

bone

morphogenetic proteins

bone
morphogenetic proteins

2001 6

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bone morphogenetic proteins

Bone morphogenetic proteins (BMPs) transforming growth factor-
(TGF-) superfamily ,

, ,
BMPs

, BMP-4 -7

BMPs I II BMP
. BMP ligand가 II
I , Smad
cascade가 . BMP
(BMPRs) I (ActR-I, BMPR-IA
BMPR-IB) II (ActR-II, ActR-IIB BMPR-II)가
, BMPR-IA, BMPR-IB BMPR-II가 BMP-specific
receptors .

BMPs

BMP-4, -6 -7

BMPs ,

BMP .

112, 117 181 ,

BMP-6

112

112 pSBE4 (plasmid containing luciferase reporter gene under the control of 4 tandem repeats of Smad binding element) transient transfection , BMP-6 (0 ng/ml) (100 ng/ml, 16) luciferase activity , BMP-6 luciferase activity가 가 BMP-6 Smad cascade .

117 181 BMP-6 luciferase activity 가 , BMPR-II pSBE4 transient co-transfection BMP-6 (0ng/ml) (100ng/ml, 16) luciferase activity , BMP-6 luciferase activity가 가 Northern blot analysis Western blot analysis BMPR-II mRNA BMPR-II 112 , 117 181 .

112 BMPRs가 BMP-6-BMPR ligation Smad cascade pathway가 가 , 112 BMP-6 .

117 181 BMPR-II Smad cascade pathway 가 117 181 BMP-6 BMPR-II . BMPR-II

가 , 가

: , bone morphogenetic protein, bone morphogenetic protein

**bone morphogenetic
proteins**

< , >

I.

Bone morphogenetic proteins (BMPs)

1-3

transforming growth factor- (TGF-), activin/inhibins, Müllerian
inhibiting substance TGF- superfamily , 30-38 kDa
dimeric protein 가

10 BMPs subfamily가⁴ BMPs

3 subgroup: (a) BMP-2 BMP-4; (b) BMP-5, BMP-6
BMP-7; (c) BMP-3⁴

BMPs alkaline phosphatase activity,
collagen , proteoglycan

5

chemotaxis, osteoblast , BMPs^{6,7}

apoptosis⁸ ,

가

9-10

BMPs BMP (BMP receptors; BMPRs) TGF-
transmembrane serine-threonine kinase receptor family ligand
receptor complex I II 가 heteromeric
cross-phosphorylation , Smad cascade
target gene (1).¹¹ BMP
I (ActR-I, BMPR-IA BMPR-IB)
II (ActR-II, ActR-IIB BMPR-II)가 .
BMPR-IA BMPR-IB BMPR-II TGF-
superfamily BMPs가 ,¹¹ ActR-II
ActR-IIB, ActR-I BMP-4 ¹² BMPR-IA, BMPR-IB
BMPR-II가 BMP-specific receptors .
BMP family가

¹³ ¹⁵ , ¹⁶ , ¹⁷ ¹⁸

BMP 가 .

