MCF-7E breast cancer cells express transforming growth factor-β (TGF-β) receptors RI and RII in comparison to MCF-7L cells. We present data showing that Sp3 acts as a transcriptional repressor of RI and RII in MCF-7L cells and GEO colon cancer cells. MCF-7L and GEO cells express high levels of Sp3 protein. Gel shift analysis indicated enhanced binding of Sp3 from MCF-7L cells to a consensus Sp1 oligonucleotide. Southwestern data indicated increased binding of Sp3 to RI and RII promoters in MCF-7L cells, suggesting a correlation between Sp3 binding and reduced expression of TGF-β receptors in MCF-7L cells. Cotransfection of CMV-Sp3 cDNA with RI and RII promoter-luciferase reporter constructs decreased RI and RII promoter activities by 70% in MCF-7E and GEO cells. Southwestern analysis detected the binding of transiently expressed Sp3 to RI and RII promoters in MCF-7E cells. Significantly, ectopic Sp3 expression led to repression of RI and RII transcripts in MCF-7E cells. This report demonstrates that inappropriate overexpression of Sp3 is a mechanism that contributes to repression of TGF-β receptors.

TGF-β plays a vital role in the regulation of cell proliferation, differentiation, and extracellular matrix re-modeling in various cell types (1, 2). TGF-β carries out its biological effects through three cell surface receptors, which are referred to as type I (RI), type II (RII), and type III (RIII). RI and RII are serine/threonine kinases, and an active receptor complex consists of two molecules each of RI and RII, which are essential for TGF-β signal transduction and inhibition of cell growth (3–6).

One of the crucial roles of TGF-β is the growth inhibition of normal epithelial cells as well as some cancer cells. Because RI and RII are necessary for TGF-β-mediated growth arrest, mutational inactivation of either receptor has been reported to generate TGF-β resistance and hence the loss of the tumor suppressive function of TGF-β in cancer cells (7–9). DNA methylation of the RI promoter has been reported in a subset of gastric cancer cells (10). Another mechanism for loss of TGF-β-mediated tumor suppression is transcriptional repression of RI due to decreased binding of stimulatory nuclear proteins to the RI promoter in keratinocytes and breast cancer cells (11, 12). RI and RII replacement in cells that lack, or show reduced levels of, TGF-β receptors led to restoration of TGF-β response and subsequent reversal of malignancy, as seen in breast and colon cancer cells (13, 14).

The promoters for RI and RII have been characterized (15, 16). Both RI and RII promoters lack distinct TATA boxes and are highly GC-rich and depend on Sp1 transcription factor for the initiation of transcription. The RI promoter contains four consensus and several putative Sp1 sites, whereas the RII promoter contains two Sp1 sites. The Sp gene family of transcription factors consists of four members, which are referred to as Sp1–Sp4. Whereas Sp1, Sp2, and Sp4 are known to be activators of gene transcription, Sp3 is generally considered to be a repressor (17). Sp1 and Sp3 transcription factors recognize the same DNA element and have similar binding affinities. Sp3 has been shown to repress Sp1-mediated trans-activation of several genes (18–20).

MCF-7 early passage (MCF-7E) breast cancer cells express RI and RII and are responsive to the growth inhibitory effects of TGF-β. However, MCF-7 late passage cells (MCF-7L) lack RII, show reduced levels of RI, and are TGF-β-resistant. Loss or reduced expression of TGF-β receptors was due to low Sp1 protein levels in MCF-7L cells in comparison to MCF-7E cells (21, 22). Sp1 deficiency was reversed by 5-aza-2’-deoxycytidine treatment of these cells, leading to the restoration of TGF-β receptor expression and signal transduction (12). We now show that in addition to Sp1 deficiency, MCF-7L breast cancer cells express higher levels of Sp3 than do MCF-7E cells, which express adequate amounts of both receptors and are consequently sensitive to TGF-β. Sp3 acts as a transcriptional repressor of TGF-β receptors. Furthermore, the transient expression of CMV-Sp3 cDNA into TGF-β-responsive MCF-7E cells resulted in dose-dependent down-regulation of TGF-β receptor mRNA, thus confirming transcriptional repression by Sp3. Similar results were also found for TGF-β receptor expression in a colon cancer cell line designated GEO. Taken together, the previous and present results in the MCF-7 model system indicate that Sp1/Sp3 ratios dictate expression versus repression of TGF-β receptors RI and RII.

