Transcriptional Regulation of the Transforming Growth Factor β Type II Receptor Gene by Histone Acetyltransferase and Deacetylase Is Mediated by NF-Y in Human Breast Cancer Cells*

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Seok Hee Park‡‡, Sae Ra Lee‡, Byung Chul Kim‡, Eun Ah Cho‡, Sejal P. Patel‡, Hee-Bum Kang‡, Edward A. Sausville‡, Osamu Nakanishi‡, Jane B. Trepel‡‡, Byoung Ick Leel ‡‡, and Seong-Jin Kim‡‡‡‡

From the ‡Laboratory of Cell Regulation and Carcinogenesis, the §Developmental Therapeutics Program, and the ¶Division of Basic Science, National Cancer Center, Madu-dong, Goyang-Si, Gyeonggi-do, 411-764, Korea, and ||Mitsui Pharmaceuticals, Chiba 297-0017, Japan

Transcriptional repression of the transforming growth factor-β (TGF-β) type II receptor (TβRII) gene is one of several mechanisms leading to TGF-β resistance. Previously, we have shown that MS-275, a synthetic inhibitor of histone deacetylase (HDAC), specifically induces the expression of the TβRII gene and restores the TGF-β signaling in human breast cancer cell lines. However, little is known about the mechanism by which inhibition of HDAC activates TβRII expression. MS-275 treatment of cells expressing a wild-type TβRII promoter/luciferase construct resulted in a 10-fold induction of the promoter activity. DNA transfection and an electrophoretic mobility shift assay showed that the induction of the TβRII promoter by MS-275 requires the inverted CCAAT box and its cognate binding protein, NF-Y. In addition, a DNA affinity pull-down assay indicated that the PCAF protein, a transcriptional coactivator with intrinsic histone acetyltransferase (HAT) activity, is specifically recruited to the NF-Y complex upon treatment with MS-275, specifically recruiting the HDAC inhibitor induces TβRII promoter activity by the recruitment of the PCAF protein to the NF-Y complex, interacting with the inverted CCAAT box in the TβRII promoter.

The histone-modifying enzymes histone acetyltransferase (HAT) and histone deacetylase (HDAC) have been proposed to play an important role in transcriptional regulation by altering chromatin structure (1, 2). HATs specifically catalyze the acetylation of the ε-amino group of lysine residues at the N-terminal domain of histone H2A, H2B, H3, and H4, leading to a destabilization of the nucleosome structure whereas HDACs remove the acetyl group, resulting in a compact chromatin configuration. Hyperacetylation of chromatin is generally associated with transcriptional activation, whereas hypoacetylation of chromatin is associated with transcriptional repression (3, 4). HATs and HDACs thus constitute important links between chromatin structure and transcriptional output.

Transforming growth factor β (TGF-β) has been implicated in a wide variety of cellular processes, including regulation of the cell cycle, cell differentiation, and extracellular matrix synthesis (5, 6). TGF-β primarily exerts its biological effects through interactions with the TGF-β type II receptor (TβRII) (7, 8). Much work (9–12) has shown that inactivation of TβRII contributes to malignant transformation at an early step of tumorigenesis and that it can occur through mutation or transcriptional repression of the TβRII gene. Interestingly, many human cancer cell lines express normal TβRII and downstream signaling intermediates, but express significantly low or undetectable levels of TβRII mRNA, suggesting that transcriptional repression of the TβRII gene might be a more common mechanism leading to TGF-β resistance (9, 13, 14).

We have previously demonstrated (15) that MS-275, a histone deacetylase inhibitor, induces the accumulation of acetylated histones in the chromatin of the TβRII gene and that this induction is associated with an increase of TβRII mRNA in human breast cancer cell lines, contributing to the restoration of TGF-β signaling. In this study, we have expanded upon this early observation and examined the molecular mechanism of the induction of the TβRII gene by MS-275 treatment in human breast cancer cell lines. We first show that the inverted CCAAT box and its cognate binding protein, NF-Y, play an important role in the induction of TβRII gene expression. Second, we found that PCAF, a protein with an intrinsic HAT activity, is recruited to the NF-Y complex upon treatment with MS-275, leading to the increase of TβRII gene expression. These findings demonstrate the mechanism by which the TβRII gene, which is transcriptionally repressed by hypoacetylation, is induced by an HDAC inhibitor.

