Alteration of gene expression in response to bone morphogenetic protein-2 in androgen-dependent human prostate cancer LNCaP cells

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Abstract. Bone morphogenetic protein (BMP)-2, a multifunctional member of the transforming growth factor (TGF)-ß superfamily with powerful osteoinductive effects, has various biological activities in a variety of cells. We observed that BMP-2 inhibits cell proliferation in the androgen-dependent human prostate cancer cell line, LNCaP. To investigate the mechanism of inhibition of androgen-dependent growth by BMP-2, we compared the gene expression in LNCaP cells treated with dihydrotestosterone (DHT) to that of LNCaP cells treated with DHT and BMP-2, using DNA microarray analysis. Of 8,400 human genes on the gene chip, 38 genes were up-regulated by >2.0-fold and 48 genes were down-regulated by <0.5-fold by treatment with BMP-2. These genes were involved in a variety of cellular functions, including signal transduction, transcription regulation, enzymes, transporters, structural molecules and translation. RT-PCR analysis showed that CH1CL and BMX were up-regulated and DACH1 and WNT5A were down-regulated by treatment with BMP-2. Furthermore, we detected an increase of WNT5A protein in the medium by DHT and inhibition of the increase by BMP-2. In the present study, we identified several BMP-2-responsive genes in LNCaP cells. Further studies of the roles of these genes may clarify the mechanisms underlying the inhibition of cell proliferation by BMP-2 and identify better approaches for the prevention and treatment of prostate cancer.

Introduction

Bone morphogenetic proteins (BMPs) are secreted signaling molecules that belong to the transforming growth factor (TGF)-ß superfamily (1,2) and were originally identified from extracts of bone on the basis of their ability to induce ectopic bone formation *in vivo* (3,4). In addition to functions as a possible bone-inducing factor, BMPs have been shown to play important roles in various biological processes such as chemotaxis of monocytes (5), migration of osteoblasts (6) and differentiation of neural cells (7). BMPs and their receptors are widely distributed not only in bone and cartilage but also in other tissue. BMP receptor (BMPR) mRNA is expressed at higher levels in the prostate than in other organs (8), and the expression of BMPRs (9) and BMP-2 (10) decreases with the progression of prostate cancer. We were interested in the relationship between BMP-2 and prostate cancer and, therefore, investigated the effect of BMP-2 on prostate cancer cell lines. BMP-2 inhibited the androgen-dependent growth of human prostate cancer LNCaP cells; however, it had no effect on the growth of androgen-independent prostate cancer cell lines. Ide *et al* (11) reported a similar result. Recently, we suggested that the alteration of some cell cycle-related proteins is associated with growth inhibition (12). However, the mechanism underlying the inhibitory effect of BMP-2 on prostate cancer cell proliferation is not fully understood.

In the present study, to investigate the mechanism underlying the inhibitory effect of BMP-2 on the growth of androgen-dependent prostate cancer cells, we examined the pattern of global gene expression in LNCaP cells treated with dihydrotestosterone (DHT) alone or with a combination of DHT and BMP-2 using microarray analysis. DNA microarrays are useful for the study of the regulation of gene expression in cancer cells, particularly in examining the changes in gene expression associated with the development and progression of many cancers, including prostate cancer. We compared the gene expression in LNCaP cells treated with DHT to that of LNCaP cells treated with DHT and BMP-2 and identified various novel putative BMP-2-responsive genes involved in several cellular functions, including signal transduction, transcription regulation, transport, structure, and translation regulation.

Materials and methods

Cell culture and treatment. The human prostate cancer cell line, LNCaP [American Type Culture Collection (ATCC), VA, USA], was maintained in RPMI-1640 medium (Gibco, NY, USA) supplemented with 10% fetal bovine serum (FBS)

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(ATCC) at 37˚C in a humidified 5% CO_2 incubator. To measure the effect of recombinant human BMP-2 (rhBMP-2) on the androgen-induced proliferation of LNCaP cells, $5.0x10^6$ cells were seeded in a 100-mm dish in phenol red-free RPMI-1640 containing 5% charcoal-dextran-treated FBS. After 1 day of growth in androgen-depleted medium, cells were treated with dihydrotestosterone (DHT; Sigma-Aldrich, MO, USA) at a final concentration of 1 nM and rhBMP-2 at a final concentration of 100 ng/ml. rhBMP-2 was provided by Yamanouchi Pharmaceutical Co., Ltd. (Japan).