**Experimental Procedures**

Cell Culture—MCF-7E cells were obtained from Dr. Soule (23), and MCF-7L cells were obtained from the American Type Culture Collection. Cells were grown in McCoy’s 5A medium supplemented with 10% fetal bovine serum (Sigma), amino acids, antibiotics, pyruvate, and vitamins (Life Technologies, Inc.). GEO colon cancer cells were grown in

---

*This work was supported by National Institutes of Health Grants CA 38173 and CA 72001. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Surgery, University of Texas Health Science Center, San Antonio, Texas 78229.

**From the Departments of Surgery and Biochemistry, University of Texas Health Science Center, San Antonio, Texas 78229**
Western Immunoblot Analysis of Sp3—Nuclear extracts (5 μg) were obtained from MCF-7E and MCF-7L breast cancer cells and GEO colon carcinoma cells, and Western analysis was performed as described previously (12). Rabbit anti-human Sp1, Sp3, and c-Jun polyclonal antibodies were purchased from Santa Cruz Biotechnology.

Southwestern Blotting—Southwestern analysis was performed as described previously (20). Briefly, nuclear extracts were resolved by 7.5% SDS-polyacrylamide gel electrophoresis and electrophoretically transferred to a nitrocellulose membrane. Following transfer, the membrane was blocked overnight with 2.5% (w/v) nonfat dried milk in 25 mM HEPES, pH 8.0, 1 mM dithiothreitol, 10% (v/v) glycerol, 50 mM NaCl, and 1 mM EDTA. The membrane was then incubated with [α-32P]dCTP-labeled RI (618 bp to the start site) and RII (274 bp to the start site) promoter probes and poly(dI-dC) as a nonspecific competitor for 4 h. Later, the membrane was washed with wash buffer (10 mM Tris, pH 7.5, 50 mM NaCl, 1 mM EDTA, and 1 mM dithiothreitol), dried, and autoradiographed.

Transfections and Luciferase Assay—The RI and RII promoter-luciferase reporter constructs (RI-Luc and RII-Luc, respectively) were used to determine RI and RII promoter activities, respectively (15, 24). The Sp1-dependent thymidine kinase promoter-Luc (TK-Luc) and non-Sp1-dependent NFκB-Luc constructs were obtained from Promega and Stratagene, respectively. The CMV-Sp3 cDNA was described previously (18). The CMV-Sp3 cDNA vector or CMV control vector without Sp3 cDNA, along with the constructs mentioned above and a β-galactosidase plasmid for normalization (12), was transiently transfected into MCF-7E breast cancer cells and GEO colon cancer cells using the Fugene 6 method (Roche Molecular Biochemicals). The CMV-Sp1 cDNA vector or CMV control vector without Sp1 cDNA, along with RI-Luc, RII-Luc, NFκB-Luc, and a β-galactosidase plasmid, was transiently transfected into MCF-7L cells. Cells were harvested at 60 h following transfection, and promoter activities were determined using a commercial luciferase assay (Luciferase Assay System, Promega). To determine RI and RII mRNA expression, transfected MCF-7E cells were harvested at 60 h following transfection, and RNA was isolated to perform reverse transcriptase-PCR analysis using RI and RII primers as described below. Total RNA from CMV-control vector- or CMV-Sp3 cDNA vector-transfected MCF-7E cells was reverse transcribed into cDNA. PCR analysis was then performed to determine the RI and RII expression levels in control and CMV-Sp3 cDNA transfected-MCF-7E cells using the respective cDNAs as templates. It was previously reported that Sp3 could not affect the activities of Sp1-dependent thymidine kinase promoter (19). Thus, primers for TK were used to demonstrate the specificity of Sp3 effects on RI and RII expression. Primers for TK were used as an internal control to normalize the expression levels. A total of 30 cycles of amplification were performed. Primers for RI generate an 865-bp fragment (sense primers, TTG TGG CAC GGT GAG AGT GT; antisense primers, GCC AAC AAC ATC AAC CAC AAC ACA; antisense primers, TAG TGT TTA GGG AGC GGT CTT CAG). Primers for RII generate a 1003-bp fragment (sense primers, GCC AAC AAC ATC AAC CAC AAC ACA; antisense primers, TAG TGT TTA GGG AGC GGT CTT CAG). Primers for Sp1 generate a 340-bp fragment (sense primers, GCC AAC AAC ATC AAC CAC AAC ACA; antisense primers, TAG TGT TTA GGG AGC GGT CTT CAG). Primers for Sp3 generate a 754-bp fragment (sense primers, GCC AAC AAC ATC AAC CAC AAC ACA; antisense primers, TAG TGT TTA GGG AGC GGT CTT CAG).

