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Advances in Brief

Expression of Transforming Growth Factor β Type II Receptor Reduces Tumorigenicity in Human Gastric Cancer Cells[1]

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Abstract

Expression of transforming growth factor β (TGF-β) receptor type II (RII) is required for the growth-inhibitory effects of TGF-β on proliferating epithelial cells. TGF-β RII mutations have been identified in a broad spectrum of human epithelial malignancies, including colon and gastric cancers, and are highly correlated with development of TGF-β resistance in cell lines derived from these tumors. In this study, the role of TGF-β RII in regulating the tumorigenic potential of the SNU-638 human gastric cancer cell line was investigated by infecting these cells with retroviral construct (MFG) expressing TGF-β RII. The SNU-638 cell line displays the DNA replication error phenotype and encodes a truncated, inactive TGF-β RII protein. Infection of these cells with retroviral constructs expressing wild-type TGF-β RII led to significant increases in TGF-β RII mRNA and protein expression. These cells responded to exogenous TGF-β with reduced proliferation compared to that of control cells infected with retroviral vector expressing chloramphenicol acetyltranferase. Addition of TGF-β-neutralizing antibodies led to increased proliferation of wild-type TGF-β RII-expressing SNU-638 cells but had no effect on control cells. The latter finding suggests that TGF-β acts in an autocrine fashion to inhibit cell proliferation in SNU-638 cells. When transplanted into athymic nude mice, wild-type TGF-β RII-expressing SNU-638 cells showed decreased and delayed tumorigenicity compared with control cells. This study suggests a strong association between the expression of wild-type TGF-β RII and the degree of malignancy in human gastric cancer cells.

Introduction

The molecular events of gastric carcinogenesis constitute a multistep pathway that, analogous to the well-described model for colon cancer (1), involves the sequential mutation of various oncogenes and tumor suppressor genes. Mutation of the *p53* gene, commonly observed in many different human malignancies, is considered a relatively late step in carcinogenesis and has been identified in four of six human gastric cancer cell lines previously studied by our group (2). More recently, the prevalence of TGF-β[4] RII mutations in several epithelial-type human malignancies has been reported. These tumor cells, which include colon, stomach, head and neck, and endometrial cancers, demonstrate decreased sensitivity to the growth-inhibitory effects of TGF-β. We have examined one human gastric cancer cell line, SNU-16, that possesses a p53 mutation but expresses wild-type TGF-β RII protein and is appropriately growth-inhibited by TGF-β. These observation suggest that TGF-β RII mutation may occur at a later point in the carcinogenesis pathway than does p53 mutation.

Numerous other mutations have been associated with gastric carcinoma, including point mutation of the *ras* oncogene and amplification of c-*met*, K-*sam*, and c-*erbB-2/neu* as well as loss of heterozygosity at the *bcl-2*, *APC*, and *DCC* gene loci. However, the TGF-β RII mutation seems to occur more frequently than any other single mutation previously associated with human gastric carcinomas. TGF-β RII mutation and the resulting escape from TGF-β-mediated growth inhibition may therefore represent a threshold-achieving event determining the rate of progression of a gastric tumor toward malignancy.

TGF-β plays an important role in many critical cellular processes, including regulation of the cell cycle, differentiation, and extracellular matrix synthesis (3, 4). One of its most prominent activities is inhibition of cell proliferation. Biological activity of TGF-β is mediated through binding to a heteromeric receptor complex comprised of both RI and RII proteins (5–8). Both RI and RII present transmembrane serine/threonine kinases (5, 7). The accepted mechanism of action begins with the binding of TGF-β ligand to RII, which then allows cooperative binding to RI.

Transphosphorylation of RI by RII then allows the signaling pathway to proceed. Because of the prominent role played by TGF-β in the regulation of cell growth and differentiation, it has been predicted that defects in the TGF-β receptor system would be identified as a principal cause of TGF-β resistance. This prediction has been borne out by the discovery of absent or deficient TGF-β receptor expression in a large number of malignant cell lines resistant to TGF-β (2, 9–11). For example, most tumors derived from epithelial tissue, as well as osteosarcoma and lymphomas, express markedly reduced levels of TGF-β receptor proteins and demonstrate resistance to TGF-β-mediated growth inhibition (2, 9–12).