Crystal violet assay. LNCaP cells were seeded into 24-well plates ($2x10^4$ cells/well). LNCaP cells were cultured as described above. After cells were treated with 1 nM DHT alone or with a combination of 1 nM DHT and 100 ng/ml BMP-2 for 1, 2 or 4 days, formaldehyde was added to a final concentration of 2% for fixation, the medium was removed from the wells and the plate was washed with 0.9% NaCl. Crystal violet solution was added, and cells were incubated for 30 min at room temperature. Cells were then washed with 0.9% NaCl followed by addition of 1% SDS to solubilize the cells. The solution was transferred to 96-well plates, and the absorbance of each well at 595 nm was determined.

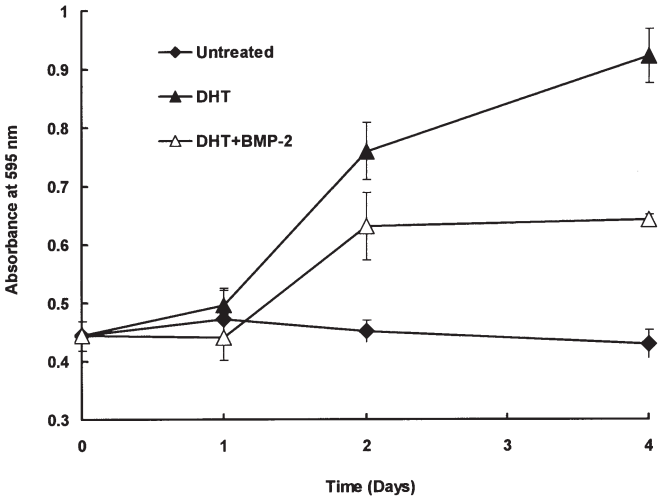

Figure 1. Effect of BMP-2 on androgen-dependent growth of LNCaP cells. Cells were seeded into 24-well plates ($2x10^4$ cells/well) and cultured in phenol red-free RPMI-1640 containing 5% charcoal-dextran-treated FBS for 24 h. Cells were then treated with 1 nM DHT alone or with a combination of 1 nM DHT and 100 ng/ml BMP-2. After treatment for 1, 2 or 4 days, cell growth was analyzed as described in Materials and methods. The absorbance at 595 nm is correlated with the number of living cells.

RNA extraction. LNCaP cells grown to 70% confluence were treated with 1 nM DHT in the absence or presence of 100 ng/ml BMP-2 for 24 h. Total RNA from LNCaP cells was extracted using isogen (Wako, Japan). RNA concentrations were measured at 260 nm. For DNA microarray assay, poly(A)$^+$ RNA was extracted using an Oligotex TM-dT30 Super mRNA purification kit (Takara, Japan). Poly(A)$^+$ RNA quality was assessed using an Agilent BioAnalyzer (Agilent Technologies, CA, USA) and poly(A)$^+$ RNA quantity was assessed using ND-1000 (NanoDrop Technologies, DE, USA). For reverse transcription polymerase chain reaction (RT-PCR), poly(A)$^+$ RNA was extracted using an Oligotex-dT30 (Takara).

DNA microarray and data analysis. mRNAs were labeled with different fluorescent labels for application on gene chips. For assessing the quality of biotin-labeled RNA, actin and GAPDH were examined using a test chip and GeneChip Operating Software (GCOS; Affymetrix Inc., CA, USA). For additional information, see www.biomatrix.co.jp. Samples were processed on the chips. The human genome focus GeneChip array (Affymetrix, Inc.) was used to study changes in expression of 8,400 human genes. GCOS was used for initial data analysis. GeneSpring 5.0 (Silicon Genetics, CA, USA) software was used to compare changes in gene expression between treatments. More than 2.0-fold and <0.5-fold changes in gene expression were the cut-offs used for determination of significant changes in expression.

