Restoration of Bone Morphogenetic Protein Receptor Type II Expression Leads to a Decreased Rate of Tumor Growth in Bladder Transitional Cell Carcinoma Cell Line TSU-Pr1

Isaac Yi Kim,1,4,5 Dong-Hyeon Lee,2 Dug Keun Lee,1 Wun Jae Kim,3 Moses M. Kim,4 Ronald A. Morton,4 Seth P. Lerner,4 and Seong Jin Kim1

1Laboratory of Cell Regulation and Carcinogenesis, National Cancer Institute, Bethesda, Maryland; 2Department of Urology, Ewha Women’s University, Seoul, Korea; 3Department of Urology, Changbuk National University Hospital, Cheongju, Korea; 4Scott Department of Urology, Baylor College of Medicine, Houston, Texas; and 5Department of Urology, University of California, Irvine, Irvine, California

ABSTRACT

Bone morphogenetic proteins (BMPs), potential regulators of cellular growth and metastasis that signal through an interaction with plasma membrane receptors, have been suggested to be important regulators of malignant cells. The present study was carried out to evaluate the potential role of BMP receptor (BMP-R) types IA, IB, and II in bladder transitional cell carcinoma (TCC) cells. Initially, we investigated the expression of these BMP-Rs in 30 archival tissues of human bladder TCC using immunohistochemistry; 10 benign bladder specimens were used for comparison. The results demonstrated that the expression of BMP-Rs is localized preferentially to the transitional epithelium and that there was a significant association between loss of BMP-RII expression and tumor grade. To find a cell line that can serve as a model system for clinical observation, we subsequently examined sensitivity to BMP-4 and expression of BMP-RII in three human bladder cancer cell lines, TCC-Sup, RT4, and TSU-Pr1. Of the three cell lines, TSU-Pr1 exhibited a decreased level of BMP-RII expression and was resistant to the growth-inhibitory effect of BMP-4. Overexpression of BMP-RII in TSU-Pr1 cells not only restored BMP-4 responsiveness but also significantly decreased tumorigenicity in vivo. Taken together, these results demonstrate that human bladder TCC tissues have a frequent loss of BMP-RII expression and that overexpression of BMP-RII leads to restoration of BMP signaling and decreased tumor growth in the human bladder TCC cell line TSU-Pr1.

INTRODUCTION

Transitional cell carcinoma (TCC) of the urinary bladder is the second most frequently diagnosed urologic malignancy. In 2003, there were an estimated 57,400 new cases and 12,500 deaths (1). At initial presentation, 70% to 80% of cases are superficial, whereas the remainder of cases present with muscle-invasive disease. As many as 50% of patients with invasive disease have either detectable or occult metastatic disease at the time of diagnosis. Once diagnosed, Ta and T1 tumors are treated with a local resection, whereas Tis disease usually requires intravesical Bacillus Calmette-Guerin therapy. Low-grade papillary disease (Ta and T1) rarely progresses to muscle-invasive disease; however, up to 30% of patients with Tis or high-grade papillary tumor are refractory to treatment with intravesical therapy and progress to a more advanced disease (reviewed in ref. 2). For patients with Bacillus Calmette-Guerin–refractory Tis and muscle-invasive disease, radical cystectomy is the recommended treatment. Nevertheless, 14% to 33% of patients with lymph node-negative muscle-invasive disease will eventually die of disease within 5 years of surgery. To optimize currently available treatment options and develop novel therapies, additional information concerning the biology of bladder TCC is necessary.

Bone morphogenetic proteins (BMPs), the largest subgroup within the transforming growth factor (TGF)-β superfamily, were originally isolated as factors that induce bone and cartilage formation (3, 4). Recent work has demonstrated that normal BMP function is critical during mammalian development, cellular chemotaxis, and cellular differentiation (reviewed in ref. 5). Based on sequence homology, BMPs are divided into several subgroups. Investigation of BMP knockout mice suggests that each type of BMP may function independently (6). However, the specific role(s) for each type of BMP remains unclear. BMPs signal through an interaction with the membrane receptors where ligand binding results in cross-phosphorylation of type I receptor by type II receptor; type I receptor, in turn, propagates BMP signaling (7). Currently, three type I receptors [Act-R1, BMP receptor (BMP-R) Iα, and BMP-RIB] and three type II receptors (Act-RII, Act-RIIB, and BMP-RII) have been identified. In vitro experiments have shown that all BMPs that belong to the TGF-β superfamily bind to BMP-RI in combination with BMP-RIA or BMP-RIB (8). In contrast, Act-R1, Act-RIIB, and Act-R1 do not bind BMP-4 (9). Thus, in the present study, we focused our initial efforts on BMP-RII, BMP-RIA, and BMP-RIB.