The discovery that hereditary nonpolyposis colon cancer cells possess defects in DNA RER leading to high rates of TGF-β RII mutation represented a key breakthrough in the understanding of how TGF-β RII mutation occurs (9, 13). A 10-nucleotide polyadenine repeat sequence adjacent to the transmembrane domain of the gene renders it vulnerable to the acquisition of frameshift mutations in cells with defective DNA repair and the phenotype of microsatellite instability (14). Transfecting human colon cancers as well as hepatoma cells lacking TGF-β RII with wild-type TGF-β RII restores sensitivity to TGF-β and decreases tumorigenicity (15, 16).

TGF-β RI is a less common target for mutation in human malignancies. LNCap human prostate cancer cells demonstrate genetic alterations of TGF-β RI, resulting in no detectable expression of TGF-β RI protein and insensitivity to TGF-β (17). Transient transfection of these cells with RI cDNA restored TGF-β sensitivity.

The identification of high-frequency mutations in TGF-β RII leading to unrestrained clonal growth in human gastric cancers suggests that TGF-β RII may be an important tumor suppressor gene. This study describes the infection of retroviral vector expressing wild-type

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[4] The abbreviations used are: TGF-β, transforming growth factor β; RI, receptor type I; RII, receptor type II; CAT, chloramphenicol acetyltransferase; RER, replication error repair.

TGF-β RII into SNU-638 human gastric cancer cells possessing disabling mutations in TGF-β RII and unresponsive to the growth-inhibitory effects of TGF-β. It is demonstrated that this transfection restores TGF-β sensitivity as well as reduces tumorigenicity in transplanted cells. These results strongly indicate that the degree of malignancy of human gastric cancer is directly associated with loss of TGF-β RII expression.

Materials and Methods

Cell Culture. The SNU-638 human gastric cancer cell line was maintained as described previously (2, 18) and generously supplied by Dr. Jae-Gahb Park (Cancer Research Center, Seoul National University College of Medicine). Cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. To study autocrine growth inhibition, cells were plated in 24-well dishes at a density of 5×10^4 cells/well in 0.5 ml of assay medium (RPMI 1640 and 0.2% fetal bovine serum). TGF-β1 monoclonal antibody (Genzyme) or control mouse IgG was added to the medium at a final concentration of 15 μg/ml to determine the autocrine TGF-β activity. After a 24-h incubation, cells were pulse-labeled with 0.5 μCi of [^{3}H]thymidine for 2 h at 37°C.

Generation of RII Stable Cell Line. The coding region of the TGF-β RII (5) and CAT fragments was PCR amplified, restriction-digested, and purified to be subcloned into the MFG vector (19, 20). An IRES-NEO cassette was also subcloned into the constructs to obtain the stable transfectants.

Northern Blot Analysis. Total RNA was isolated with guanidinium isothiocyanate-phenol chloroform. Total RNA (10 μg) was electrophoresed on a 1.0% agarose gel containing 0.66 M formaldehyde, transferred to a Duralon-UV membrane, and cross-linked with UV Stratalinker (Stratagene). Blots were hybridized with cDNA probes for neomycin and TGF-β RII cDNA (5).

