Expression of Bone Morphogenetic Protein Receptors Type-IA, -IB, and -II Correlates with Tumor Grade in Human Prostate Cancer Tissues¹

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Abstract

Bone morphogenetic proteins (BMPs) are potential regulators of prostate cancer cell growth and metastasis that signal through an interaction with BMP membrane receptors (BMPRs) type I and type II. In the present study, Western blot and immunohistochemical analysis of BMPRs were carried out in benign and malignant human prostate tissues to explain the loss of BMP response in human prostate cancer cells. The results demonstrated that the benign prostate specimens expressed high levels of all three BMPRs. In normal prostate, BMPRs were localized predominantly to epithelial cells. Among prostate cancer specimens, well-differentiated cancers were positive for the expression of BMPR-II, BMPR-IA, and BMPR-IB, for the most part. In contrast, only 1 of 10 poorly differentiated prostate cancer cases was positive for each of the three BMPRs (P < 0.005 for all three receptors). Taken together, these results indicate that human prostate cancer cells frequently exhibit loss of expression of BMPRs and suggest that loss of BMPRs may play an important role during the progression of prostate cancer.

Introduction

Prostate cancer is the most common malignancy and the second leading cause of male cancer deaths in the United States (1). At the present time, the precise mechanism involved in prostate carcinogenesis remains uncertain. Nevertheless, it is likely that there are certain genetic alterations in prostatic epithelial cells that permit them to proliferate and metastasize. In this regard, it has been suggested that BMPs play a role during prostate carcinogenesis (2, 3). BMPs, the largest subgroup within the TGF-β superfamily, are isolated as factors that induce bone and cartilage formation (4–6). Recent work has demonstrated that normal BMP function is critical during mammalian development, cellular chemotaxis, and cellular differentiation (7–9). Based on sequence homology, BMPs are divided into three subgroups: (a) BMP-2 and -4; (b) BMP-5, -6, -7, and -8; and (c) BMP-3 (10). Investigation of BMP knockout mice suggests that each type of BMP may function independently (11, 12). However, the specific role(s) for each type of BMP remains unclear. BMP signal transduction follows the paradigm established by TGF-β signaling. As with TGF-β, BMPs signal through an interaction with a heteromeric complex of BMPRs, BMPR-I and -II. Specifically, ligand binding results in cross-phosphorylation of BMPR-I by BMPR-II; BMPR-I, in turn, propagates BMP signaling (13). In vitro experiments have shown that all members of BMP that belong to the TGF-β superfamily bind to BMPR-II in combination with BMPR-IA or -IB (14, 15). In contrast, BMP-4 does not bind to ActR-II or ActR-I (13). Thus, it has been suggested that BMPR-IA, -IB, and -II are BMP-specific receptors. In prostate cancer, it has been shown that BMP-2 decreases the rate of proliferation of LNCaP but not of TSU-PR1, PC3, and DU145 cells (16). Because these three prostate cancer cell lines are insensitive to the growth-inhibitory effect of BMP-2, it is possible that some primary human prostate cancer cells are also insensitive to BMPs. The exact mechanism for rendering insensitivity to BMPs has not been established. As an initial attempt to investigate the mechanism underlying the loss of sensitivity to BMPs, we have determined the expression of BMP-specific receptors (BMPR-II, -IA, and -IB) in archival human prostate cancer specimens. We report that the expression of BMPRs is frequently lost in high-grade prostate cancer cells.

Materials and Methods

Tissue Specimens. Formalin-fixed and paraffin-embedded tissue specimens of 40 histopathologically diagnosed prostate cancer samples and 10 samples of benign prostate were obtained from the archives of the Scott Department of Urology, Baylor College of Medicine (Houston, TX) and the Department of Urology, University of Ulsan (Seoul, Korea). Tumor specimens were divided into 10 cases of well-differentiated cancer, 20 cases of moderately differentiated cancer, and 10 cases of poorly differentiated cancer, according to the Gleason score (17). Sections were cut at 4.0 µm and kept at room temperature until use. The first and last section from each specimen was stained with H&E to verify histopathological diagnosis.