In malignant cells, BMP-2 has been shown to induce apoptosis in human myeloma cells (10) and decrease the rate of proliferation of prostate cancer cells (11), whereas we have demonstrated a frequent loss of expression of BMP-Rs in human prostate cancer tissues (12). More recently, Hallahan et al. (13) reported that BMP-2 mediates the retinoid-induced apoptosis in medulloblastoma cells. These observations as a whole suggest that BMPs are potentially important regulators of cellular proliferation in malignant cells. In normal bladder tissues, the expression of BMP-5 has been localized to the mesenchymal cells beneath the transitional epithelial layers (14). In addition, it has been reported that the human bladder TCC cell line T-24 expresses BMP-2 (15). In this study, we report that the human bladder TCC tissues frequently have a preferential loss of expression of BMP-RII and that the overexpression of BMP-RII in a BMP-resistant cell line leads to a restoration of BMP signaling and a decreased rate of tumor growth.

MATERIALS AND METHODS

Tissue Specimens. Thirty formalin-fixed and paraffin-embedded tissue specimens of histopathologically diagnosed urinary bladder TCC were obtained from the archives of the Scott Department of Urology and the Department of Pathology, Baylor College of Medicine (Houston, TX) and the Department of Urology, Ewha Women’s University (Seoul, Korea). Four-micrometer–thick sections were made and kept at room temperature until use. One of the sequential sections from each specimen was stained with hematoxylin and eosin to confirm the histopathologic grading.

Immunohistochemistry. Archival bladder TCC specimens fixed in neutral buffered formalin were sectioned at a thickness of 4 μm, deparaffinized in
xylene, and rehydrated in PBS. Endogenous peroxidase activity was inactivated by incubation in 3% H2O2 for 10 minutes. After a preincubation with 2% normal serum to block nonspecific sites, the sections were incubated with primary antibodies in a humidified chamber for 18 hours at 4°C. All three primary antibodies were purchased from R&D Systems (Minneapolis, MN). Antigenic binding sites were visualized with serial incubation with a biotinylated secondary antibody, followed by avidin-biotin-horseradish peroxidase complex and diaminobenzidine tetrahydrochloride before counterstaining with Gill’s Hematoxylin (ABC Kit; Vector Laboratories, Burlingame, CA). Negative control sections were processed in an identical manner by substituting the primary antibody with a normal goat IgG fraction. As an initial analysis, all cases were classified as either positive or negative for BMP-R staining. Specimens were classified as positive if >10% of cells had a staining intensity greater than that of negative control slides per high-power field. Negative cases were confirmed with at least two independent staining experiments. All staining results were reviewed by at least two people independently (J.-Y. K. and D.-H.L.).

Cell Culture and Mitogenic Assay. Human bladder TCC cell lines were purchased from American Type Culture Collection (Manassas, VA). All cells used in this study were from passages 50 through 55. Cells were routinely maintained in RPMI 1640 containing 10% fetal bovine serum (FBS), penicillin (100 units/ml), and streptomycin (100 μg/ml). Human BMP-4 (R&D Systems) was diluted to 2 μg/ml and added to culture medium at preselected concentrations.

For cell counts, cells were plated at 20,000 cells per well in 24-well culture plates in RPMI 1640 supplemented with 10% FBS and allowed to adhere for 24 hours. Then the cultures were washed twice with PBS, and the cells from previously selected wells were counted to determine plating efficiency. Cells in the remaining wells were cultured for 4 days in RPMI 1640 supplemented with 1% FBS containing BMP-4 at 0, 10, 100, and 500 ng/ml. The medium was changed at day 2. After removing the medium, cells were trypsinized with 0.5 ml of 0.05% trypsin and counted using a hemocytometer.