BMP-6

,

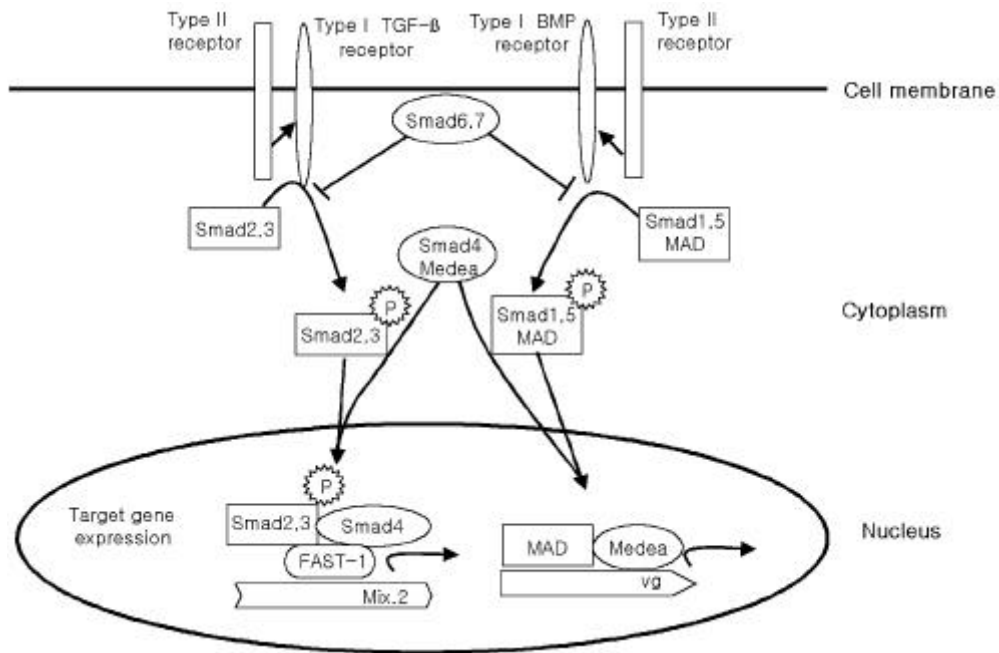
가 ,¹⁴ Thomas ¹⁵

BMP-4 BMP-7 mRNA ,

BMP-7

BMP-7

. androgen withdrawal therapy



1. BMP TGF- signaling pathway.

Smad TGF- superfamily

. Ligand-binding type II type I

, type I Smad1, 2, 3 5 .

Smads Smad4 vg (vestigial promoter)

DNA ,¹⁹ Mix.2 nuclear transcription factor

.²⁰ Smad6 7 type I 가 Smad1, 2, 3 5

²¹ Medea Smad4 Drosophila homologue

Media가 vg 가 .²²

BMPR-IA BMPR-II mRNA
 , BMPR-IB mRNA 가
 , BMPR-IB
¹³
 가 가
 BMPs 가
 BMP-2 BMP-4 ,
¹⁶ BMP-2 BMPR-IA
 BMPR-II mRNA 2 12 가
 , BMP-2
¹⁸
 BMPs가
 , Jena ²³ BMP-7 (knock-out)
 ,
 BMP-7
 , Miyazaki ²⁴ BMP-4
 .
 BMP-2 ²⁵
 BMP-4 BMP-7 branching
 morphogenesis ²⁴ BMP-7
 가 , ²⁶ BMPs
 가 .
 BMPs
 , BMP-4 BMP-7,
 BMP-7

BMP-6

.

BMP-4, BMP-6 BMP-7

, BMPs가

BMPRs

Smad

BMPRs

Smad

.

II.

1.

112, 117, 181 (1)²⁷

(National Cancer Institute, Bethesda, MD, USA)

RPMI- 1640 (Gibco BRL, Grand Island, NY, USA) 10%

(Gibco BRL) penicillin (100 U/ml), streptomycin (100 µg/ml,

Gibco BRL) 가 .
가 1:5 .

1.	112, 117	181	
	112	117	181
Histologic type	Carcinoma, papillary cell	Carcinoma, mixed cell	Carcinoma, clear cell
Tumorigenicity	*	*	*
Chromosome 3p allelic loss			

*: nude mice colony

2. BMP-4, BMP-6 BMP-7

가.

0.5% trypsin (Gibco BRL)
1200 rpm 10
3 pipeting
, 15 $\mu\ell$
0.4% trypan blue 15 $\mu\ell$ 95%
90% 24 well
2 4 5 $\times 10^3$, 10 $\times 10^3$, 20 \times
10³, 50 $\times 10^3$, 37, 5% CO₂ 4
hemocytometer 4

. BMP-4, BMP-6 BMP-7

24 well
well 20 $\times 10^3$ 24
1% RPMI-1640 BMP-4, BMP-6, BMP-7
(R&D Systems, Minneapolis, MN, USA) well BMPs
가 10 ng/ml, 100 ng/ml, 500 ng/ml가 4

2 1 , 4 0.5%
 trypsin (Gibco BRL)
 hemocytometer BMPs

$$= \frac{\quad}{\quad} \times 100$$

3 , BMPs

3. Transient transfection luciferase activity

가. BMP-6 Smad
 binding element promoter activity

6 well plate 1×10^6 ,
 luciferase reporter gene pSBE4
 (plasmid containing luciferase reporter gene under the control of 4
 tandem repeats of Smad binding element) lipofectin (Gibco-BRL)
 37 , 5% CO₂ 24 transient
 transfection .²⁸ 가 24