RT-PCR. A subset of high- and low-expressed genes was confirmed by RT-PCR with sequence-specific primers. Reverse transcription of mRNA into cDNA was performed using a Bulk First-Strand cDNA reaction mix (Amersham Biosciences, NJ, USA). Briefly, 1 μg of mRNA was reverse transcribed with pd(N)6 primer in a final volume of 30 μl, and the resulting cDNA was amplified by PCR. The program used requires suitable cycles with the following conditions: 95˚C for 30 sec (10 min for the first cycle); 55˚C for 1 min; and 72˚C for 2 min (10 min for the last cycle). PCR products were separated by 2% agarose gel electrophoresis. PCR primers were as follows: BMX sense, 5'-GTTGCCAGCAGAGCTGTAAA-3' and antisense, 5'-GGAGATGTTACCAGCAAACCA-3'; CH1CL sense, 5'-AGATGACTGAATACTGGGTTCCAGA-3' and antisense, 5'-GGCAGTCCCTTTGTCAACTTTTC-3'; DACH1 sense, 5'-TGCTGCTACCAATGCAGCTAT-3' and antisense, 5'-TGTTCACGCCGTTTCGTCT-3'; GAPDH sense, 5'-ATC ATCAGCAATGCCTCCTG-3' and antisense, 5'-CTGCTTC ACCACCTTCTTGA-3'; JAK-2 sense, 5'-ACCTCTAAGTG CTCTGGATT-3' and antisense, 5'-TCTCTTAGGTGCTC TTCAGT-3'; RARA sense, 5'-CAACAGCTCAGAACAAC GTG-3' and antisense, 5'-GATCTCCATCTTCAGCGTGA-3'; TGFBR2 sense, 5'-TGTGTGACTTTGGGCTTTCC-3' and antisense, 5'-TGTTTAGGGAGCCGTCTTCA-3'; and WNT5A sense, 5'-CCGATTTAGCAGTGTCAGCGT-3' and antisense, 5'-CCTGTGCCTTCGTGCCTATTT-3'.

Western blot analysis. LNCaP cells grown to 70% confluence were treated with 1 nM DHT in the absence or presence of 100 ng/ml BMP-2 for 24 h. After treatment, cell cultures were washed twice with phosphate-buffered saline (PBS) and incubated for 24 h at 37˚C in fresh phenol red-free RPMI-1640 (serum-free). Conditioned medium was then harvested, centrifuged to remove cells, and concentrated using Microcon-YM10 tubes (Millipore, Bedford, MA, USA). Cell number-standardized conditioned medium was analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were transferred to a polyvinylidene difluoride membrane. The blots were blocked at 4˚C with 5% non-fat milk overnight in PBS-T (10 mM PBS and 0.05% Tween-20) and incubated for 2 h at room temperature with a polyclonal anti-Wnt5a antibody (Santa Cruz Biotechnology, CA, USA). The blots were then washed three times and incubated for 1 h with HRP-

Table I. Genes up-regulated in LNCaP cells after treatment with DHT and BMP-2 rather than DHT alone.

Gene category	Gene name	Gene symbol	Affymetrix ID	Fold change
Cell cycle	Cell division cycle 25A	CDC25A	204695_at	3.743
Enzyme	Poly(ADP-ribose) glycohydrolase	PARG	205060_at	3.673
	Enoyl-coenzyme A, hydratase/3-hydroxyacyl coenzyme A dehydrogenase	EHHADH	205222_at	2.419
	Cytochrome P450, family 20, subfamily A, polypeptide 1	CYP-M	219565_at	2.368
	IMP (inosine monophosphate) dehydrogenase 1	IMPDH1	204169_at	2.079
	Glucose-6-phosphatase, catalytic, 2	IGRP	221453_at	2.032
Signal transducer	Platelet derived growth factor C	PDGFC	218718_at	21.046
	Transforming growth factor, ß receptor II	TGFBR2	208944_at	12.357
	RAS protein activator like 2	RASAL2	219026_s_at	4.969
	BMX non-receptor tyrosine kinase	BMX	206464_at	3.352
	Janus kinase 2 (a protein tyrosine kinase)	JAK2	205841_at	2.974
	Muscle, skeletal, receptor tyrosine kinase	MUSK	207633_s_at	2.402
	Protein tyrosine phosphatase-like, member a	PTPLA	219654_at	2.249
	Transient receptor potential cation channel, subfamily C, member 3	TRPC3	206425_s_at	2.249
	Frizzled homolog 3 (*Drosophila*)	FZD3	219683_at	2.227
	Cannabinoid receptor 2 (macrophage)	CNR2	206586_at	2.176
	Transmembrane 4 superfamily member tetraspan	NET-7	218693_at	2.167
	Norrie disease (pseudoglioma)	NDP	206022_at	2.089
	Low density lipoprotein receptor-related protein 6	LRP6	34697_at	2.030
	Inositol 1,4,5-triphosphate receptor, type 1	ITPR1	203710_at	2.015
Structural molecule	Tubulin, α 8	TUBA8	220069_at	11.805
Transcription regulator	Hairless homolog (mouse)	HR	220163_s_at	2.926
	T-cell acute lymphocytic leukemia 1	TAL1	206283_s_at	2.306
	Retinoic acid receptor, α	RARA	203749_s_at	2.148
	Zinc finger protein 165	ZNF165	206683_at	2.133
Translation	Mitochondrial translational release factor 1	MTRF1	219822_at	5.145
	Chromosome condensation 1-like	CHC1L	204759_at	2.110
	CUG triplet repeat, RNA binding protein 2	CUGBP2	202157_s_at	2.075
Transporter	ATPase, Ca^{++} transporting, cardiac muscle, fast twitch 1	ATP2A1	205444_at	10.292
	Rattus norvegicus sodium/calcium/potassium exchanger	SLC24A1	206081_at	3.369
	ATP-binding cassette, subfamily A (ABC1), member 3	ABCA3	204343_at	2.001
Unclassified	SMT3 suppressor of mif two 3 homolog 2 (yeast)	SMT3H2	213879_at	2.529
	Ecotropic viral integration site 2A	EVI2A	204774_at	2.473
	Low density lipoprotein-related protein 2	LRP2	205710_at	2.473
	Chromosome 21 open reading frame 107	WDR9	219280_at	2.302
	Cytidine and dCMP deaminase domain containing 1	NYD-SP15	221015_s_at	2.137
	Hypothetical protein LOC340562	LOC340562	213282_at	2.127
	Nudix (nucleoside diphosphate linked moiety X)-type motif 13	LOC51055	207917_at	2.060