MATERIALS AND METHODS

Cell Culture, Transfections, and Reporter Assay—The human breast cancer cell lines, MCF-7 and ZR-75, were cultured in RPMI 1640 medium without phenol red with 10% charcoal-treated fetal bovine serum and were incubated at 37 °C with 5% CO2. MS-275, an inhibitor of histone deacetylase, was provided by Mitsui Pharmaceuticals (16). MCF-7 and ZR-75 cells were transfected using Lipofectin reagent (In-vitrogen) according to the manufacturer’s protocol. Briefly, for transient transfection, cells were seeded in six-well plates at a density of 3 × 105 cells/well. The following day cells were transfected with the indicated...
Transcriptional Repression of the TβRII Gene

Results

TβRII promoter construct (1.0 µg/well) or cotransfected with 1.0 µg of a TβRII promoter construct and 1.0 µg of PCAF expression vector. Cells were incubated for 24 h prior to treatment with MS-275 and treated for 24 h before harvesting. For stable transfection, either pGL3-basic, pTβRII, or pTβRII – 102/+2M4-luc (Fig. 4) was cotransfected with pCIneo (Promega) plasmids into MCF-7 cells using Fugene 6 reagent (Roche Molecular Biochemicals) according to the manufacturer’s protocol. After 2 days, stable transfectants were selected using G418 (800 µg/ml; Calbiochem) for 3 weeks. Resultant colonies were picked for further analysis. Luciferase assay was performed with commercially available reagents and normalized relative to protein concentration as determined by the Bradford assay kit (Bio-Rad). All experiments were repeated at least three times with similar results.

Plasmids and Site-directed Mutagenesis—Deletion mutants of the TβRII promoter in this study were cloned into the promoterless luciferase vector (pGL3-basic) using HindIII and SacI sites. Site-directed mutagenesis of the region from −102 to −50 as shown in Fig. 4 was performed by the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The PCAF expression plasmid was kindly provided by Dr. Y. Nakatani.

RNA Extraction and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)—Total RNAs were isolated with the Triazol reagent (Invitrogen) according to the manufacturer’s protocol. RT was performed using the SuperScript kit (Invitrogen) according to the manufacturer’s instructions. For the PCR reaction, the PCR reagent system kit from Invitrogen was used according to the manufacturer’s instructions. The sequences of the luciferase primers were 5’TCAAA-GAGCCGAACCTGTTCG-3’ and 5’TTCCTCGTACATGGCTTCTTC-3’. As a control, β-actin primers were 5’-TGCCTGCAGACAAGTCGAC-3’ and 5’-AGGATGCTCCTGCTTCG-3’. The luciferase and β-actin cDNAs were amplified in separate PCR reactions. Samples that lacked RT were also amplified to control for the presence of any contaminating genomic DNA.

Nuclear Extracts, Electrophoretic Mobility Shift Assay (EMSA), and Antibody Supershift Assay—Preparation of nuclear extracts, EMSA, and antibody supershift assay were performed as described previously (18). MCF-7 cells were cultured with and without either MS-275 (0.5 µM), TSA (0.3 µM), or NaBu (2 mM) for 24 h and harvested to obtain nuclear extracts. For EMSA, double-stranded oligonucleotides containing the CCAAT box region (−100 to −62) (18) were labeled with [γ-32P]ATP and polynucleotide kinase and purified using a 10% nondeaturing polyacrylamide gel. To perform a competition assay, unlabeled oligonucleotides were used as competitors. For the antibody supershift assay, the reactions were performed by preincubating nuclear extracts with 5 µg of antibody at 4 °C. The anti-NF-YA and anti-NF-YB antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