EMSA—EMSA was performed using 32P-labeled consensus Sp1 oligonucleotide and nuclear extracts from MCF-7E and MCF-7L cells. Wherever Sp3 antibody was used, the nuclear extracts were pre-incubated with 2 μg of Sp3 antibody prior to the addition of 32P-labeled oligonucleotide.

Detection of Sp3 binding to RI and RII promoters. Southwestern analysis was performed by resolving MCF-7E and MCF-7L nuclear extracts using 7.5% SDS-polyacrylamide gel electrophoresis and probing the nitrocellulose membrane following protein transfer with radiolabeled RI (a) and RII (b) promoter probes.

RESULTS

Expression of Sp3 Protein—To determine Sp3 protein expression levels, Western immunoblot was performed using 5 μg each of nuclear extracts from MCF-7E, MCF-7L, and GEO cells. Western analysis showed three protein species of 115, 70, and 68 kDa (Fig. 1). The 68–70-kDa species was the result of differential internal translation initiation (25). Only the 115-kDa species has been reported to be biologically active. MCF-7L and GEO cells showed significantly higher levels of all the three Sp3 isoforms in comparison to MCF-7E cells, whereas there were no differences in the c-Jun levels, indicating selectivity of Sp3 modulation.

EMSA—EMSA were performed using MCF-7E and MCF-7L nuclear extracts and 32P-labeled consensus Sp1 oligonucleotide to determine the DNA binding activities of Sp3 (Fig. 2). Enhanced binding of a high mobility complex was observed...
in MCF-7L nuclear extracts in comparison to MCF-7E extracts (Fig. 2, lanes 2 and 4). To identify whether the high mobility complex contains Sp3, the nuclear extracts were pre-incubated with 2 μg of Sp3 antibody to delete Sp3 protein from the extracts prior to the addition of 32P-labeled oligonucleotide. The high mobility complex was eliminated by the Sp3 antibody in MCF-7L extracts, confirming that the protein-DNA complex contains Sp3 (Fig. 2, lane 5). This Sp3 antibody has previously been reported to cause similar depletion of the protein-DNA complexes following incubation with endothelial cell nuclear extracts prior to the addition of 32P-labeled kinase domain receptor promoter of the vascular endothelial growth factor (26).

**Transcriptional Repression of RI and RII by Sp3—** Southwestern analysis using nuclear extracts from MCF-7E and MCF-7L cells and radiolabeled RI and RII promoters was carried out to demonstrate Sp3 binding in MCF-7L cells. Higher Sp3 binding to RI and RII promoters was observed in nuclear extracts from MCF-7L cells, suggesting the plausible role of Sp3 as a transcriptional repressor of TGF-β receptors (Fig. 3, a and b).