Receptor Cross-Linking. Cells were plated at a density of 1×10^6 cells/well in 6-well dishes. Cells were washed twice with cold binding buffer containing 1× DMEM, 25 mM HEPES (pH 7.4), and 1 mg/ml BSA fraction V. Binding was carried out with 100 pM ^{125}I-labeled TGF-β in the presence and absence of 100-fold molar excess of unlabeled TGF-β, and cells were incubated on a rotating platform at 4°C for 2.5 h. Cells were washed twice with cold wash buffer containing 1× DMEM and 25 mM HEPES (pH 7.4). One ml of 300 μM disuccinimidyl suberate was added to cross-link associated proteins, and cells were incubated for 15 min at 4°C. Cells were washed twice with cold wash buffer containing 250 mM sucrose, 10 mM Tris (pH 7.4), and 1 mM EDTA. Cellular protein was solubilized with buffer containing 1% Triton X-100, 10 mM Tris (pH 7.4), 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, and 1 μg/ml each of pepstatin and leupeptin while incubating on a rotating platform at 4°C. Lysate was centrifuged, and the supernatant was electrophoresed on a 4–10% linear SDS polyacrylamide gel (Novex).

Transient Transfection and Luciferase Assay. Cells were seeded in 6-well plates at 2×10^5 cells/well and transiently transfected with p3TP-Lux using LipofectAMINE (Life Technologies, Inc.). After 12 h, complete media were added, and cells were incubated for an additional 24 h. Cells were treated with 5 ng/ml TGF-β1 for an additional 24 h. Luciferase activity was determined in the cell lysate using an assay kit (Analytic Luminescence Lab), and a Dynatech Laboratories ML3000 luminometer. Activities were normalized on the basis of β-galactosidase expression from pSVβ-galactosidase in all luciferase reporter experiments. All experiments were repeated at least three times, and similar results were obtained each time.

Tumorigenicity Study. Cells (5×10^6) from exponential cultures of SNU-638 cells expressing wild-type TGF-β RII and CAT were inoculated s.c. into nude mice (Samsung Medical Center Research Institute, Seoul, Korea). Mice were maintained in a pathogen-free environment. Tumor growth rate was determined by measuring the xenografts in three dimensions. Volume (V) was determined using the equation $V = (L \times W^2) \times 0.5$, in which V = volume, L = length, and W = width.

Results and Discussion

SNU-638 cells, shown previously to express only a truncated, inactive form of TGF-β RII and to be insensitive to TGF-β, were infected with MFG-TGF-β RII or MFG-CAT. Both MFG-TGF-β RII (-neo) and MFG-CAT (-neo) SNU-638 clones were pooled after retroviral delivery of expression constructs using the replication-defective MFG vector. Northern analysis revealed that wild-type TGF-β RII-expressing SNU-638 cells consistently express high levels of TGF-β RII mRNA compared with both parental SNU-638 and control CAT (-neo)-transfected cells (Fig. 1*A*). The high expression of neo fusion mRNA in both CAT and TGF-β RII clones confirmed successful transfection of these clones. Receptor cross-linking assays further demonstrated that TGF-β RII clones express significantly higher TGF-β RII protein levels compared with those of the control (Fig. 1*B*). It seems that restoration of TGF-β RII expression also increases cell surface expression of TGF-β RI, which supports the