RNA Isolation and Northern Blot Analysis. Cells were harvested, and total RNA was isolated using TRIzol reagent (Gibco-BRL, Grand Island, NY) according to the manufacturer’s protocol. Once isolated, 15 μg of total RNA were separated by electrophoresis in a 1% formaldehyde-agarose gel and transferred to a nylon membrane (Zeta-Probe GT membrane; Bio-Rad, Hercules, CA). The membrane was subsequently rinsed in 2x SSC [1× SSC = 0.15 mol/L NaCl and 0.015 mol/L sodium citrate (pH 7.0)] and cross-linked using ultraviolet light. Prehybridization was performed in 50% formamide, 0.12 mol/L Na2 HPO4 (pH 7.2), 0.25 mol/L NaCl, 7% (w/v) SDS, and 250 μg/ml heat-denatured salmon sperm DNA for 2 hours at 42°C. Hybridization was performed overnight at 42°C in the prehybridization solution containing probe labeled with [32P]dCTP using a random oligonucleotide priming kit (Prime It; Stratagene, La Jolla, CA). The membrane was washed sequentially in 2x SSC-0.1% SDS for 15 minutes, 0.5x SSC-0.1% SDS for 15 minutes, and 0.1x SSC-0.1% SDS for 30 minutes. Autoradiography was carried out at -70°C.

Immunoblot Analysis. Cells were harvested, placed in sample buffer (0.0625 mol/L Trizma base, 2% SDS, and 5% 2-mercaptoethanol), and boiled for 5 minutes. Electrophoresis was carried out using 50 μg of total protein in each lane. After electrophoresis, protein was transferred to a 0.2-μm nitrocellulose membrane (Bio-Rad). After the transfer, the membrane was incubated in blocking buffer (5% nonfat dry milk, Tris-buffered saline, and 0.1% Tween) for 1 hour and incubated with the appropriate primary antibody at a concentration of 0.2 μg/ml overnight at 4°C. As with immunohistochemistry, all three primary antibodies were purchased (R&D Systems). Anti-Flag antibody was purchased from Sigma-Aldrich (St. Louis, MO). Subsequently, the membrane was incubated in the presence of appropriate secondary antibody at a dilution of 1:3,000 for 2 hours. After washing several times with TBST, immunoreactive bands were visualized by enhanced chemiluminescence.

Transfection and Luciferase Activity Assay. For transient transfections, cells were seeded in 6-well plates at 100,000 cells per well. After allowing the cells to adhere, they were transfected with pSBE4 (16), the plasmid containing the luciferase reporter gene under the control of four tandem repeats of smad-binding element (SBE), with or without BMP-RI using Lipofectin according to the manufacturer’s directions (Gibco-BRL). Briefly, 1 μg of pSBE4 with or without BMP-RII and 12 μL of Lipofectin were added with 1 mL of transfection medium (Opti-mem Gibco-BRL) to each well, and cells were incubated for 24 hours. Subsequently, fresh medium was added, and the cells were incubated for an additional 24-hour period. Finally, 100 ng/mL BMP-4 in RPMI 1640 supplemented with 1% FBS was added, and the cultures were maintained for 16 hours. The extent of the promoter activity of pSBE4 was assayed by measuring luciferase activity using a commercial luciferase assay kit (Enhanced Luciferase Assay Kit; Analytical Luminescence Laboratory, San Diego, CA). To correct for varying transfection efficiencies, cells were cotransfected with 1 μg of pSV-β-galactosidase (Promega, Madison, WI), and the lysates were divided into two aliquots. Luciferase activity was normalized to the level of β-galactosidase activity that was determined using the β-galactosidase Enzyme Assay System (Promega). For stable transfections, cells were transfected in an identical manner as transient transfections. Subsequently, stable clones were selected in the presence of G418 at 400 μg/mL. The plasmid encoding BMP-RII protein tagged with the Flag epitope was kindly provided by Dr. Kohei Miyazono (The Cancer Institute, Tokyo, Japan).

Animals and Demethylation Assays. Adult male nude mice were obtained from the National Cancer Institute and housed according to the National Institutes of Health standards established in the guidelines for the care and use of experimental animals. One million cells were inoculated into each animal (5 mice per group) for the tumor xenograft studies. For demethylation studies, 5-aza-2’-deoxycytidine was added to cell culture at designated concentrations and incubated overnight. The following day, cells were harvested.

Statistical Analysis. All tissue culture experiments were repeated at least three times, and all numerical data are expressed as a mean ± SE of triplicate observations. Differences of means among different treatments were compared by χ² test. A value of P < 0.05 was considered statistically significant.

