression and function; (iii) Pak1 regulates cyclin D1 transcription by means of an NF-kB-dependent pathway; and (iv) Pak1 is highly expressed in breast tumors. These data provide evidence that up-regulation of the Pak1 pathway in breast cancer epithelial cells may have functional implications in the enhancement of the growth-rate of breast cancer cells. Any potential up-regulation of cyclin D1 by Pak1 and its upstream activators in breast cancer epithelial cells is likely to sustain and perhaps further promote the ability of tumor cells to grow by supporting the putative functions of cyclin D1 and might contribute toward the noticed hyperplasia in mammary epithelium from kinase-active Pak1 transgenic mice (19).

Acknowledgment—We thank Bruce Mayer for the pEBG-GST vector.

REFERENCES
1. Narumiya, S., Ishizaki, T., and Watanabe, N. (1997) FEBS Lett. 410, 68–72
2. Ridley, A. J., and Hall, A. (1992) Cell 70, 389–399
3. Kozma, R., Ahmed, S., Best, A., and Lim, L. (1995) Mol. Cell. Biol. 15, 1942–1952
4. Nobes, C. D., and Hall, A. (1995) Cell 81, 53–62
5. Zhao, Z. S., Maner, E., Chen, X. Q., Chong, C., Leung, T., and Lim, L. (1998) Mol. Cell. Biol. 18, 2153–2163
6. Manser, E., Huang, H. Y., Loo, T. H., Chen, X. Q., Dong, J. M., Leung, T., and Lim, L. (1997) Mol. Cell. Biol. 17, 1129–1143
7. Galisteo, M. L., Chernoff, J., Su, Y. C., Skalnik, E. Y., and Schlessinger, J. (1996) J. Biol. Chem. 271, 21097–21100
8. Bagheri-Yarmand, R., Vadlamudi, R. K., Wang, R. A., Mendelsohn, J., and Kumar, R. (2000) J. Biol. Chem. 275, 39453–39457
9. Vadlamudi, R. K., Li, F., Adam, L., Nguyen, D., Okta, Y., Stossel, T. H., and Kumar, R. (2002) Nat. Cell. Biol. 4, 681–690
10. Sells, M. A., and Chernoff, J. (1997) Trends Cell Biol. 7, 162–167
11. Bagrodia, S., Derijard, B., Davis, R. J., and Cerione, R. A. (1995) J. Biol. Chem. 270, 27995–27998
12. Zhang, S., Han, J., Sells, M. A., Chernoff, J., Knaus, U. G., Ulevitch, R. J., and Bokoch, G. M. (1995) J. Biol. Chem. 270, 23934–23936
13. Adam, L., Vadlamudi, R. K., Kendapaka, S. B., Chernoff, J., Mendelsohn, J., and Kumar, R. (1998) J. Biol. Chem. 273, 28238–28246
14. Adam, L., Vadlamudi, R. K., Mandal, M., Chernoff, J., and Kumar, R. (2000) J. Biol. Chem. 275, 12041–12050
15. Vadlamudi, R. K., Adam, L., Wang, R. A., Mandal, M., Nguyen, D., Sahin, A., Chernoff, J., Hung, M. C., and Kumar, R. (2000) J. Biol. Chem. 275, 36239–36244
16. Schraml, P., Schwerdtfeger, G., Burkhalter, F., Raggi, A., Schmidt, D., Buffalo, T., King, W., Wilber, K., Mihatsch, M. J., and Moch, H. (2003) Am. J. Pathol. 163, 985–992
17. Bekri, S., Adelaide, J., Merscher, S., Gregoire, J., Caroli-Base, F., Perucca-Lostanlen, D., Kelley, P. M., Pribuske, M. J., Theillet, C., Birnbaum, D., and Gaudray, P. (1997) Cytogenet. Cell Genet. 79, 125–131
18. Salt, R., Marotta, A., Wagey, R., Sayed, M., and Pelech, S. (2002) Int. J. Cancer 98, 145–154
19. Wang, R. A., Mazumdar, A., Vadlamudi, R. K., and Kumar, R. (2002) EMBO J. 21, 5437–5447
20. Wang, T. C., Cardiff, R. D., Zakerberg, L., Lees, A., Arnold, A., and Schadt, E. V. (1994) Nature 369, 669–671
21. Matsuura, I., Kitamura, T., Wako, H., Tanaka, H., Hashimoto, K., Albanese, C., Dowward, J., Pestell, R. G., and Kanakura, Y. (1999) EMBO J. 18, 1367–1377
22. Henry, D. O., Moskalenko, S. A., Kaur, J. K., Fu, M., Pestell, R. G., Camonis, J. H., and White, M. A. (2000) Mol. Cell. Biol. 20, 8084–8092
23. Joyce, D., Bouzahzah, B., Fu, M., Albanese, C., D’Amico, M., Steer, J., Klein,
J. U., Lee, R. J., Segall, J. E., Westwick, J. K., Der, C. J., and Pestell, R. G. (1999) J. Biol. Chem. 274, 25245–25249
24. Wilcken, N. R., Prall, O. W., Musgrove, E. A., and Sutherland, R. L. (1997) Clin. Cancer Res. 3, 849–854
25. Prall, O. W., Rogan, E. M., Musgrove, E. A., Watts, C. K., and Sutherland, R. L. (1998) Mol. Cell. Biol. 18, 4499–4508
26. Lukas, J., Bartkova, J., and Bartek, J. (1996) Mol. Cell. Biol. 16, 6917–6925
27. Carroll, J. S., Prall, O. W., Musgrove, E. A., and Sutherland, R. L. (2000) J. Biol. Chem. 275, 38221–38229
28. Wang, C., Pattabiraman, N., Zhou, J. N., Fu, M., Sakamaki, T., Albanese, C., Li, Z., Wu, K., Hultt, J., Neumeister, P., Novikoff, P. M., Brownlee, M., Scherer, P. E., Jones, J. G., Whitney, K. D., Donohower, L. A., Harris, E. L., Rohan, T., John, D. C., and Pestell, R. G. (2003) Mol. Cell. Biol. 23, 6159–6173
29. Michaelsen, J. S., and Leder, P. (2001) Oncogene 20, 5093–5099
30. Kumar, R., Mandal, M., Eatskin, B. J., Liu, N., and Lipton, A. (1996) J. Cell. Biochem. 62, 102–112
31. Marumdar, A., Wang, R. A., Mishra, S. K., Adam, L., Bagheri-Yarmand, R., Mandal, M., Vadlamudi, R. K., and Kumar, R. (2001) Nat. Cell. Bio. 3, 30–37
32. Bagheri-Yarmand, R., Mandal, M., Talukder, A. H., Wang, R. A., Vadlamudi, R. K., Kung, H. J., and Kumar, R. (2001) J. Biol. Chem. 276, 28405–28409
33. Yu, J. Y., DeRutyer, S. L., and Turner, D. L. (2000) Proc. Natl. Acad. Sci. U. S. A. 99, 6047–6052
34. Frost, J. A., Swantek, J. L., Stippec, S., Yin, M. J., Gaynor, R., and Cobb, M. H. (2000) J. Biol. Chem. 275, 19693–19699
35. Foryst-Ludwig, A., and Naumann, M. (2000) J. Biol. Chem. 275, 39779–39785
36. Harbaj, E. W., Maggirwar, S. B., Good, L., and Sun, S. C. (1996) Mol. Cell. Biol. 16, 6736–6743
37. Motokura, T., and Arnold, A. (1993) Genes Chromosomes Cancer 7, 89–95