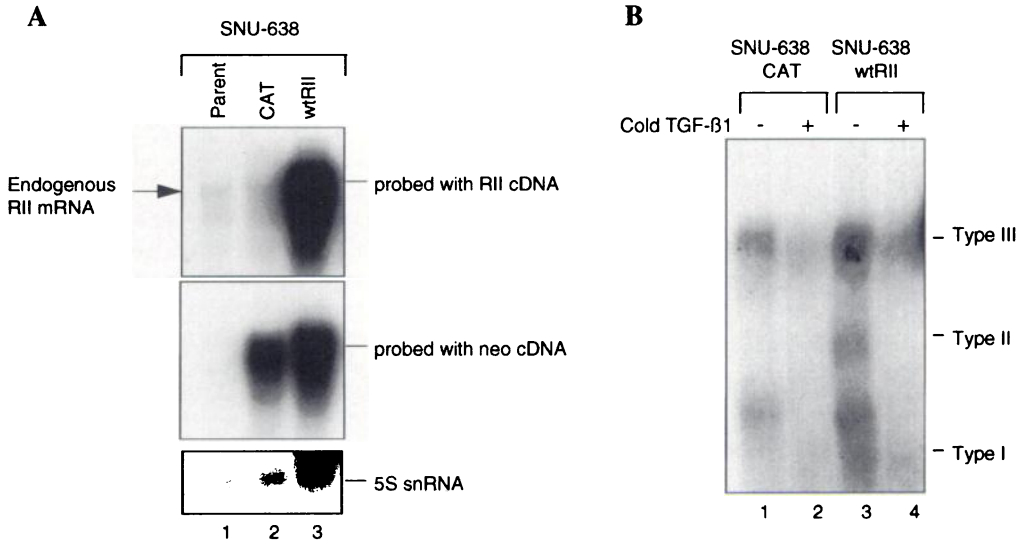

Fig. 1. Expression of TGF-β RII mRNA and protein in SNU-638 cells infected with MFG-TGF-β RII. *A*, Northern blot analysis of TGF-β RII mRNA in the MFG-TGF-β RII and MFG-CAT-SNU-638 cell lines. Total RNA was isolated from the MFG-TGF-β RII and MFG-CAT-SNU-638 human gastric cancer cell lines and analyzed by Northern analysis using ^{32}P-labeled TGF-β RII and neomycin probes. A radiolabeled probe for 5SRNA was used to control for loading. *B*, receptor protein cross-linking assay using iodinated TGF-β1. Receptor-ligand binding was performed with 100 pM ^{125}I-labeled TGF-β1 in the presence (*Lanes 2* and *4*) or absence (*Lanes 1* and *3*) of 100-fold molar excess of unlabeled TGF-β1. Bound proteins were cross-linked using 300 μM disuccinimidyl suberate, solubilized, and separated with gel electrophoresis. Bands representing unknown species are seen in both control and RII-infected cells. Assays were repeated three times, and a representative gel is shown. Levels of TGF-β RII were significantly increased in the TGF-β RII-transfected cell line compared with those of the control CAT-transfected cell line.