RESULTS

Expression of Bone Morphogenetic Protein Receptors in Human Bladder Transitional Cell Carcinoma Tissues. Initially, the expression of BMP-Rs was investigated in 30 archival human bladder TCC tissues using immunohistochemistry; 10 normal bladder specimens were used for comparison. The results, shown in Table 1 and Fig. 1, demonstrated that the expression of BMP-RIA, BMP-RIB, and BMP-RII in normal bladder tissues localized preferentially to the transitional epithelium and that bladder TCC cells frequently have a loss of expression of BMP-RII but not BMP-RIA and BMP-RIB. When the status of BMP-R expression was analyzed with respect to the tumor grade, a statistically significant association was observed again only between BMP-RII and tumor grade. Three of 10 well-differentiated tumors (grade 1) showed a selective loss of expression of BMP-RII, whereas 8 of 10 poorly differentiated tumors (grade 3) demonstrated a loss of expression of BMP-RII.

Effect of Bone Morphogenetic Protein-4 and Expression of Its Receptors in Human Bladder Transitional Cell Carcinoma Cell Lines. To investigate the potential biological consequence of the loss of expression of BMP-RII detected in human bladder TCC tissues, we studied the effect of BMP-4 and the expression of the three BMP-Rs in three human bladder TCC cell lines, RT4, TCC-Sup, and TSU-Pr1. Of these three cell lines, TSU-Pr1 was initially characterized as a prostate cancer cell line but was eventually shown to be a bladder TCC cell line (17). The results demonstrated that TCC-Sup and TSU-Pr1 were insensitive to BMP-4 whereas RT4 exhibited a significant inhibition of cellular proliferation in the presence of BMP-4 in a dose-dependent manner (Fig. 2A). At 100 ng/mL BMP-4, the number of RT4 cells was approximately 61% of that of the control. Because the activation of a BMP-responsive promoter that is present in the reporter construct pSBE4 has been reported to reflect the gene transcriptional activity of BMPs (16), the three bladder cancer cell lines were transiently transfected with pSBE4. After transfection, cells were treated with 100 ng/mL BMP-4 for 16 hours. Fig. 2B shows that the BMP-4 treatment resulted in an induction of luciferase activity in RT4 cells only. As with the results of the cellular proliferation assay,
BMP-4 treatment did not significantly alter the level of luciferase activity in TCC-Sup and TSU-Pr1 cells. Next, Northern blot analysis for BMP-RII, BMP-RIA, and BMP-RIB was carried out in the three bladder TCC cell lines (Fig. 3). As expected TSU-Pr1 cells did not express a detectable level of BMP-RII. These results, taken together, demonstrate that TSU-Pr1 cells have a decreased level of expression of BMP-RII.

To show that the decreased level of expression of BMP-RII is the mechanism responsible for the observed resistance to BMP-4, TSU-Pr1 cells were transiently cotransfected with a BMP-RII expression vector and pSBE4. After treatment with 100 ng/mL BMP-4, luciferase activity was measured again. The results, shown in Fig. 3C, demonstrate that transfection with BMP-RII resulted in a significant level of induction of luciferase activity in TSU-Pr1 cells.

To determine the mechanism of loss of expression of BMP-RII in TSU-Pr1 cells, Southern blot analysis was initially carried out to detect any gross genetic changes. The results demonstrated no significant genetic alteration of the BMP-RII gene in TSU-Pr1 cells (data not shown). Next, each exon of BMP-RII was cloned and sequenced using polymerase chain reaction. Again, no mutation was detected (data not shown). Because promoter suppression through methylation is another potential mechanism for the loss of expression of BMP-RII in TSU-Pr1 cells, we subsequently investigated the potential role of methylation in regulating BMP-RII expression in TSU-Pr1 cells by treating them with the demethylating agent 5-aza-2′-deoxycytidine. The result, shown in Fig. 3D, demonstrates a dramatic increase in the level of expression of BMP-RII in TSU-Pr1 cells after treatment with the demethylating agent. Interestingly, only one of the three splice variants of BMP-RII was detected in TSU-Pr1 cells after treatment with 5-aza-2′-deoxycytosine. These results, taken together, suggest that the mechanism for the decreased level of expression of BMP-RII in TSU-Pr1 cells is due, in part, to DNA methylation of the promoter.