conjugated secondary antibodies. Immunoreactive proteins were visualized using an enhanced chemiluminescence detection system (Amersham Biosciences).

Results

DHT-induced growth of LNCaP cells was inhibited by BMP-2. To analyze the effect of BMP-2 on DHT-induced proliferation of LNCaP cells, cells grown for 24 h in androgen-depleted medium were treated with 1 nM DHT alone and with 1 nM DHT plus 100 ng/ml BMP-2. After treatment for 1, 2 and 4 days, cell growth was analyzed by crystal violet assay (Fig. 1). The number of cells treated with 1 nM DHT was approximately 2.1-fold greater on day 4 than the number of untreated control cells. However, treatment with a combination of DHT and BMP-2 yielded only a 1.5-fold increase. In other words,

Table II. Genes down-regulated in LNCaP cells after treatment with DHT and BMP-2 rather than DHT alone.

Gene category	Gene name	Gene symbol	Affymetrix ID	Fold change
Apoptosis regulator	Baculoviral IAP repeat-containing 7 (livin)	BIRC7	220451_s_at	0.475
Cell adhesion molecule	Vinexin ß (SH3-containing adaptor molecule-1)	SCAM-1	209253_at	0.342
	Vasodilator-stimulated phosphoprotein	VASP	202205_at	0.457
Enzyme	Glucosaminyl (N-acetyl) transferase 3, mucin type	GCNT3	219508_at	0.203
	Transmembrane protease, serine 3	TMPRSS3	220177_s_at	0.281
	A disintegrin and metalloproteinase domain 18	ADAM18	207597_at	0.360
	Cytochrome b-561	CYB561	210816_s_at	0.447
	Sialyltransferase 7D	SIAT7D	220937_s_at	0.478
Enzyme regulator	Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 13	SERPINB13	211362_s_at	0.212
Motor	Kinesin family member 5A	KIF5A	205318_at	0.400
Signal transducer	Toll-like receptor 7	TLR7	220146_at	0.110
	Toll-like receptor 3	TLR3	206271_at	0.204
	Leukemia inhibitory factor receptor	LIFR	205876_at	0.235
	Inositol polyphosphate-4-phosphatase, type II, 105 kDa	INPP4B	205376_at	0.246
	Tachykinin receptor 1	TACR1	208048_at	0.276
	IL2-inducible T-cell kinase	ITK	211339_s_at	0.328
	Endothelin receptor type B	EDNRB	204271_s_at	0.340
	Taste receptor, type 2, member 16	TAS2R16	221444_at	0.363
	Rho GDP dissociation inhibitor (GDI) α	ARHGDIA	213606_s_at	0.372
	vav 2 oncogene	VAV2	205536_at	0.397