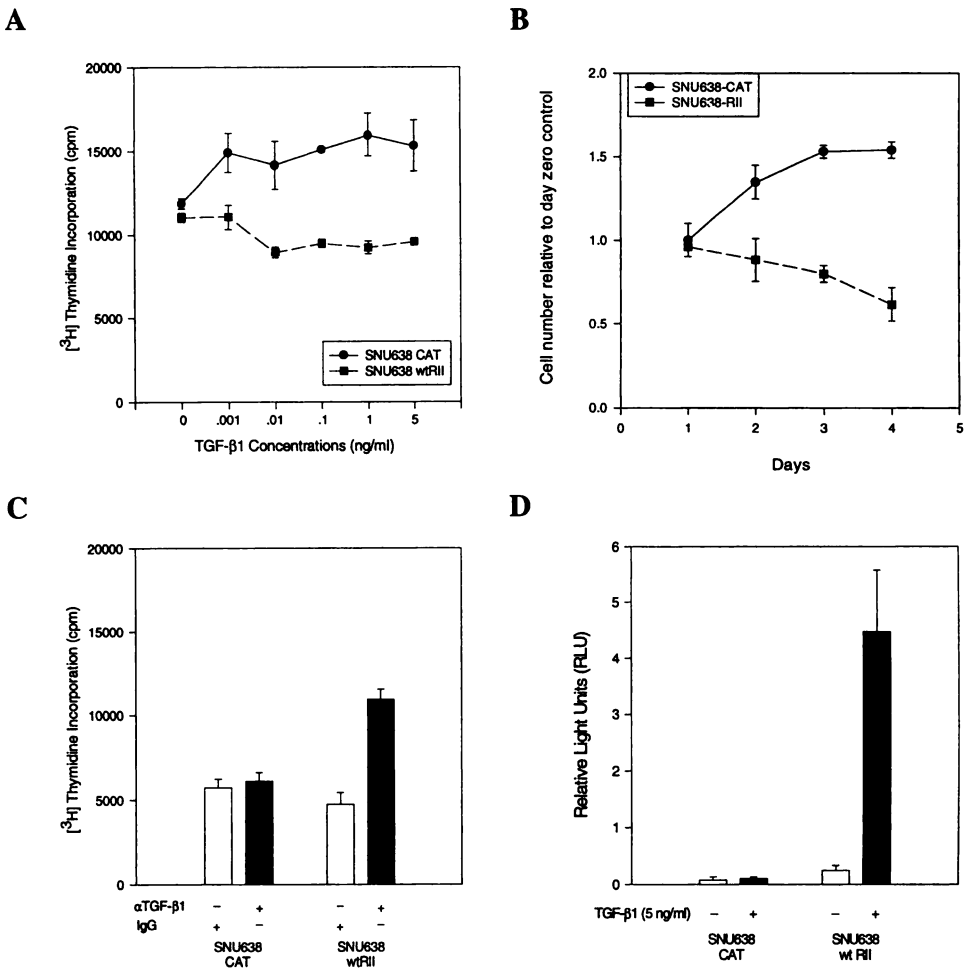

Fig. 2. TGF-β1 sensitivity in wild-type TGF-β RII-expressing SNU-638 cells. *A*, wild-type TGF-β RII-expressing SNU-638 cells and CAT-expressing SNU-638 cells were treated with increasing doses of TGF-β1 (0–5 ng/ml), and [^{3}H]thymidine incorporation was examined. *B*, changes in cell number in DMEM + 10% fetal bovine serum were examined as a function of time with 10 ng/ml TGF-β1 in SNU-638-CAT cells (●) or SNU-638-RII cells (■). *C*, autocrine TGF-β activity in wild-type TGF-β RII-expressing SNU-638 cells. SNU-638 cell lines expressing either CAT or wild-type TGF-β RII were plated in 24-well plates at 0.5×10^5 cells/well in the presence of 30 μg/ml normal IgG or 30 μg/ml monoclonal antibody against TGF-β1, and [^{3}H]thymidine incorporation was examined. *D*, effect of TGF-β1 on transcriptional activation of p3TP-Lux. MFG-TGF-β RII and MFG-CAT SNU-638 cells were seeded at 0.2×10^6 cells/well in 6-well plates and transfected transiently with p3TP-Lux. After transfection, cells were treated with 5 ng/ml TGF-β1 for 24 h. Luciferase activity was measured and expressed in relative light units.

theory that TGF-β RII recruits TGF-β RI in ligand-receptor-induced signaling (6).

To investigate whether expression of TGF-β RII could restore TGF-β sensitivity in SNU-638 cells, we compared growth curves for both the TGF-β RII-transfected and control CAT-transfected clones. Both clones were treated with increasing concentrations of TGF-β1, and proliferation was assessed using a [^{3}H]thymidine incorporation assay. This experiment revealed that the proliferation rates of the control cells (MFG-CAT-SNU-638) actually increase in response to exogenous TGF-β, whereas the addition of TGF-β1 inhibits the proliferation of cells infected with MFG-TGF-β RII (Fig. 2*A*). Next, growth rates of SNU-638-CAT and SNU-638-RII were investigated. Fig. 2*B* shows that the growth rate of SNU-638-RII cells was significantly lower than that of the control cells. After 4 days of culture, the cell count of SNU-638-RII cells was about 40% of that of the control cells.

Another observation was that basal proliferation rates for TGF-β RII-transfected cells were consistently lower than those for the control CAT-transfected cells. Consequently, another [^{3}H]thymidine incorporation assay was performed comparing proliferation of TGF-β RII-transfected and control cells in the presence of TGF-β1-neutralizing monoclonal antibody (Fig. 2*C*). The presence of antibody had no effect on proliferation rates of the MFG-CAT-infected cells, whereas the addition of anti-TGF-β1 antibody led to a marked increase in the growth rate of the MFG-TGF-β RII-infected cells. This suggests that TGF-β-mediated growth inhibition of MFG-TGF-β RII SNU-638 cells occurs through an autocrine mechanism.