100 ng/ml BMP-6가
 16 enhanced luciferase activity kit (Analytical
 Luminescence Laboratory, CA, USA) luciferase activity
 pSBE4 promoter activity

BMPr-II Smad
 binding element promoter activity

6 well plate 1×10^6 , Dr. Kohei Miyazono
 (Department of Biochemistry, The Cancer Institute, Japanese Foundation
 for Cancer Research, Tokyo, Japan) BMPr-II cDNA 1 μ g 1
 μ g pSBE4, 12 μ l lipofectin 1 Ml transfection medium
 (Gibco-BRL) well 24 37, 5% CO₂
 24

, 100 ng/ml BMP-6가
 16 enhanced luciferase activity kit (Analytical
 Luminescence Laboratory) luciferase activity
 pSBE4 promoter activity

4. mRNA

mRNA MiniRiboSepTH mRNA
 isolation kit (Becton Dickinson Labware, Bedford, MA, USA)

1 x 10⁷ 37 lysis
 buffer 10 ml proteinase K 50 μℓ 45 2
 resin elution buffer 2 Mℓ 3000 x
 g elution buffer 2
 Mℓ 3000 x g 1
 1 Mℓ binding buffer 2
 pellet resin 1 Mℓ binding buffer lysate
 600 μℓ 5M NaCl 60
 3000 x g 5 5 Mℓ
 binding buffer 3000 x g
 2 pellet 250
 μℓ binding buffer . Microfuge tube column
 pellet 5000 x g 10
 microfuge tube column 300 μℓ binding buffer 5000 x g
 10 3 . Microfuge tube
 column microfuge tube 250
 μℓ elution buffer 5000 x g 10
 microfuge tube 250 μℓ elution buffer
 column 5000 x g 10 . Microfuge tube
 500 μℓ -70 5000 x g
 30 2
 pure mRNA diethylpyrocarbonate water
 spectrophotometer 240 nm

5. Northern blot analysis

Dr. Kohei Miyazono BMPR-IA, BMPR-IB BMPR-II

cDNA [^{-32}P]dCTP (3000 Ci/mmol, Amersham, Buckinghamshire, UK
) , Prime-It^R RmT random primer labelling kit (Stratagene, La Jolla, CA,
USA) probe labelling probe

specific-radioactivity 1×10^9 dpm/ μg .

112 117, 181 2 μg mRNA 100 V 3 1%

formaldehyde agarose gel 20 x SSC (1 x SSC =
0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) capillary

transfer nylon membrane (Amersham) .

membrane cross-linking mRNA

membrane . Membrane prehybridization (50%
formamide, 0.12 M Na_2HPO_4 , pH 7.2, 0.25 M NaCl, 7% wt/vol SDS 250
 $\mu\text{g/ml}$ heat-denatured salmon sperm DNA) 42 , 2

prehybridization [^{-32}P] dCTP-labelled BMPRs probe

 42 24 hybridization . Hybridization

 membrane washing solution I (2 x SSC, 0.1% SDS)

 15 , washing solution II (0.5 x SSC, 0.1% SDS)

15 , washing solution III (0.1 x SSC, 0.1% SDS) 30

 . membrane -70 X-ray film 24

 BMPRs mRNA .

6. Western blot analysis

112 117, 181 RIPA buffer [50 mM Tris ·
 HCl, pH 8.0, 150 mM NaCl, 1% NP40, 0.1% sodium deoxysulfate (SDS),
 10 mM sodium deoxycholate] 13,000 rpm
 5 .

Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond,
 CA, USA) . Bradford ²⁹ 595nm
 spectrophotometer , (Gibco BRL) standard
 curve . 50 µg
 12% SDS-PAGE (polyacrylamide gel electrophoresis) (Hoefer
 miniVE, Pharmacia biotech, Piscataway, NJ, USA) .

, Hybond-ECL nitrocellulose filter paper (Amersham)
 . filter paper blocking solution (25 mM Tris ·HCl,
 pH 8.0, 125 mM NaCl, 0.1% Tween20, 5% dry milk) 1
 , membrane TBS (0.5% tween20) 1
 BMPR-IA, BMPR-IB BMPR-II (1:400, R&D systems,
 0.1% nonfat dry milk) 4 . PBS (0.5%
 tween20) 1 horseradish peroxidase-labeled goat
 antirabbit antibody (Bethyl Laboratories Inc., Montgomery, TX, USA)
 1:5000(0.1% nonfat dry milk) 1
 . TBS (0.5% tween 20) 1 membrane
 enhanced chemiluminescence (Pharmacia biotech) .