	Protein kinase, cGMP-dependent, type II	PRKG2	207505_at	0.411
	Mitogen-activated protein kinase 8 interacting protein 1	MAPK8IP1	213014_at	0.444
	InaD-like protein	INADL	214705_at	0.453
	Calcitonin receptor-like	CALCRL	206331_at	0.455
	Mitogen-activated protein kinase kinase kinase 8	MAP3K8	205027_s_at	0.462
	Wingless-type MMTV integration site family, member 5A	WNT5A	205990_s_at	0.491
Structural molecule	Amelogenin (amelogenesis imperfecta 1, X-linked)	AMELX	208410_x_at	0.112
	Peanut-like 1 (*Drosophila*)	PNUTL1	209767_s_at	0.322
	Myosin, heavy polypeptide 13, skeletal muscle	MYH13	208208_at	0.326
	Myosin binding protein C, slow type	MYBPC1	214087_s_at	0.367
Transcription regulator	Histone 1, H4f	H4FC	208026_at	0.031
	Chromodomain helicase DNA binding protein 2	CHD2	203461_at	0.280
	ets variant gene 7 (TEL2 oncogene)	ETV7	221680_s_at	0.290
	Histone 1, H4g	H4FL	208551_at	0.400
	Homeo box B6 (HOXB6)	HOXB6	205366_s_at	0.442
	SWI/SNF related, matrix associated, actin dependent regulator of chromatin	SMARCA1	203874_s_at	0.446
	TEA domain family member 1	TEAD1	214600_at	0.481
	Dachshund homolog 1 (*Drosophila*)	DACH	205471_s_at	0.487
Translation	Ribosomal protein S20	RPS20	216247_at	0.045
	Polypyrimidine tract binding protein 1	PTBP1	212016_s_at	0.469
Transporter	Solute carrier organic anion transporter family, member 1B1	SLCO1B1	210366_at	0.063
	Solute carrier family 7, member 1	SLC7A1	206566_at	0.449
	Sodium channel, voltage-gated, type I, ß	SCN1B	205508_at	0.489
	Adaptor-related protein complex 4, mu 1 subunit	AP4M1	209837_at	0.500
Unclassified	Spermatogenesis associated 1	SPATA1	221057_at	0.303
	Chromosome 13 open reading frame 24	PIBF1	213239_at	0.348
	Transgelin	TAGLN	205547_s_at	0.357
	Chromosome 8 open reading frame 1	C8orf1	41553_at	0.493

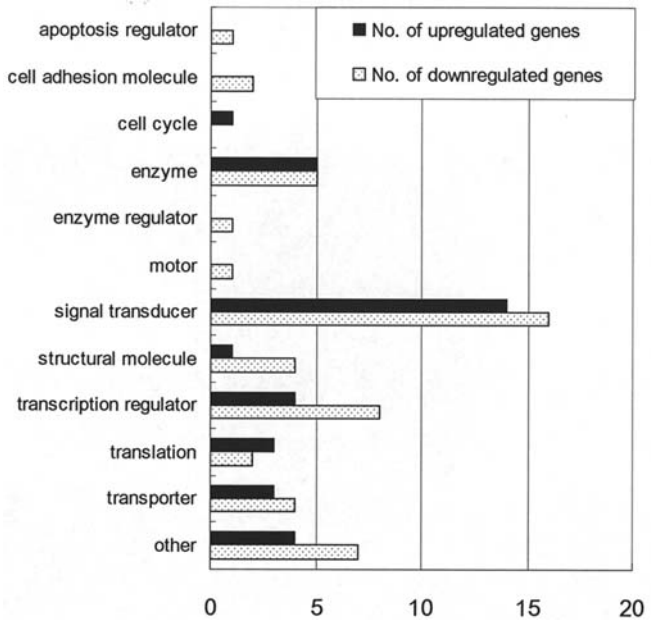

Figure 2. Categories of genes showing differential expression in response to BMP-2. Genes expressed in LNCaP cells treated with DHT alone or with a combination of DHT and BMP-2 were assigned to categories according to biological functions per the Gene Ontology database. The numbers of up- and down-regulated genes are shown for each gene category.