**Effect of Exogenous Sp1 on RI and RII Promoter Activities—** MCF-7L cells, which lack, or show reduced levels of, TGF-β receptors, express high levels of Sp3 and low levels of Sp1 protein. To determine whether the modulation of Sp1/Sp3 protein levels leads to induction of RI and RII promoter activities, we transfected varying amounts (0–8 μg) of CMV-Sp1 cDNA into MCF-7L cells. Western analysis showed increasing amounts of the Sp1 in MCF-7L cells (Fig. 4a). c-Jun was used as a loading control. Cotransfection of RI and RII promoter-luciferase reporter constructs with the varying amounts of CMV-Sp1 cDNA resulted in a dose-dependent increase in the RI and RII promoter activities, thus suggesting that Sp1/Sp3 ratios control RI and RII promoter activities (Fig. 4b). Sp1 expression enhanced Sp1-dependent TK promoter activity but not the non-Sp1-dependent NFκB-Luc control construct, thus confirming the specificity of Sp1 effects on the Sp1-dependent RI, RII, and TK promoters.

**Effect of Exogenous Sp3 on RI and RII Promoter Activities—** MCF-7E cells, which express TGF-β receptors, contain high levels of Sp1 and low levels of Sp3. Consequently, we transfected varying amounts (0–8 μg) of CMV-Sp3 cDNA into MCF-7E cells to modulate the Sp1/Sp3 protein levels and evaluated the effects on RI and RII promoter activities. Western analysis showed increasing levels of Sp3 in MCF-7E cells (Fig. 4c). c-Jun was used as a loading control. Cotransfection of RI and RII promoter-luciferase reporter constructs with varying amounts of CMV-Sp3 cDNA showed a dose-dependent decrease in the RI and RII promoter activities, thus confirming that Sp1/Sp3 ratios control RI and RII promoter activities (Fig. 4d). The transfection efficiencies at 0, 2, 4, and 8 μg of CMV-Sp3 cDNA were 100, 86.4, 84.6, and 85.2%, respectively, for the RI-Luc construct and 100, 80.3, 81.1, and 82.4%, respectively, for the RII-Luc construct. Sp3 expression did not affect the activity of Sp1-dependent TK-Luc and the non-Sp1-dependent NFκB-Luc control construct, thus confirming the specificity of Sp3 on the non-Sp1-dependent RI, RII, and TK promoters.

**Fig. 4.** Effects of ectopic Sp1 and Sp3 on RI and RII promoters. A, Western analysis using rabbit anti-human Sp1 antibody was performed on the nuclear extracts of CMV-Sp1 cDNA-transfected MCF-7L cells. C-Jun was used as a loading control. B, MCF-7L cells were transiently cotransfected with CMV-Sp1 cDNA along with RI-Luc, RII-Luc, TK-Luc promoter-reporter, RII-Luc promoter-reporter, and control NFκB-Luc constructs as described under "Experimental Procedures." Cells were harvested at 60 h following transfection, and luciferase activity was determined and presented as relative units. C, Western analysis using rabbit anti-human Sp3 antibody was performed on the nuclear extracts of CMV-Sp3 cDNA-transfected MCF-7E cells. C-Jun was used as a loading control. D, MCF-7E cells were transiently cotransfected with CMV-Sp3 cDNA along with RII-Luc promoter-reporter, RII-Luc promoter-reporter, and control NFκB-Luc constructs, and E, GEO cells were transiently cotransfected with CMV-Sp3 cDNA along with RII-Luc promoter-reporter, RI-Luc promoter-reporter, and control NFκB-Luc constructs as described under "Experimental Procedures." Cells were harvested at 60 h following transfection, and luciferase activity was determined and presented as relative units. RLU, relative luciferase units.
Sp3 effects on RI and RII promoter activities. Although GEO cells express significant levels of Sp3 (Fig. 1), low levels of RI and RII can still be detected in these cells. Consequently, we determined the effect of ectopic Sp3 expression on the RI and RII promoter activities in GEO cells as well. Sp3 expression down-regulated RI and RII promoter activities, indicating that Sp3 acts as a transcriptional repressor of TGF-β receptors in GEO colon cancer cells as well (Fig. 4e).