7.

,
unpaired Student's t-test, BMPs

² trend test , p

0.05 .

III.

1. BMPs

가.

BMPs가

4 5 50 x 10³
112, 117 181 doubling time 24
, well 20 x 10³ 4
20 x 10³

. BMPs

BMPs가

BMP-4, BMP-6 BMP-7

4

112, 117 181 BMP-4
(2). BMP-7 100 ng/ml 500 ng/ml
112
, 117 181
(3). BMP-6 117 181

2. BMP-4

(ng/ml)			
	112	117	181
0	100 ± 8.1	100 ± 8.4	100 ± 9.6
10	97.2 ± 7.7	97.2 ± 5.2	101.3 ± 10.5
100	103.4 ± 4.3	105.4 ± 10.3	98.9 ± 8.0
500	102.9 ± 4.7	104.2 ± 12.8	106.7 ± 16.4

4 112, 117 181 BMP-4 가 3

3. BMP-7

(ng/ml)			
	112	117	181
0	100 ± 9.7	100 ± 7.4	100 ± 5.2
10	102.3 ± 9.2	100.1 ± 8.1	98.2 ± 5.9
100	86.9 ± 6.7	98.1 ± 13.7	101.5 ± 8.5
500	82.7 ± 4.6	107.8 ± 12.9	102.6 ± 12.3

4 112, 117 181 BMP-7 가 3

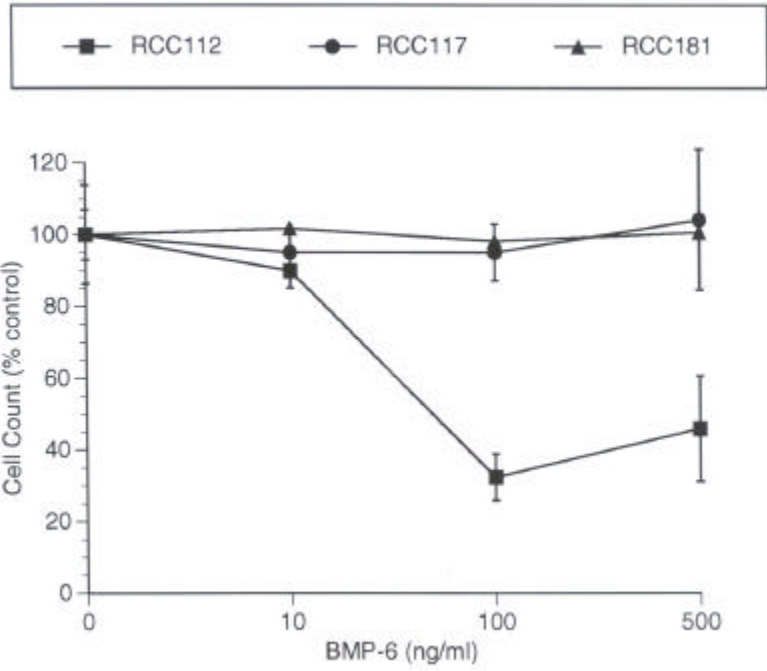
(4, 2), 112
(p<0.05).

4. BMP-6

(ng/ml)	112*	117	181
0	100 ± 9.7	100 ± 6.9	100 ± 13.7
10	89.9 ± 9.2	95.1 ± 4.8	101.7 ± 5.9
100	32.3 ± 6.7	95.1 ± 6.6	98.3 ± 7.9
500	45.9 ± 4.6	104.2 ± 14.8	100.7 ± 19.6

4 112, 117 181 BMP-6 가
3 112
BMP-6

* p < 0.05, ² trend test



2. BMP-6

112, 117, 181 BMP-6 가
 4 . 3
 181 BMP-6
 , 112 BMP-6
 (p < 0.05, ² trend test).