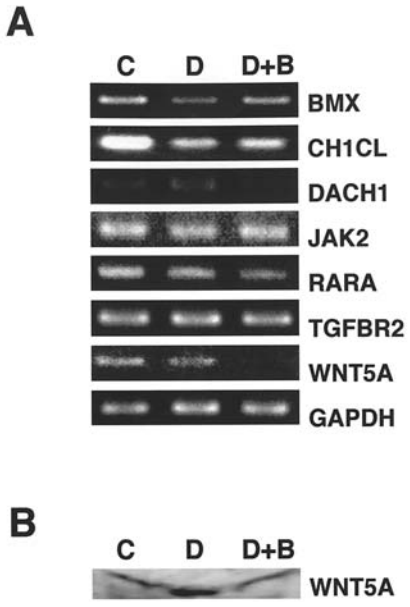

Figure 3. (A) Expression levels of various mRNAs in LNCaP cells treated with DHT alone and with a combination of DHT and BMP-2. mRNAs from untreated (control) LNCaP cells, cells treated with DHT alone and cells treated with both DHT and BMP-2 for 24 h were extracted and used as templates for the synthesis of cDNAs. After normalization of cDNA concentrations, RT-PCR analysis was performed as described in Materials and methods. The data shown are control (C), DHT (D), and DHT plus BMP-2 (D+B). (B) Effect of DHT and BMP-2 on the secretion of WNT5A protein in LNCaP cells. Cells were treated with DHT alone and a combination of DHT and BMP-2 for 24 h. After treatment, cells were incubated in fresh phenol red-free RPMI-1640 (serum-free) for 48 h. Conditioned medium was concentrated with Microcon-YM10 tubes and subjected to Western blot analysis. Western blot analysis was performed as described in Materials and methods. The data shown are control (C), DHT (D), and DHT plus BMP-2 (D+B).

treatment with BMP-2 resulted in a 57% reduction of DHT-induced proliferation of LNCaP cells on day 4.

Analysis of gene expression by DNA microarray analysis. To investigate the mechanism underlying the action of BMP-2 on androgen-dependent prostate cancer LNCaP cells, RNA samples were prepared from cells treated with 1 nM DHT alone and with 1 nM DHT plus 100 ng/ml BMP-2 for 24 h and analyzed on the Affymetrix Human Genome Focus GeneChip Array. To fully understand the biological significance of the global changes in gene expression, microarray expression data were analyzed by GeneSpring 5.0 software and categorized by the Gene Ontology database. Genes up-regulated and down-regulated by co-treatment with BMP-2 and DHT compared with treatment with DHT alone are listed in Tables I and II. Thirty-eight genes were up-regulated by >2.0-fold, and 48 genes were down-regulated by <0.5-fold. There were relatively few up-regulated genes, approximately 79% of the number of down-regulated genes.

These 86 differentially expressed genes were assigned to various functional gene categories by the GeneOntology database. Thirty (34.9%) genes were classified as signal transducers, 12 (14.0%) were transcription regulators, 10 (11.6%) were enzymes, 7 (8.1%) were transporters and 5 (5.8%) were structural molecules and translations (Fig. 2). The inhibitory effect of BMP-2 on the androgen-dependent growth of LNCaP cells may be associated with modulation of a variety of signal transducers and transcription regulators. Signaling molecules in a variety of pathways were modulated in LNCaP cells treated with BMP-2 for 24 h. These signaling molecules include components of the mitogen-activated protein kinase (MAPK) pathway, such as MAPK8IP1 and MAP3K8; the inositol phosphorylation pathway, such as INPP4B and ITPR1; and the Ras-related pathway, such as CHC1L, GDI and RASAL2. Furthermore, the expression of other signaling proteins involved in cell proliferation and differentiation, such as WNT5A, DACH1, BMX and JAK2, was altered. Interestingly, receptors regulating cell proliferation, including TGFBR2 and RARA, were up-regulated by co-treatment with BMP-2. In addition, BMP-2 regulated the expression of molecules related to chromatin remodeling, such as CHD2 and SMARCA1.