DNA Binding Activities of Transiently Expressed Sp3—To determine whether transiently expressed Sp3 was binding to RI and RII promoters, Southwestern analysis was performed using radiolabeled RI and RII promoter fragments exposed to nuclear extracts from CMV-Sp3-transfected MCF-7E cells as well as CMV-control vector-transfected MCF-7E cells. Transiently expressed Sp3 bound to RI promoter as well as RII promoter (Fig. 5, a and b).

Effect of Exogenous Sp3 on RI and RII Expression—To evaluate whether transiently expressed Sp3 binding to RI and RII promoters in MCF-7E cells results in down-regulation of RI and RII transcripts, reverse transcriptase-PCR analysis using RI and RII primers was performed on total RNA from varying amounts (0–8 μg) of CMV-Sp3 cDNA-transfected MCF-7E cells as well as CMV-control vector-transfected MCF-7E cells. The transfection efficiencies at 0, 2, 4, and 8 μg of CMV-Sp3 cDNA were 100, 80.1, 78.9, and 79.4%, respectively. Sp3 dose-dependent repression of RI and RII expression levels was observed, thus confirming the role of Sp3 as a transcriptional repressor of TGF-β receptors RI and RII. However, Sp3 transfection did not affect thymidine kinase mRNA expression. Because the thymidine kinase promoter contains an Sp1 site important for transcription of the gene, this result indicated that Sp3 did not affect this site, thus reflecting the selective repression of TGF-β receptors by Sp3 (Fig. 6).

DISCUSSION

Loss or reduced expression of TGF-β receptors has been implicated in TGF-β resistance leading to tumor formation and progression (8, 9, 13, 14, 27, 28). Whereas MCF-7E breast cancer cells express TGF-β receptor RI as well as RII and are responsive to growth inhibition by TGF-β, MCF-7L cells lack RII and show reduced levels of RI. MCF-7L cells are TGF-β-resistant. In addition, MCF-7L cells were found to be tumorigenic, and RII replacement reduced malignancy in athymic nude mice (13). The RI and RII promoters lack distinct TATA boxes but do contain multiple GC elements and depend on Sp1 for the initiation of transcription (15, 16). Loss or reduced expression of TGF-β receptors in MCF-7L cells in comparison to MCF-7E cells was related to low expression levels of Sp1 transcription factor (22). However, in this report we present data suggesting that another member of the Sp gene family, Sp3, which has GC element binding affinities similar to those of Sp1, acts as a transcriptional repressor of TGF-β receptors RI and RII.

Sp gene family members Sp1, Sp2, and Sp4 are generally known as activators of gene transcription, whereas Sp3 is considered to be a repressor of gene transcription (17). MCF-7L cells express higher levels of Sp3 in comparison to MCF-7E cells (Fig. 1). The 115-kDa Sp3 protein is the biologically active form, and the inactive 68–70-kDa species present in Western blots arise as a result of differential internal translocation initiation (25). However, as opposed to direct promoter repression, the Sp3-derived 68–70-kDa species can bind GC elements and thus act as inhibitors of Sp1-mediated gene activation. Electrophoretic mobility shift assays revealed the DNA-binding activities of Sp3 from MCF-7L nuclear extracts (Fig. 2). Southwestern analysis confirmed the binding of the 115-kDa nuclear Sp3 protein to the RI promoter as well as the RII promoter in MCF-7L cells (Fig. 3, a and b), which lack RII expression and show reduced levels of RI. Significantly, nuclear Sp3 binding to the RII promoter in MCF-7E cells was not observed, and only a trace of Sp3 bound to the RI promoter was found (Fig. 3, a and b). Taken together, the Southwestern data from MCF-7E and MCF-7L nuclear extracts indicate a possible role for Sp3 in transcriptional repression of TGF-β receptors RI and RII in MCF-7L cells.