DNA Affinity Pull-down Assay—A DNA affinity pull-down assay using M280 magnetic beads (Dynal) was performed as previously described by Sasaki et al. (19) with minor modification. Four copies of the region from −100 to −67 containing the CCAAT box were cloned into pUC18 and biotinylated by PCR, using a biotin-labeled M13 reverse primer and a non-biotin-labeled M13 forward primer. The purified PCR fragments (40 µg) were conjugated to 10 mg of M280 magnetic beads according to the manufacturer’s protocol (Dynal). DNA-conjugated beads (50 µl) were mixed with 1.0 mg of MCF-7 nuclear extracts in binding buffer (10 mM Tris, pH 7.5, 100 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 5% glycerol) for 4 h at 4 °C with constant rotation. The suspension was precipitated with a magnetic plate (Dynal MPC-S), washed in binding buffer three times, and reprecipitated with centrifugation. The bound proteins were eluted with 20 µl of BC500 buffer as described by Sasaki et al. (19). The eluted proteins were analyzed by Western blotting with anti-NF-YB and anti-PCAF antibodies (Santa Cruz Biotechnology).

Results

TGF-β Type II Receptor Promoter Is Activated by the HDAC Inhibitors, Including MS-275—Because MS-275 increased the accumulation of acetylated histones H3 and H4 in the TβRII promoter and induced expression of TβRII mRNA in human breast cancer cell lines (15), we investigated whether the histone deacetylase inhibitors MS-275, TSA, and sodium butyrate (NaBu) activate TβRII promoter activity in the MCF-7 human breast cancer cell TβRII/luciferase reporter construct (pTβRII – 219/+35-luc), showing the strongest response to MS-275, and control vector pGL3-basic were transiently transfected and treated with MS-275 (0.5 µM), TSA (0.3 µg/ml), and NaBu (2 mM) for 24 h. As shown in Fig. 1a, TβRII promoter activity was dramatically increased about 60- to 70-fold following treatment with MS-275 or TSA and about 30-fold after treatment with NaBu. No significant induction in promoter activity of the control vector (pGL3-basic) was found after treatment with the inhibitors. These results were consistent with the previous report that the HDAC inhibitor, MS-275, induces TβRII gene expression at a transcriptional level.

Several reports have shown that only promoters that are integrated into the chromosome could be regulated by HATs and HDACs, whereas several other reports have shown that the activity of transiently transfected promoters could be efficiently modulated by HATs and HDACs (20–25). Therefore, we constructed stable cell lines expressing the TβRII promoter/reporter gene to examine whether the TβRII promoter/reporter gene stably integrated into chromosomal DNA is also regulated by HATs and HDACs. The MCF-7 cell line was transfected with either the TβRII/luciferase reporter constructs (pTβRII – 102/+2-luc or pTβRII – 102/+2M4-luc) or its control vector as described under “Materials and Methods,” and stable transfectants were selected. The cell lines in which either the pTβRII – 102/+2-luc or pTβRII – 102/+2M4-luc gene were stably integrated were treated with 0.5 µM MS-275 for 24 h. As a control, the stable cell line, which contained pGL3-basic, a promoterless luciferase vector, was also treated with MS-275 under the same conditions. Expression of the luciferase gene was studied by RT-PCR. As shown in Fig. 2a, a DNA band of about 328 bp corresponding to a luciferase fragment of expected size was detected in MCF-7 cell lines. However, the level of luciferase expression in cell lines expressing control vector and pTβRII – 102/+2M4-luc was very low, and MS-275 treatment did not increase the expression level. In the stable cell lines expressing pTβRII – 102/+2-luc gene, the activity of the TβRII promoter was increased 5-fold following treatment with MS-275 (Fig. 2a). The pTβRII – 102/+2M4-luc stable cell lines showed high basal luciferase activity, and MS-275 treatment further induced its luciferase activity (Fig. 2b). These results indicate that MS-275 has the same effect in stable cell lines expressing the TβRII promoter/luciferase gene as in cells expressing the transiently transfected TβRII promoter.