Much of the cellular regulatory effects attributed to TGF-β may occur through the activation of specific TGF-β-responsive promoter elements in critical target genes. To assess whether this intracellular mechanism was present and functional in SNU-638 cells, a reporter construct, p3TP-Lux, that contains multiple TGF-β response elements (6) was transiently transfected into both wild-type TGF-β RII-expressing cells and control cells. Exogenous TGF-β1 induced luciferase activity greater than 12-fold in MFG-TGF-β RII SNU-638 cells with no observable induction in control cells (Fig. 2*D*). The fact that the introduction of a wild-type TGF-β RII expression vector restores TGF-β sensitivity to SNU-638 cells indicates that the remaining regulatory apparatus is essentially intact.

The reduced proliferative capacity of MFG-TGF-β RII-infected SNU-638 cells introduces the possibility that the restoration of expression of wild-type TGF-β RII and of autocrine TGF-β activity might render these cells less tumorigenic. To test this hypothesis, exponentially growing CAT- and TGF-β RII-expressing SNU-638 cells were inoculated into athymic nude mice at a dose of 5×10^6 cells/site, and the mice were monitored for progression of xenograft formation. The appearance of xenografts was significantly delayed after inoculation of MFG-TGF-β RII-infected cells compared to that of control cells (Fig. 3). Moreover, when tumor burden was measured at day 27, the xenograft size of MFG-TGF-β RII-infected cells was less than 30% of that of control tumors. These results suggest that expression of wild-type TGF-β RII inhibits *in vivo* growth of SNU-638 human gastric cancer cells.

This study demonstrates that expression of TGF-β RII in the TGF-β-unresponsive gastric cancer cell line SNU-638 restored sensitivity to TGF-β and significantly reduced the growth rate. TGF-β RII expression reconstitutes a functional TGF-β-mediated autocrine

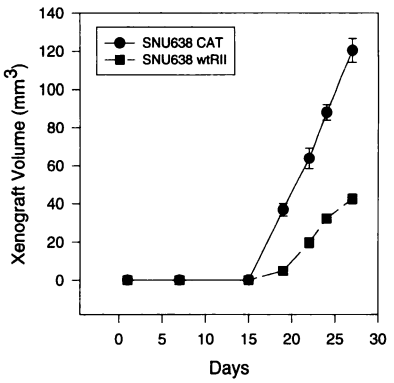

Fig. 3. Tumorigenicity of MFG-TGF-β RII and MFG-CAT SNU-638 cells. Five × 10^6 cells each were injected s.c. into nude mice (n = 10). Tumor volume was followed for 5 weeks. In mice that were inoculated with TGF-β RII-expressing SNU-638 cells, the tumor growth rate was reduced dramatically. Values are the means ± SE of established tumors.

inhibitory loop in SNU-638 cells that operates to restrain baseline growth rates. Finally, transplantation of wild-type TGF-β RII-expressing SNU-638 cells into athymic nude mice results in markedly reduced tumorigenic potential compared to transplantation of control cells. Taken together, these experiments suggest that reduced or aberrant expression of TGF-β RII may play a crucial role in determining the malignant behavior of human gastric cancer cells.

Previous studies have indicated that a majority of human gastric cancer cell lines have developed resistance to the growth-inhibitory effects of TGF-β. This resistance correlates well with the presence of clearly discernible structural defects in the TGF-β *RII* gene. This laboratory has characterized and reported the presence of specific TGF-β RII genetic mutations in six of seven gastric cancer cell lines that are resistant to growth inhibition by TGF-β. Two cell lines, SNU-5 and SNU-668, contain deletions within the TGF-β RII kinase domain,[5] and two cell lines, SNU-601 and SNU-719, display abnormal amplification of the structural gene. Mutation of the TGF-β *RII* gene has also been associated with the phenomenon of microsatellite instability, a phenotypic marker for defective DNA RER (RER+). This association was first observed in human colon cancer (9, 13). Recently, this laboratory has investigated several RER+ human gastric cancer cell lines, and a similar correlation has been identified between the presence of microsatellite instability and mutation of the polyadenine repeat region of the TGF-β *RII* gene (14). The high incidence of TGF-β RII mutation in both RER− and RER+ gastric cancer indicates the existence of multiple potential mechanisms for TGF-β RII mutation and suggests that inactivation of the TGF-β RII may present a pivotal event in the overall process of carcinogenesis.