The Sp1 dose-dependent up-regulation of RI and RII promoter-luciferase reporter activities after cotransfection with varying amounts of CMV-Sp1 cDNA into Sp1-deficient MCF-7L cells suggested that Sp1/Sp3 protein levels/activities control RI and RII promoter activities (Fig. 4b). This was confirmed by the Sp3 dose-dependent down-regulation of RI and RII promoter-luciferase reporter activities after cotransfection with varying amounts of CMV-Sp3 cDNA into Sp3-deficient MCF-7E cells (Fig. 4d). Southwestern analysis confirmed that the transiently expressed Sp3 bound to the RI and RII promoters in MCF-7E cells (Fig. 5, a and b). Most significantly, Sp3 binding to the RI and RII promoters in CMV-Sp3 cDNA-transfected MCF-7E cells resulted in repression of RI and RII mRNA expression, as indicated by the reverse transcriptase-PCR analyses (Fig. 6). However, thymidine kinase, which was used as a control, was not affected. It was previously reported that Sp3 could repress the activity of multiple Sp1 sites contained in the dihydrofolate reductase promoter but not the single Sp1 site contained in the thymidine kinase promoter (19). The RI promoter contains four consensus Sp1 sites, whereas the RII promoter contains two (15, 16). Ornithine decarboxylase promoter activity was also repressed by Sp3 (20). However, Sp3 was able to trans-activate c-fos and c-myc promoters (29). Hence, the availability of specific coactivators, corepressors, or other transcription factors may dictate whether Sp3 activates or inhibits transcription of
a specific gene. Moreover Sp3 effects may also depend on the context as well as the number of Sp1 binding sites.

It has been reported previously that MCF-7E cells express high levels of Sp1 in comparison to MCF-7L cells (21, 22). Our present data indicate that MCF-7E cells express low levels of Sp3 in comparison to MCF-7L cells (Fig. 1). Consequently, the high Sp1/Sp3 ratios in MCF-7E cells appear to support RI and RII expression, whereas low Sp1/Sp3 ratios contribute to receptor repression in MCF-7L cells. This hypothesis was confirmed by the ectopically driven increase of Sp1/Sp3 ratios in MCF-7 cells by CMV-Sp1 cDNA expression in MCF-7L cells, and the decrease of Sp1/Sp3 ratios in MCF-7E cells by Sp3 cDNA expression, which resulted in repression of RI and RII expression (Fig. 6). The correlation between Sp1/Sp3 ratios and expression of RI and RII was also observed in GEO human colon carcinoma cells. Thus, transcriptional control of TGF-β receptor repression is dependent upon the ratio of Sp1/Sp3, and cancer cells can gain a growth advantage by favoring receptor repression through a combination of reduced Sp1 and elevated Sp3 expression.

Acknowledgments—We thank Drs. Guntram Suske, Richard Goldstein, and Seong-jin Kim for kindly providing CMV-Sp3 cDNA, RI-Luc, and RII-Luc, respectively.