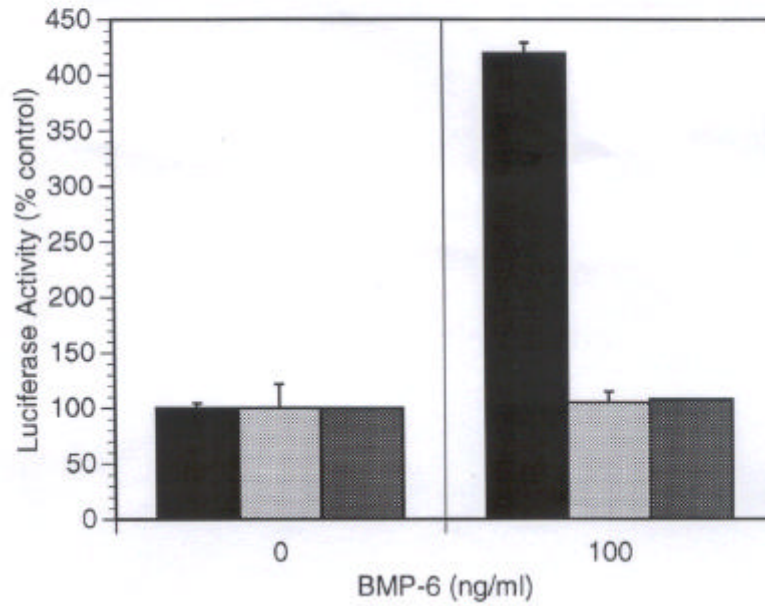
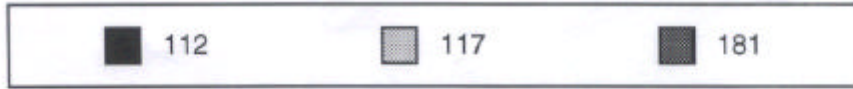
2. Smad pathway BMP - 6

가. BMP-6 Smad binding element promoter activity

BMP-6 Smad pathway Smad binding element 4 tandem repeat luciferase reporter gene , pSBE4 transient transfection , BMP-6 luciferase activity . 117 181 100 $\pm 13\%$, $100 \pm 21.5\%$ $104.6 \pm 9.3\%$, $107.1 \pm 9.9\%$ ($p > 0.05$).

112 BMP-6 $100 \pm 4.1\%$ $419.5 \pm 10.5\%$ 가 ($p < 0.05$)(3). BMP-6 Smad cascade .

BMP-6 luciferase activity 가 117 181 BMPR-II cDNA pSBE4 transient transfection BMP-6 luciferase activity . BMPR-II cDNA control DNA pSBE4 transient transfection



3. BMP-6 luciferase activity.
 1 × 10⁶ 112, 117 181 Smad-binding element가 4
 tandem repeat construct pSBE4 luciferase reporter construct transient
 transfection BMP-6 (0 ng/ml) (100 ng/ml, 16) luciferase
 activity . BMP-6 luciferase activity 100%
 . 3
 . 112 BMP-6 luciferase
 activity가 가 (p<0.05, unpaired Student's t-test).

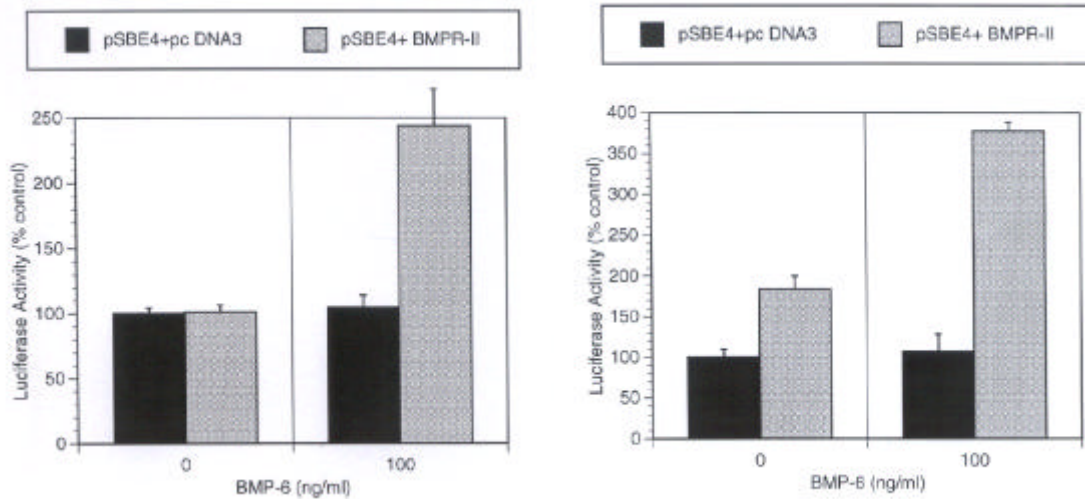
, 117 181 BMP-6 luciferase activity 가 . BMPR-II cDNA pSBE4 transient transfection , BMP-6 2 4 luciferase activity 가가 (: 101.0 ± 5.3%, 183.5 ± 16.2%, : 243.8 ± 28.3%, 377.5 ± 10.7%; 117, 118, p<0.05). BMP-6 BMPR-II 가 (4).