Immunohistochemistry. Anti-BMPR antibodies were kindly provided by Dr. Kohsei Miyazono of The Cancer Institute (Tokyo, Japan). The specificity of the antibodies has been established previously (18, 19). Archival specimens fixed in neutral buffered formalin were sectioned at a thickness of 4 µm, deparaffinized in Xylene (Fisher Scientific Co., Pittsburgh, PA), and rehydrated in PBS. Endogenous peroxidase activity was inactivated by incubation in 0.3% H2O2 for 10 min. After a preincubation with 2% normal goat serum to block nonspecific sites, the sections were incubated with primary antibodies in a humidified chamber for 18 h at 4°C. Anti-BMP-IA, anti-BMP-IB, and anti-BMPR-II antibodies were used at a concentration of 4 µg/ml. Antigenic binding sites were visualized with a serial incubation with 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 primary antibody with a normal rabbit IgG fraction. All negative control sections showed no color reaction.

All cases were classified into groups that showed either positive or negative staining for BMPRs. Specimens were classified as positive if >10% of cells had a staining intensity greater than that of negative control slides per high-power field. At the least three high-power fields were reviewed for each case. All cases were confirmed with at least two independent staining experiments. In addition, all staining specimens were reviewed by two blinded independent investigators.

Western Blot Analysis. Frozen normal and malignant prostate tissues were homogenized with PBS at 4°C, and the protein concentration was determined. Samples were placed in sample buffer (0.0625 M Trizma base, 2% SDS, and 5% 2-mercaptoethanol) and boiled for 5 min. Electrophoresis was carried out...
in a 10% SDS-polyacrylamide gel using 100 μg of total protein in each lane. After electrophoresis, proteins were transferred to a 0.2-μm nitrocellulose membrane (Bio-Rad). After the transfer, the membranes were incubated overnight in blocking buffer (5% nonfat dry milk, PBS, and 0.1% Tween). Subsequently, the membranes were incubated with appropriate antibodies at a dilution of 1:400 overnight at 4°C. After washing with PBS-0.1% Tween, the membranes were incubated in the presence of goat antirabbit horseradish peroxidase-labeled secondary antibody (Bio-Rad) at a dilution of 1:3000 for 2 h at room temperature. After washing several times, immunoreactive bands were visualized by enhanced chemiluminescence (Amersham).

Statistics. The correlation between BMPR expression and tumor grade was evaluated by the χ² test. P < 0.05 was considered statistically significant.

Results

Expression of BMPRs in Benign Prostate Tissues. Initially, the expression of BMPRs was investigated in benign human prostate tissues by immunohistochemistry. In benign tissues, the expression of BMPR-IA, -IB, and -II was predominantly localized in the epithelial cells (Fig. 1). As described previously (18, 19), the specificity of the antibodies was confirmed by successfully neutralizing the color reactions when the antibodies were preincubated with 100-fold molar excess of the corresponding peptide antigen (data not shown).

Expression of BMPRs in Malignant Prostate Tissues. To determine whether or not a significant portion of human prostate cancer cases have reduced levels of BMPRs, 40 archival samples were screened for BMPR expression using immunohistochemistry (Table 1). For comparison, 10 samples of benign prostatic tissues were used. Fig. 2, A–C, shows representative immunohistochemical staining for BMPR-IA, -IB, and -II, respectively. There was a wide variation in staining intensity for BMPR expression among the human prostate cancer specimens. The tissue samples were classified into groups that showed either positive or negative staining for BMPR expression. No attempt was made to quantitate the levels of expression of BMPRs among positively stained specimens. All benign prostate tissues were positive for expression of all three BMPR proteins. However, with increasing tumor grade, prostate cancers demonstrated a progressive loss of BMPR protein expression. Table 2 shows the relationship between the loss of expression of BMPRs and grade of prostate malignancy by dividing the cancer tissue specimens into three classes, based on the Gleason score (16): (a) 10 well-differentiated cancers (Gleason score, 2–4); (b) 20 moderately differentiated cancers (Gleau-
son score, 5–7); and (c) 10 poorly differentiated cancers (Gleason score 8–10). In 10 of 10 benign prostate samples, all three BMPRs were readily detected. Similarly, seven, eight, and nine samples of well-differentiated cancers expressed BMPR-II, BMPR-IA, and BMPR-IB, respectively. In contrast, only 1 of 10 samples of poorly differentiated cancers expressed detectable levels of the three BMPRs investigated in this study. The frequency of loss of expression of BMPRs in prostate cancer cells was statistically significant in each group ($P < 0.005$ for all three BMPRs).