An Inverted CCAAT Box Plays an Important Role in Activation of the TβRII Promoter by MS-275—To characterize the promoter

![Fig. 1. Activation of the TβRII promoter by MS-275, TSA, and sodium butyrate in MCF-7 cells.](image-url)
region responsible for the induction of the TβRII promoter by MS-275, serial deletion mutants of the TβRII promoter, as shown in Fig. 3, were transiently transfected into MCF-7 and ZR-75 breast cancer cell lines, and the cells were then treated with MS-275. When the promoter was deleted to −172, induction of the TβRII promoter by MS-275 was decreased to 50% in both cell lines, suggesting the presence of an element responsible for the induction within the region from −219 to −172. Interestingly, deletion of the region from −100 to −47 dramatically decreased the induction of the TβRII promoter to only about 2-fold upon treatment of MS-275, whereas expression of pTβRII−100/+35-luc was still induced −20-fold (Fig. 3). These results suggest that the region from −100 to −47 contains a major element(s) required for the induction by MS-275. Therefore, the region from −100 to −47 was focused on in order to study the mechanism of MS-275-mediated induction of the TβRII promoter.

To further investigate the MS-275 responsive sequence between −100 and −47, we constructed site-directed mutants within this region, as shown in Fig. 4. Following transfection, the MCF-7 cells were exposed to MS-275 for 24 h and analyzed for luciferase activity. As shown in Fig. 4b, M3 and M4 mutants were only slightly affected by treatment with MS-275, whereas the wild type and other mutants of the TβRII promoter were dramatically induced. M3 and M4 contain mutations of the inverted CCAAT box (−82 to −78), which had previously been reported to be involved in v-SRC-mediated induction of the TβRII promoter (18). Thus, two different mutations in the CCAAT box abolished promoter activity induced by MS-275, indicating that this CCAAT box plays a critical role in the induction of the TβRII promoter by the HDAC inhibitor, MS-275.

NF-Y Protein Binds to the Inverted CCAAT Box and Its Binding Activity Is Not Changed by MS-275—To identify specific binding of proteins to the sequences from −100 to −62, EMSA was performed as described above using a double-stranded 32P-labeled oligonucleotide containing the sequences between −100 and −62. The reaction mixture was then electrophoresed on a polyacrylamide gel and viewed by autoradiography (Fig. 5). In the absence of unlabeled competitor oligonucleotide (Fig. 5b, lane 1), three strong bands (complexes a, b, and c) were apparent. It is clear that these bands represent specific binding of protein(s) to the target oligonucleotide sequence because binding to the labeled probe diminishes with a mutant unlabeled competitor (Fig. 5b, lane 2). Mutant oligonucleotides derived from the sequences between −100 and −62 were used to identify the target sequences for complexes a, b, and c (Fig. 5a). M1 and M2 mutants failed to compete for binding to these complexes, whereas the M3 mutant decreased competition for binding to all three complexes (Fig. 5b, lanes 3–5). This region contains the inverted CCAAT consensus sequences. The binding of complexes a, b, and c to the M3 mutant was also markedly reduced (Fig. 5c).

To characterize which transcription factors interact with the CCAAT box in response to MS-275, an EMSA was performed with MCF-7 nuclear extracts prepared from MS-275 treated or untreated cells. Three protein-DNA complexes (complexes a, b, and c) from MCF-7 cells interacted with a 34-mer oligonucleotide probe of the region around the wild-type CCAAT box (Fig. 6a). Treatment with MS-275 prior to preparation of nuclear extract had no apparent effect on the complex formation (Fig. 6a). EMSA with the nuclear extract of ZR-75 human breast cancer cells showed the same protein-DNA complexes, which again were not affected by MS-275 treatment (data not shown).

In addition, competition assay showed that all three protein-DNA complexes were specific for the inverted CCAAT box (Fig. 5a). The addition of unlabeled wild-type CCAAT box sequences completely abolished the formation of all three complexes, whereas both unlabeled M4 oligonucleotides containing the mutation of the CCAAT box and nonspecific oligonucleotides did not affect complex formation (Fig. 6a).

To identify transcription factors interacting with this inverted CCAAT box in the TβRII promoter, antibody supershift assays were performed. Because it had been reported previously (18) that the NF-Y protein binds to the CCAAT box of the
TβRII promoter, we determined whether the NF-Y protein interacts with the CCAAT box in MCF-7 breast cancer cell lines. NF-Y is a complex composed of three subunits, NF-YA (CBF-B), NF-YB (CBF-A), and NF-YC (CBF-C), which are highly conserved throughout evolution, and all are required for DNA binding (26–28). As shown in Fig. 6b, the NF-YA and NF-YB antibodies were found to selectively supershift the complex a in both MS-275-untreated and -treated nuclear extracts, whereas the antibody against the other CCAAT box-binding protein, C/EBP, did not show any shifted band. Interestingly, complexes b and c were not changed by the supershift assay, suggesting that these complexes are formed by other nuclear proteins. Consequently, these results indicate that complex a represents the NF-Y protein bound to the CCAAT boxes of the TβRII promoter in human breast cancer cell lines and that the binding activity of NF-Y protein is not affected by MS-275 treatment. However, we do not yet understand the reason why the supershifted band by NF-YA has lower mobility than by NF-YB.