3. BMP

BMP-6 BMPR-II 가 가 , BMPRs Northern blot analysis Western blot analysis .

가. Northern blot analysis

mRNA Northern blot analysis 112 BMPR-IA, BMPR-IB BMPR-II mRNA . 117 181 BMPR-IA BMPR-IB mRNA , BMPR-II mRNA (5).



4. BMPR-II

BMP -6

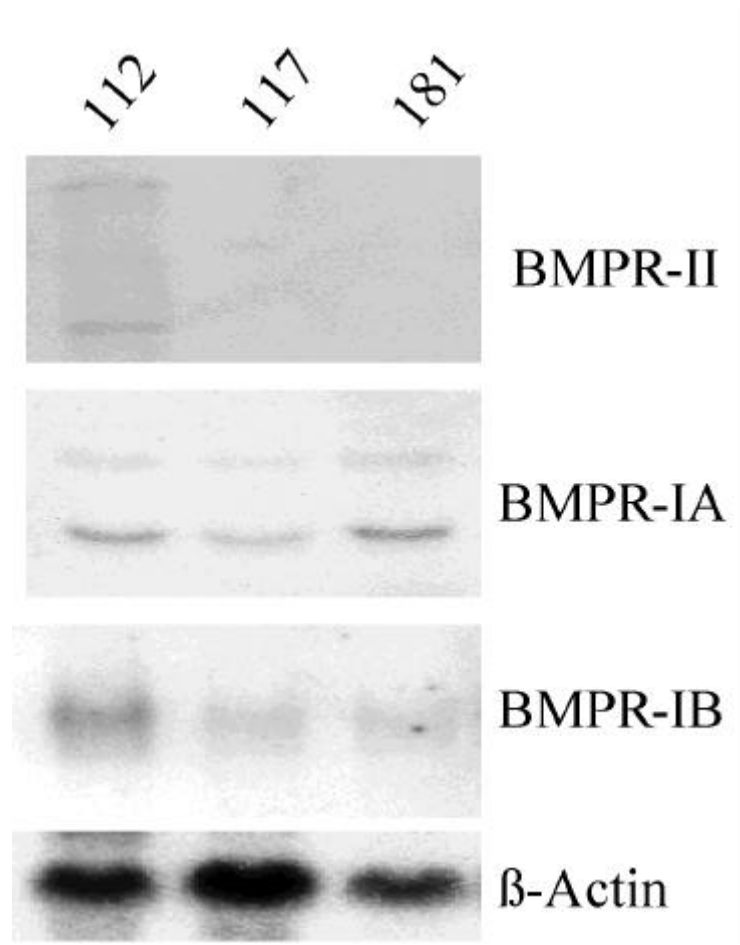
117

181 luciferase activity.

1 × 10⁶ (pcDNA3)
 BMPR-II (BMPR-II) pSBE4 transient co-transfection BMP-6
 (0ng/ml) (100ng/ml, 16) luciferase activity
 pSBE4 co-transfection BMP-6
 luciferase activity 100% . 3

117 181

, BMPR-II pSBE4 co-transfection , BMP-6
 luciferase activity가 가 (p<0.05, unpaired Student's t-test).