To further demonstrate that prostate cancer tissues have decreased levels of BMPR expression, Western blot analysis was carried out. As shown in Fig. 3, normal prostate tissues had readily detectable levels of the three BMPRs (Lanes 1 and 2). On the other hand, prostate cancer tissues frequently showed loss of BMPR expression (Lanes 3–7). Specifically, three of five prostate cancer tissues examined had decreased levels of BMPR-II expression. With regard to type I receptors, three of five and five of five malignant prostate tissues had decreased levels of expression of BMPR-IA and BMPR-IB, respectively.

Discussion

These data demonstrate that BMPRs are preferentially expressed by epithelial cells in the human prostate and that human prostate cancer cells frequently have reduced levels of expression of BMPR-IA, BMPR-IB, and BMPR-II. The results also show an inverse correlation between the levels of BMPR expression and tumor grade in human prostate malignancy. Taken together, these observations provide a valuable insight regarding the potential role of BMPs during carcinogenesis in the human prostate.

It has been suggested that members of TGF-β superfamily mediate stromal-epithelial cell interaction in the normal prostate. Specifically, TGF-β is predominantly expressed by prostate stromal cells, whereas TGF-β receptors are present in high concentrations among prostate epithelial cells (20, 21). In the present study, the expression of BMPRs was localized mainly to the epithelial compartment. Such preferential expression of BMPRs by the prostatic epithelial cells also suggests that BMPs may be a mediator of stromal-epithelial interaction. Further work is necessary to verify this hypothesis.

The accepted mechanism of carcinogenesis is a process involving multiple molecular genetic alterations. To date, the exact molecular mechanism of prostate carcinogenesis is incompletely understood. Nevertheless, the development and progression of prostate cancer likely involve multiple steps and factors. In this regard, the results of the present study suggest that loss of BMPRs may play a role during prostate carcinogenesis. Of the 40 prostate cancer cases investigated in this study, 32 (80%) exhibited loss of expression of one or more BMPR. Of the 32 cases, 25 had either loss of BMPR-II or loss of BMPR-I (BMPR-IA or BMPR-IB). Since BMP signaling requires both type I and type II receptors for BMP signaling, 25 of 40 cases (62%) investigated in the present study likely had defective BMP signaling. Recently, it has been...
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proliferation (22), prostate cancer cells that retain BMP signaling will 

demonstrated that BMP-2 inhibits the proliferation of a prostate cancer cell line, LNCaP (16). Taken together, these observations suggest that BMPs are growth-inhibitory factors and that some prostate cancer cells acquire resistance to the growth-inhibitory effect of BMPs through down-regulation of receptor expression.

The frequent loss of BMPRs in high-grade prostate cancer cells also suggests a potential mechanism for the high frequency of metastasis to bone in prostate cancer patients. Because the normal bone has relatively high concentrations of BMPs, which normally inhibit cellular proliferation (22), prostate cancer cells that retain BMP signaling will not be able to proliferate in such a microenvironment. On the other hand, prostate cancer cells that have a loss of BMPRs are released from the growth-inhibitory effect of BMPs and will be able to proliferate in the bone. Further analysis is necessary to confirm this hypothesis.

Finally, the results of the present study suggest that the loss of BMPRs may be used as a prognostic marker in prostate cancer patients. Specifically, there was an inverse correlation between the loss of BMPR expression and increasing tumor grade (Gleason score) because 10 of 10 poorly differentiated prostate cancer cases had a loss of one of the three BMPRs; on the other hand, only 5 of 10 well-differentiated prostate cancer cases had loss of expression of one of these receptors. Because Gleason score is one of the best available predictors of outcome among prostate cancer patients, an association between Gleason score and the loss of BMPR expression suggests that the status of BMPRs may also be used as a prognostic marker in prostate cancer patients. Currently, the possibility that the status of BMPR expression functions as an independent prognostic marker in prostate cancer patients is under investigation.

In conclusion, the results of the present study have demonstrated that human prostate epithelial cells preferentially express BMPRI-A, BMPRI-B, and BMPRII and that prostate cancer cells frequently have loss of expression of these receptors. In the future, the specific roles and expression of each BMPR during the progression and metastasis of prostate cancer will be the subject of investigation.

### References

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Cancer Res 2000;60:2840-2844.

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