A recent study of heterozygous TGF-β1 knockout mice revealed a high incidence of abnormal hyperplastic lesions in the gastric mucosa of mice harboring germ-line mutations of a single *TGF-β1* allele (21), whereas abnormal lesions were not identified in any other organ system studied. The presence of potentially preneoplastic lesions in the stomach alone in association with allelic loss of TGF-β1 suggests that the stomach may be uniquely vulnerable to the development of malignancy in response to defects in the TGF-β signaling pathway.

The results from this study support the central role of TGF-β receptor inactivation in the development and progression of human gastric cancer. One important consequence of receptor inactivation is the interruption of a negative autocrine pathway serving to restrict the growth of gastric cancer cells. This TGF-β-mediated autocrine mechanism may represent one of the principal restraints on cell proliferation that must be overcome during the process of carcinogenesis or progression to metastatic disease.

[5] Unpublished observations.

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References

1. Fearon, E. R., and Vogelstein, B. A genetic model for colorectal tumorigenesis. Cell, *61:* 759–767, 1990.
2. Park, K., Kim, S-J., Bang, Y-J., Park, J-G., Kim, N. K., Roberts, A. B., and Sporn, M. B. Genetic changes in the transforming growth factor β (TGF-β) type II receptor gene in human gastric cancer cells: correlation with sensitivity to growth inhibition by TGF-β. Proc. Natl. Acad. Sci. USA, *91:* 8772–8776, 1994.
3. Roberts, A. B., and Sporn, M. B. Peptide growth factors and their receptors. *In:* A. B. Robert and M. B. Sporn (eds.), Handbook of Experimental Pharmacology, pp. 419–472. Heidelberg, Germany: Springer-Verlag, 1990.
4. Massagué, J., Attisano, L., and Wrana, J. The TGF-β family and its composite receptors. Trends Cell Biol., *4:* 172–178, 1994.
5. Lin, H., Wang, X-F., Ng-Eaton, E., Weinberg, R., and Lodish, H. Expression cloning of the TGF-β type II receptor a functional transmembrane serine/threonine kinase. Cell, *68:* 775–785, 1992.
6. Wrana, J. L., Attisano, L., Carcamo, J., Zentella, A., Doody, J., Laiho, M., Wang, X., and Massagué, J. TGF-β signals through a heteromeric protein kinase receptor complex. Cell, *71:* 1003–1014, 1992.
7. Franzén, P., ten Dijke, P., Ichijo, H., Yamashita, H., Schulz, P., Heldin, C-H., and Miyazono, K. Cloning of a TGF-β type I receptor that forms a heteromeric complex with the TGF-β type II receptor. Cell, *75:* 681–692, 1993.
8. Wrana, J. L., Attisano, L., Wieser, R., Ventura, F., and Massagué, J. Mechanism of activation of the TGF-β receptor. Nature (Lond.), *370:* 341–347, 1994.
9. 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. Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability. Science (Washington DC), *268:* 1336–1338, 1995.
10. Capocasale, R., Lamb, R., Vonderheid, E. C., Fox, F. E., Rook, A. H., Nowell, P. C., and Moore, J. S. Reduced surface expression of transforming growth factor β receptor type II in mitogen-activated T cells from Sézary patients. Proc. Natl. Acad. Sci. USA, *92:* 5501–5505, 1995.