PCAF Is Only Recruited to NF-Y upon Treatment with an Inhibitor of HDAC—These results, however, did not clearly reveal the mechanism of induction of the TβRII promoter by MS-275 because binding of the NF-Y protein was not changed in MCF-7 nuclear extracts, whether untreated or treated by MS-275. Recent reports show that the NF-Y protein is connected with histone acetyltransferase activity (24, 29, 30). It was reported that the NF-Y complex possesses histone acetyltransferase activity through physical association with the related histone acetyltransferases, human GCN5 and PCAF, in vivo (29). In two other reports, TSA, a potent inhibitor of HDAC, increased the activity of NF-Y-dependent promoters such as human MDR1 and Xenopus HSP70 in vivo (24, 30). In the case of the MDR1 gene, overexpression of PCAF with intrinsic histone acetyltransferase activity induced the wild-type MDR1 promoter but not a promoter containing a mutation in the CCAAT box. Moreover, it was shown that NF-YA interacts with PCAF in vitro. In the HSP70 promoter it was reported that NF-YB is a substrate of p300 acetylation and recruits p300 to modulate transcriptional activity (30).

To investigate whether the NF-Y complex is truly involved in recruiting histone acetyltransferase activity to the TβRII promoter in response to either MS-275 or TSA, a DNA affinity pull-down assay was performed. Four copies of the region containing the wild-type CCAAT box were conjugated with mag-
netic beads and incubated with nuclear extracts of MCF-7 cells, which were treated or untreated with either MS-275 or TSA. As a negative control, four copies of the region in which the CCAAT box was mutated were simultaneously conjugated. Bound materials were eluted, and immunoblot analysis was performed with PCAF, NF-Y, and p300 antibodies. Interestingly, only PCAF, not p300, was detected in the nuclear extracts treated with MS-275 and NF-Y protein bound to the wild-type CCAAT box, not the mutant CCAAT box (Fig. 7a).

However, we could not observe the presence of PCAF in untreated cells. This result suggests a novel mechanism for the activation of the TβRII promoter by an inhibitor of HDAC in human breast cancer cell lines. In the absence of either MS-275 or TSA treatment, NF-Y does not interact with PCAF in breast cancer cell lines, whereas PCAF is recruited to the NF-Y complex upon treatment with either MS-275 or TSA, increasing the activity of the TβRII promoter.

To further support the possibility that PCAF is involved in the activation of the TβRII promoter in human breast cancer cell lines, a plasmid expressing PCAF was cotransfected with...
a wild-type (pTβRII–102/+–2-luc) or CCAAT-mutated (pTβRII–102/+–2M4–luc) TβRII promoter/luciferase construct into MCF-7 cells. Although transfection of PCAF increased the activity of the wild-type TβRII promoter about 5-fold, it had no effect on the CCAAT box mutant (Fig. 7b). However, expression of p300 did not increase the activity of the wild-type TβRII promoter in MCF-7 breast cancer cells (data not shown). Consequently, our results strongly suggest that activation of the TβRII promoter by an inhibitor of HDAC is due to the increase of HAT activity by recruiting PCAF to NF-Y tethered to the TβRII promoter in human breast cancer cell lines.

DISCUSSION

Several studies demonstrate that transcriptional repression of the TGF-β type II receptor gene is one of several mechanisms leading to TGF-β resistance. Many human cancer cell lines harbor a normal TβRII gene and downstream signaling proteins but express significantly reduced or undetectable levels of TβRII mRNA (9, 13). Transcriptional regulation of TβRII gene expression plays an important role in modulating TGF-β responsiveness. Transformation of cells by the product of the adenovirus E1A gene or overexpression of cyclin D1 in epithelial cells has been associated with down-regulation of TβRII expression and TGF-β resistance (32–34). Recently, we reported (12) that the Ewing sarcoma EWS-Fl1 fusion gene suppresses transcription of the TβRII gene. These results imply that the TβRII gene acts as a tumor suppressor gene and may be a candidate target for cancer therapy of therapeutic targets for cancer.