REFERENCES

1. Massague, J. (1990) Annu. Rev. Cell Biol. 6, 597–641
2. Roberts, A. B., and Sporn, M. B. (1991) in Peptide Growth Factors and Their Receptors (Sporn, M. B., and Roberts, A. B., eds) pp. 419–472, Springer-Verlag, Heidelberg, Germany
3. Lin, H. Y., Wang, X.-F., Ng-Eaton, E., Weinberg, R. A., and Lodish, H. F. (1992) Cell 68, 775–785
4. Wrana, J. L., Attisano, L., Carcamo, J., Zentella, A., Doody, J., Laiho, M., Wang, X.-F., and Massague, J. (1992) Cell 71, 1003–1014
5. Franzen, P., ten Dijke, P., Ichijo, H., Yamashita, H., Schulz, P., Helden, C.-H., and Miyazono, K. (1993) Cell 75, 681–692
6. Wrana, J. L., Attisano, L., Weiser, R., Ventura, F., and Massague, J. (1994) Nature 370, 341–347
7. Markowitz, S., Wang, J., Myeroff, L., Parsons, R., Sun, L.-Z., Lutterbaugh, J., Fan, R. S., Zborowska, E., Kinzler, K. W., Vogelstein, B., Brattain, M. G., and Willson, J. K. V. (1995) Science 268, 1336–1338
8. Wang, J., Sun, L., Myeroff, L., Wang, X.-F., Gentry, L. E., Yang, J., Liang, J., Zborowska, E., Markowitz, S., Willson, J. K. V., and Brattain, M. G. (1995) J. Biol. Chem. 270, 22044–22049
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. Kang, S. H., Bang, Y.-J., Im, Y.-H., Yang, H.-K., Lee, D. A., Lee, Y. H., Lee, H. S., Kim, N. K., and Kim, S.-J. (1999) Oncogene 18, 7280–7286
11. Kim, D. H., Chang, J. H., Lee, K. H., Lee, H. Y., and Kim, S.-J. (1997) J. Biol. Chem. 272, 688–694
12. Ammanamachi, S., Kim, S.-J., Sun, L.-Z., and Brattain, M. G. (1998) J. Biol. Chem. 273, 16527–16534
13. Sun, L.-Z., Wu, G., Willson, J. K. V., Zborowska, E., Yang, J., Rajakarunanayake, I., Wang, J., Gentry, L. E., Wang, X.-F., and Brattain, M. G. (1994) J. Biol. Chem. 269, 29449–29455
14. Wang, J., Han, W., Zborowska, E., Liang, J., Wang, X.-F., Willson, J. K. V., Sun, L.-Z., and Brattain, M. G. (1996) J. Biol. Chem. 271, 17366–17371
15. Bloos, B. B., Humphries, D. E., Kuang, P. F., Fine, A., and Goldstein, R. H. (1996) Biochem. Biophys. Acta 1312, 243–248
16. Bae, H. W., Geiser, A. G., Kim, D. H., Chung, M. T., Burmester, J. K., Sporn, M. B., and Kim, S.-J. (1995) J. Biol. Chem. 270, 29460–29468
17. Suske, G. (1999) Gene 238, 291–300
18. Hagen, G., Muller, S., Beato, M., and Suske, G. (1994) EMBO J. 13, 3843–3851
19. Birbauma, M. J., van Wijnen, A. J., Odgren, P. R., Last, T. J., Suske, G., Stein, G. S., and Stein, J. L. (1995) Biochemistry 34, 16560–16568
20. Kumar, A. P., and Butler, A. P. (1997) Nucleic Acids Res. 25, 2012–2019
21. Ko, Y., Banerji, S. S., Liu, Y., Li, W., Liang, J., Soule, H. D., Pauley, R. J., Willson, J. K. V., Zborowska, E., and Brattain, M. G. (1998) J. Cell Physiol. 176, 424–434
22. Liu, Y., Li, W., Zhong, X., Brattain, M. G., and Banerji, S. S. (2000) J. Biol. Chem. 275, 12231–12236
23. Soule, H. D., Vazquez, J., Long, A., Albert, S., and Brennan, M. (1973) J. Natl. Cancer Inst. 51, 1409–1416
24. Choi, S.-G., Ki, Y., Kim, Y.-S., Kato, M., Chang, J., Chung, H.-W., Hahn, K. B., Yang, H.-K., Rhee, H. H., Bang, Y.-J., and Kim, S.-J. (1998) J. Biol. Chem. 273, 110–117
25. Kennet, S. B., Udvardia, A. J., and Horowitz, J. M. (1997) Nucleic Acids Res. 25, 3110–3117
26. Hata, Y., Duh, E., Zhang, K., Rohinson, G. S., and Aiello, L. P. (1998) J. Biol. Chem. 273, 19294–19303
27. Inagaki, M., Moustakas, A., Lin, Y. H., Lodish, H. F., and Carr, B. I. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5359–5363
28. Kalkhoven, E., Roelen, B. A. J., de Winter, J. P., Mummery, C. L., Van den Eijnden-Van Raitj, A. J. M., Van der Saag, P. T., and Van der Burg, B. (1996) Cell Growth Diff. 7, 1311–1317
29. Udvardia, A.-J., Templeton, D. J., and Horowitz, J. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3953–3957