Effect of Bone Morphogenetic Protein Receptor Type II on Tumorigenic Potential. To investigate the effect of BMP-RII on tumorigenic potential in bladder TCC cells, TSU-Pr1 cells were transfected with BMP-RII, and stable clones were selected. To aid in screening the expression of the protein from the transfected gene, the plasmid containing the genetic sequence for BMP-RII protein tagged with the Flag epitope was used. To generate control cell lines, TSU-Pr1 cells were transiently cotransfected with a BMP-RII expression vector and pSBE4. After treatment with 100 ng/mL BMP-4, luciferase activity was measured again. The results demonstrated a dramatic increase in the level of induction of luciferase activity in TSU-Pr1 cells. To show that the decreased level of expression of BMP-RII is the mechanism responsible for the observed resistance to BMP-4, TSU-Pr1 cells were transiently cotransfected with a BMP-RII expression vector and pSBE4. After treatment with 100 ng/mL BMP-4, luciferase activity was measured again. The results, shown in Fig. 3C, demonstrate that transfection with BMP-RII resulted in a significant level of induction of luciferase activity in TSU-Pr1 cells.

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high level of BMP-RII, and the other clone expressed a low level of BMP-RII (these clones were designated BMP-RII3 and BMP-RII1, respectively). Immunoblot analysis using the Flag antibody demonstrated a high level of BMP-RII expression in BMP-RII3 cells and a low level of expression in BMP-RII1 cells (Fig. 4B). In tissue culture, these clones did not exhibit any difference in the rate of proliferation when compared with parental and control cell lines (data not shown).

Next, sensitivity to BMP-4 was studied in BMP-RII3 and BMP-RII1 clones. The results, shown in Fig. 4C, demonstrate that BMP-4 inhibited the proliferation of BMP-RII3 and BMP-RII1 cells in a dose-dependent manner.

Subsequently, BMP-RII3 cells were injected subcutaneously into nude mice, and tumor volume was followed for 4 weeks. As shown in Fig. 5A and B, there was a significant decrease in the rate of tumor growth in BMP-RII3 when compared with parental and control cells (pcDNA3.1); hematoxylin and eosin staining showed no obvious histologic difference (Fig. 5C). Immunoblot using Flag antibody was performed after harvesting the tumor samples and confirmed that the tumor xenografts expressed BMP-RII (Fig. 5D).

DISCUSSION

Results of the present study demonstrated a preferential expression of BMP-Rs to the transitional epithelium in normal human bladder tissues and a frequent loss of expression of BMP-RII in human bladder TCC tissues. Furthermore, there was a correlation between sensitivity to BMP-4 and the expression of its cognate receptor, BMP-RII, in the human bladder TCC cell line TSU-Pr1. When TSU-Pr1 cells were transfected with BMP-RII, BMP-4 sensitivity was restored, whereas tumorigenic potential decreased significantly. Treatment with the demethylating agent 5-aza-2'-deoxycytidine led to a restoration of the expression of BMP-RII in TSU-Pr1 cells. These results, taken together, provide a valuable insight concerning the role of BMPs and BMP-Rs in bladder TCC cells.

BMPs were originally named because they were isolated from the bone. However, recently published works clearly demonstrate that this group of growth factors is important for normal organogenesis and cellular differentiation (3, 4). More recently, multiple studies have suggested that BMPs may be important regulators of neoplastic cells. Hallahan et al. (13) reported that BMP-2 mediates retinoid-induced apoptosis in medulloblastoma cells, whereas Kawamura et al. (10) demonstrated that BMP-2 induces apoptosis in human myeloma cells. Our group has demonstrated a correlation between Gleason score and
BMP-R expression status in prostate cancer cells (12). To the best of our knowledge, the present work is the first report to examine the role of BMPs and BMP-Rs in bladder TCC cells.

In the present study, the expression of BMP-Rs was localized preferentially to the transitional epithelial compartment in normal bladder tissues. Previously, it has been reported that BMP-5 is expressed by mesenchymal cells that are located beneath the transitional epithelium (14). As with TGF-β (18), these observations as a whole suggest the interesting possibility that BMPs may play a role in stromal–epithelial interaction in the bladder. We are currently investigating the expression of BMPs in normal and malignant bladder tissues.

The present study also demonstrated that human bladder TCC cells frequently have a loss of expression of BMP-RII and that the proliferation of human bladder TCC cell line RT4 is inhibited by BMP-4 in a dose-dependent manner. Because the decreased level of expression of BMP-RII in TSU-Pr1 cells led to insensitivity to the growth-inhibitory effect of BMP-4 in tissue culture, it is likely that the altered expression of BMP-RII observed in human bladder TCC tissues signifies the loss of sensitivity to BMPs. The observed inhibitory effect of BMP-4 is consistent with the general characteristics of TGF-β superfamily members and supports the notion that BMPs are differentiating factors that regulate homeostasis and prevent abnormal proliferation of transitional epithelial cells in the bladder. Thus, the loss of sensitivity to BMPs through the loss of expression of BMP-RII in bladder TCC cells may be necessary to escape the normal physiological constraint on cellular proliferation and achieve a malignant phenotype.