We have previously reported that MS-275, a HDAC inhibitor, enhances TβRII gene expression in association with an accumulation of acetylated histones H3 and H4 with the TβRII promoter and restores TGF-β signaling in human breast cancer cell lines, suggesting the possibility that histone deacetylation of the TβRII gene is a new epigenetic mechanism to contribute to the resistance of human breast cancer cell lines to TGF-β (15). In this report, we have investigated the mechanism of the induction of the TβRII gene by an inhibitor of HDAC in human breast cancer cell lines. First, the induction of the TβRII gene by MS-275 requires an intact CCAAT box and its cognate binding factor, the NF-Y complex. Second, when cells are treated with an inhibitor of HDAC, PCAF with histone acetyltransferase activity is recruited into the NF-Y complex, increasing the activity of the TβRII promoter.

Our findings that the CCAAT box and NF-Y are required for activation of the TβRII promoter are similar to the data described in the induction of the MDR1 gene by TSA (24) and consistent with previous reports (29) that NF-Y is connected with histone acetyltransferase activity through physical association with human GCN5 and PCAF. However, the study of MDR1 did not clearly provide the mechanism of the induction of the MDR1 gene by TSA. They showed that PCAF interacts with NF-YA in vitro, suggesting that the direct interaction of PCAF and NF-YA mediate the transcriptional activation. Interestingly, the interaction of PCAF and the NF-Y complex on the TβRII promoter was only shown in the presence of MS-275 treatment (Fig. 7a). Under normal conditions, NF-YA tethered to the TβRII promoter did not interact with PCAF in the human breast cancer cell line. Upon treatment with either MS-275 or TSA, the interaction between the two proteins was increased as shown in the DNA affinity pull-down assay (Fig. 7a), whereas the DNA binding activity of the NF-Y complex was not changed in the absence or presence of an inhibitor of HDAC treatment. These findings suggest a novel mechanism for the activation of the TβRII promoter by an inhibitor of the HDAC. One possibility is that transcription of the TβRII