11. Kadin, M., Cavaille-Coll, M. W., Gertz, R., Massagué, J., Cheifetz, S., and George, D. Loss of receptors for transforming growth factor β in human T-cell malignancies. Proc. Natl. Acad. Sci. USA, *91:* 6002–6006, 1994.
12. Kim, D. H., and Kim, S-J. Transforming growth factor β receptors: role in physiology and disease. J. Biomed. Sci., *3:* 143–158, 1996.
13. Parsons, R., Myeroff, L. L., Liu, B. L., Willson, J. K. V., Markowitz, S. D., Kinzler, K. W., and Vogelstein, B. Microsatellite instability and mutations of the transforming growth factor β type II receptor gene in colorectal cancer. Cancer Res., *55:* 5548–5550, 1995.
14. Myeroff, L. L., Parsons, R., Kim, S-J., Hedrick, L., Cho, K. R., Orth, K., Mathis, M., Kinzler, K. W., Lutterbaugh, J., Park, K., Bamg, Y-J., Lee, H. Y., Park, J-G., Lynch, H. T., Roberts, A. B., Vogelstein, B., and Markowitz, S. D. A transforming growth factor β receptor type II gene mutation common in colon and gastric but rare in endometrial cancers with microsatellite instability. Cancer Res., *55:* 5545–5547, 1995.
15. Inagaki, M., Moustaka, A., Lin, H. Y., Lodish, H. F., and Carr, B. I. Growth inhibition by transforming growth factor β-resistant hepatoma cells after expression of TGF-β receptor type II cDNA. Proc. Natl. Acad. Sci. USA, *90:* 5359–5363, 1993.
16. Wang, J., Sun, L., Myeroff, L. L., Wang, X., Gentry, L. E., Yang, J., Liang, J., Zborowska, E., Markowitz, S., Willson, J. K. V., and Brattain, M. G. Demonstration that mutation of the type II transforming growth factor β receptor inactivates its tumor suppressor activity in replication error-positive colon carcinoma cells. J. Biol. Chem., *270:* 22044–22049, 1995.
17. Kim, I. Y., Ahn, H-J., Zelner, D. J., Shaw, J. W., Sensibar, J. A., Kim, J-H., Kato, M., and Lee, C. Genetic change in transforming growth factor β (TGF-β) receptor type I gene correlates with insensitivity to TGF-β1 in human prostate cancer cells. Cancer Res., *56:* 44–48, 1996.
18. Park, J-G., Yang, H-K., Kim, W. H., Chung, J-K., Kang, M-S., Lee, J-H., Oh, J. H., Park, H-S., Yeo, K-S., Kang, S. H., Song, S-Y., Kang, Y. K., Bang, Y-J., Kim, Y. I., and Kim, J. P. Establishment and characterization of human gastric carcinoma cell lines. Int. J. Cancer, *70:* 443–449, 1997.
19. Ohashi, T., Boggs, S., Robbins, P., Bahnson, A., Patrene, K., Wei, F-S., Wei, J-F., Li, J., Lucht, L., Fei, Y., Clark, S., Kimak, M., He, H., Mowery-Rushton, P., and Barranger, J. A. Efficient transfer and sustained high expression of the human glucocerebrosidase gene in mice and their functional macrophages following transplantation of bone marrow transduced by a retroviral vector. Proc. Natl. Acad. Sci. USA, *89:* 11332–11336, 1992.
20. Dranoff, G., Jaffee, E., Lazenby, A., Golubek, P., Levitsky, H., Brose, K., Jackson, V., Hamada, H., Pardoll, D., and Mulligan, R. C. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte macrophage colony-stimulating factor stimulates potent, specific, and long-lasting antitumor immunity. Proc. Natl. Acad. Sci. USA, *90:* 3539–3543, 1993.
21. Boivin, G. P., Molina, J. R., Ormsby, I., Stemmermann, G., and Doetschman, T. Gastric lesions in transforming growth factor β-1 heterozygous mice. Lab. Invest., *74:* 513–518, 1996.