Confirmation of DNA microarray data. To confirm the gene expression changes observed in the microarray analysis, several genes related to proliferation or malignant changes were selected and analyzed by RT-PCR. RT-PCR analysis was performed using mRNA samples isolated from untreated (control) LNCaP cells, LNCaP cells treated with 1 nM DHT alone, and LNCaP cells treated with 1 nM DHT and 100 ng/ml BMP-2 for 24 h in phenol red-free RPMI-1640 with 5% charcoal-dextran-stripped FBS. The results are shown in Fig. 3A. RT-PCR analyses revealed patterns of expression similar to those of the microarray analysis for several genes. Increased expression of CH1CL and BMX genes by BMP-2 was confirmed by RT-PCR. A decrease in the expression of DACH1 and WNT5A by BMP-2 was also confirmed. However, the increase in expression of RARA, JAK2 and TGFBR2 was not confirmed. Next, we examined the alteration

of the WNT5A protein level in medium after treatment with DHT alone and DHT plus BMP-2 using Western blot analysis (Fig. 3B). Secretion of WNT5A was increased by treatment with DHT alone, and the increase was inhibited by co-treatment with BMP-2.

Discussion

In the present study, we showed that BMP-2 induced growth arrest of LNCaP cells is accompanied by a substantial change in gene expression. BMP-2 was first identified as a factor that induces bone and cartilage formation, and the BMP pathway was subsequently shown to be involved in cellular differentiation, organogenesis, chemotaxis and cellular proliferation (13,14). While several gene expression studies have been performed on BMP-2-treated mesenchymal-derived cells, little is known about the gene expression profile in BMP-2-treated neoplasias. Comparison of our results with those of other microarray studies of the effects of BMP-2 on mesenchymal precursor cells did not reveal much overlap in the putative target genes (15-18). This suggests that the effect of BMP-2 in different cells may involve different molecular mechanisms and that the regulation of gene expression by BMP-2 is cell-specific.

To define how BMP-2 inhibits the DHT-induced proliferation of LNCaP cells and thus affects various cellular functions, differentially expressed genes were assigned to categories according to their biological functions per the Gene Ontology database. This categorization of data revealed that genes involved in signal transduction and transcription regulation showed the greatest changes in expression, suggesting that several types of genes are involved in the growth inhibition by BMP-2. RT-PCR analysis confirmed that expression of CH1CL and BMX was up-regulated and that expression of DACH1 and WNT5A was down-regulated.

The CHC1L gene encodes a member of the RCC1-related guanine nucleotide exchange factor (GEF) family. The N-terminal half of the amino-acid sequence is similar to the regulator of chromosome condensation RCC1, which acts as a GEF protein for the Ras-related GTPase Ran (19,20). Loss of heterozygosity of chromosome 13q14.2-q14.3 is one of the most frequent aberrations observed in prostate tumors (21-25). CHC1L is located in the last region and differs significantly in expression between normal and neoplastic prostate tissue (26). CHC1L may be important in the control of nuclear-cytoplasmic transport and cell cycle progression through a Ras-related signal transduction pathway. BMX is a member of the Btk tyrosine kinase family and is preferentially expressed in epithelial and endothelial cells including prostate cancer cells (27). IL-6 induced both suppression of growth and neuroendocrine-like differentiation and BMX is required for this process (28). Although the role of pathways via CHC1L or BMX have yet to be identified in the prostate, the change in expression of these genes may be involved in the BMP-2-mediated growth inhibition of LNCaP cells.

DACH1 is a member of the Ski gene family. Ski oncoprotein dramatically affects cell growth, differentiation, and/or survival through interaction with various cofactor transcription factors including Smads. Some groups have reported that Ski represses BMP signaling by interacting with and repressing the activity of Smad complexes (29,30). Wu *et al* showed that DACH1 participates in the negative regulation of TGF-ß signaling by interacting with NCoR and Smad4 (31). The decrease in expression of the DACH1 gene may lead to the enhancement of BMP-2 signaling in LNCaP cells. WNT5A is a member of the WNT gene family, which consists of structurally related genes that encode secreted signaling molecules. Several studies suggest that WNT5A has growth-enhancing or oncogenic properties (32-35). Furthermore, up-regulation of the WNT5A mRNA level has been observed in human primary prostate cancer (36). These results indicate the possibility that down-regulation of the expression of DACH1 and WNT5A plays an important role in the process of BMP-2 induced growth inhibition of LNCaP cells.

In conclusion, our results indicate that BMP-2 increases the expression of CHC1L and BMX genes and decreases the expression of WNT5A and DACH1 genes, raising the possibility that these genes are involved in the BMP-2-mediated inhibition of LNCaP cell proliferation. Our analyses also revealed several novel putative BMP-2 responsive genes involved in a variety of cellular functions and provide support for a role of BMP-2 in the inhibition of cell proliferation. Further studies are needed to clarify the roles of these genes in the inhibition of prostate cancer cell proliferation by BMP-2.

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