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REFERENCES

1. Grunstein, M. (1997) Nature 389, 349–352
2. Paranjape, S. M., Kamakaka, R. T., and Kadonaga, J. T. (1994) Annu. Rev. Biochem. 63, 265–297
3. Hebestreit, T. C., Clayton, A. L., Throne, A. W., and Crane-Robinson, C. (1994) EMBO J. 13, 1823–1830
4. Braunstein, M., Rose, A. B., Holmes, S. G., Alis, C. D., and Broach, J. R. (1993) Genetics 27, 592–604
5. Roberts, A. B., and Sporn, M. B. (1990) Handbook of Experimental Pharmacology, pp. 419–472, Springer-Verlag, Heidelberg, Germany
6. Massague, J., Attisano, L., and Wranja, J. (1994) Trends Cell Biol. 4, 172–178
7. Lin, H., Wang, X.-F., Ng-Eaton, R., Weinberg, R., and Lodish, H. (1992) Cell 69, 775–785
8. Wranja, J., Attisano, L., Wieser, R., Ventura, F., and Massague, J. (1994) Nature 370, 341–347
9. Park, K., Kim, S.-J., Bang, Y.-J., Park, J.-G., Kim, N. K., Roberts, A. B., and Sporn, M. B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8772–8776
10. Markowitz, S., Wang, J., Myeroff, L., Parsons, R., Sun, L. Z., Lutterbaugh, J., Fan, R. S., Zborowska, E., Kinzler, K. W., Vogelstein, B., Bratman, M. G., and Willson, J. K. V. (1995) Science 268, 1331–1338
11. Myeroff, L., Parsons, R., Kim, S.-J., Hedrick, L., Cho, K. R., Orth, K., Mathis, M., Kinzler, K. W., Lutterbaugh, J., Park, K., Bang, Y.-J., Lee, H. Y., Park, J.-G., Lynch, H. T., Roberts, A. B., Vogelstein, B., and Markowitz, S. (1995) Cancer Res. 55, 5345–5357
12. Hahm, K.-B., Cho, K., Lee, C., Im, Y.-H., Chang, J., Choi, S. G., Sorensen, P. H. H., Thiele, C. J., and Kim, S.-J. (1999) Nat. Genet. 23, 222–227
13. Sun, L., Wu, G., Willson, J. K. V., Zborowska, E., Yang, J., Rajkarunanayake, I., Wang, J., Gentry, L. E., Wang, X.-P., and Bratman, M. G. (1994) J. Biol. Chem. 269, 26449–26455
14. Kim, S.-D., Im, Y. H., Markowitz, S. D., and Bang, Y.-J. (2000) Cytokine Growth Factor Rev. 11, 159–168
15. Lee, B. Y., Park, S. H., Kim, J. W., Sauville, E. A., Kim, H. T., Nakashiki, O., Trepel, J. B., and Kim, S.-J. (2001) Cancer Res. 61, 931–934
16. Saito, A., Yamashita, T., Mariko, Y., Nosaka, V., Tsuchiya, K., Ando, T., Suzuki, T., Tsuuru, T., and Nakashiki, O. A. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4592–4597
17. Deleted in proof
18. Park, S. H., Birchecnall-Roberts, M. C., Yi, Y., Lee, B. Y., Lee, D. G., Bertelotte, D. C., Fu, T., Russettii, F., and Kim, S.-J. (2001) Cell Growth Differ 12, 9–18
19. Sasaki, S., Leson-Word, L. A., Dey, A., Kuwata, T., Weinbraun, B. D., Humphrey, G., Yang, W.-M., Seto, E., Yen, P. M., Howard, B. H., and Otsu, K. (1999) EMBO J. 18, 5389–5398
20. Bartosh, T., Truss, M., Bode, J., and Beato, M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 10741–10745
21. Van Lint, C., Emiliani, S., and Verdin, E. (1996) EMBO J. 15, 4592–4597
22. Alland, L., Muhle, R., Hou, Jr., H., Potes, J., Chin, L., Schreiber-Agus, N., and DePinho, R. A. (1997) Nature 387, 49–55
23. Hassig, C. A., Fleischer, T. C., Billin, A. N., Schreiber, S. L., and Ayer, D. E. (1997) Cell 89, 341–347
24. Jin, S., and Scotto, K. W. (1998) Mol. Cell. Biol. 18, 4377–4384
25. Huang, L., Sowa, Y., Sakai, T., and Pardee, A. B. (2000) Oncogene 19, 5712–5719
26. Maity, S. N., Sinha, S., Ruteshouser, E. C., and de Crombrugghe, B. (1992) J. Biol. Chem. 267, 16574–16580

Transcriptional Repression of the TβRII Gene
Transcriptional Repression of the TβRII Gene

27. Sinha, S., Maity, S. N., Lu, J., and de Crombrugghe, B. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1624–1628
28. Mantovani, R. (1999) Gene 239, 15–27
29. Currie, R. A. (1998) J. Biol. Chem. 273, 1430–1434
30. Li, Q., Herrler, M., Landsberger, N., Kaludov, N., Ogryzko, V. V., Nakatani, Y., and Wolfe, A. P. (1998) EMBO J. 17, 6300–6315
31. Park, S. H., Kim, Y. S., Park, B.-K., Hougaard, S., and Kim, S.-J. (2001) Oncogene 20, 1235–1245
32. Missero, C., Filvaroff, E., and Dotto, G. P. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3489–3493
33. Kim, D. H., Chang, J. H., Lee, K. W., Lee, H. Y., and Kim, S.-J. (1997) J. Biol. Chem. 272, 688–694
34. Okamoto, A., Jiang, W., Kim, S.-J., Spillare, E. A., Stoner, G., Weinstein, I. B., and Harris, C. C. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11576–11580
35. Baxevanis, A. D., Arents, G., Moudrianakis, E. N., and Landsman, B. (1995) Nucleic Acids Res. 23, 2685–2691