In agreement with the paradigm of TGF-β and TGF-β receptor expression during carcinogenesis, the present study demonstrated a significant decrease in tumorigenic potential in TSU-Pr1 cells when the expression of BMP-RII was restored by transfection. Specifically, the tumor volume of clones expressing a high level of BMP-4 (BMP-RII) and one clone expressing a low level of BMP-4 (BMP-RII) were isolated for further characterization. Cells were cultured in the presence of BMP-4 for 4 days.

Fig. 4. Generation of TSU-Pr1 cell lines stably expressing Flag-tagged BMP-RII protein. Expression of BMP-RII in TSU-Pr1 cell lines stably transfected with either control vector (pcDNA3.1) or BMP-RII was determined by Northern blot analysis (A) or immunoblot analysis (B). C. BMP-4 sensitivity in TSU-Pr1 clones. One clone expressing a high level of BMP-4 (BMP-RII) and one clone expressing a low level of BMP-4 (BMP-RII) were isolated for further characterization. Cells were cultured in the presence of BMP-4 for 4 days.

Fig. 5. Tumorigenicity of TSU-Pr1 cells expressing control vector and BMP-RII. One million cells were subcutaneously inoculated into each animal (5 mice per group) for the tumor xenograft studies. Tumor volume was followed for 4 weeks. A, gross picture of mice. B, tumor volume. In mice that were inoculated with BMP-RII–expressing TSU-Pr1 (BMP-RII and BMP-RII), tumor growth was markedly suppressed. Values are the mean ± SD of established tumors. C, hematoxylin and eosin staining. D, immunoblot analysis of tumors developed from nude mice injected with TSU-Pr1 cells. Tumor tissues were harvested after inoculation of wild-type TSU-Pr1, TSU-Pr1-pcDNA3.1, and TSU-Pr1-BMP-RII cells into nude mice and sonicated. The lysates were loaded in 4% to 20% SDS-PAGE. Immunoblotting was performed with anti-Flag tag antibody. Relative levels of protein loading are shown by β-actin staining.
creased tumorigenic potential in a breast cancer cell line (19). One potential explanation for the observed discrepancy of the effect of BMP-RII between bladder and breast cancer is that the function of BMP may vary among different cell lines and types of malignancies. For example, TGF-β has been shown to have both a growth-inhibitory and a growth-promoting effect in different cell lines (20, 21). Another interesting possibility is that the effect of BMP may change with tumor progression, just as TGF-β suppresses tumor growth in the early stage of carcinogenesis and promotes tumor progression in the late stage of carcinogenesis (22). Additional work is necessary to test these hypotheses.

The precise mechanism responsible for the loss of expression of BMP-RII in human bladder TCC cells remains unclear, although the results obtained in TSU-Pr1 cells suggest epigenetic events as a strong possibility. Specifically, no alteration of the BMP-RII gene was detected, whereas treatment with the demethylating agent 5-aza-2′-deoxycytidine led to the restoration of BMP-RII expression. Currently, we are carrying out a detailed analysis of the promoter and all of the exons of BMP-RII in human bladder TCC cells obtained using laser capture microscopy.

It should be pointed out, however, that the loss of expression of BMP-Rs is not the only mechanism for the observed insensitivity to BMPs. In the present study, we demonstrated that TCC-Sup cells are clearly resistant to BMP-4, yet this cell line was shown to express a comparatively high level of BMP-Rs. Potential explanations include point mutations of the receptors that are not readily detected by Northern or immunoblot analyses. Alternatively, TCC-Sup cells may have defective molecules that signal downstream of BMP-Rs. Currently, we are investigating the expression of BMP signaling intermediates, smads, in TCC-Sup cells.

In conclusion, results of the present study demonstrate that human bladder TCC tissues frequently have a loss of expression of BMP-RII and that BMP-RII is a potent suppressor of tumor growth in the human bladder TCC cell line TSU-Pr1. One potential mechanism for the loss of expression of BMP-RII may be a transcriptional repression mediated by methylation.

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