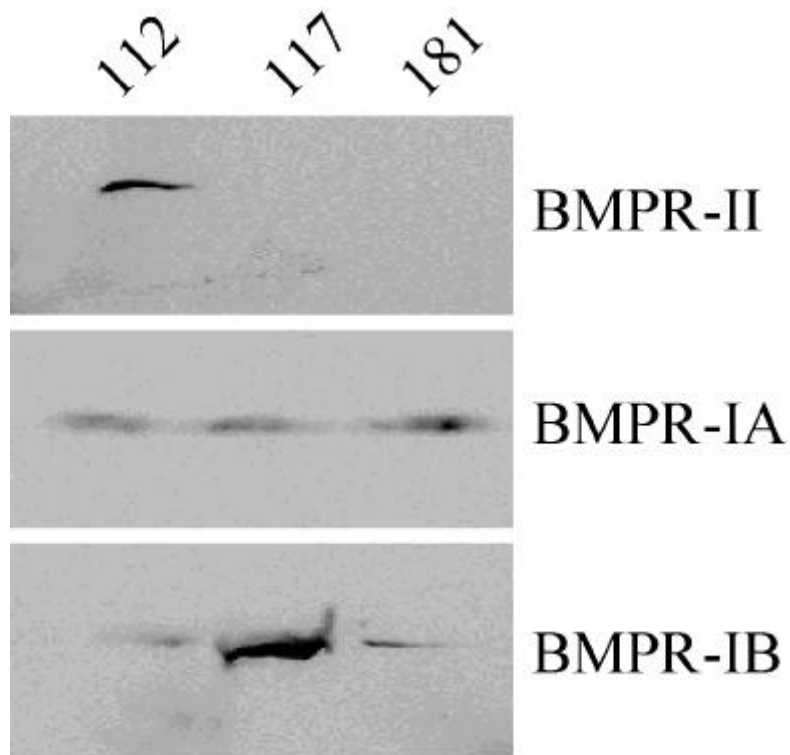


5. 112, 117 181 BMPRs Northern blot analysis.
 mRNA Northern blot analysis
 BMPR-II probe hybridization membrane stripping probe
 rehybridization . - actin, internal control.

. Western blot analysis

analysis
 ,
 BMPR-IB
 (6).
 Western blot
 BMPR-IA, BMPR-IB BMPR-II가
 112
 117 181 BMPR-IA
 , BMPR-II

luciferase reporter assay ,
 112 BMPR-IA, BMPR-IB BMPR-II가
 BMP-6-BMPR ligation Smad
 cascade pathway가 가 ,
 112 BMP-6
 . 117 181 BMPR-II
 Smad cascade pathway 가



6. 112, 117 181 BMPRs Western blot analysis.
 50 μ g Western blot analysis
 112 BMPR-IA, -IB -II
 117 181 BMPR-II가

IV.

BMPs .

, 19,22 , 13,14 9 .

가 23

, 17 , 18 , 16

가 . branching

morphogenesis BMP-7 BMP-4, BMP-2

BMPs , 19-21

가

가

BMPs

. BMPs TGF- superfamily subfamily 가

, TGF- Smad cascade

, subfamily

. BMPs ,

가 tubulogenesis BMP-7

, tumorigenesis

가 30

TGF- BMPs I

II BMP . BMP ligand가

II I .

I Smad1, 5

Smad4 DNA

.²⁶ I BMP II BMP

subtype , I ActR-I,

BMPR-IA BMPR-IB , II ActR-II, ActR-IIB

BMPR-II , *in vitro* TGF- superfamily

BMP BMPR-IA BMPR-IB BMPR-II

,¹¹ ActR-II, ActR-IIB ActR-I

BMP-4 ¹² BMPR-II BMPR-IA, BMPR-IB가

BMP-specific receptor .

¹³ ¹⁸ BMPRs

, BMPRs

BMPs oncogenesis

,¹³ ¹⁸

BMPs 가

가

. 30%

, 가

6 ³¹ vinca

alkaloid anthracyclines

³² interferon, interleukin

가
가
BMPs
molecular targeting

BMPs

BMP-4 BMP-7,

BMP-6

, BMP-4 BMP-7

, BMP-6

, BMP-6가 TGF-

BMP-6 osteoblast chondrocyte

keratinocyte

³⁴

가

³⁵

BMP-6가

가

TGF-

BMP-6가

가

가

BMP-6

BMP

BMP I II

가

BMPs가

II

Smad

²⁶ BMP-6 BMP

Smads (receptor-regulated Smads) Smad5 ³⁶

Smad1

BMP TGF- serine/threonine kinase receptor

family I II , TGF-

가 가

³⁷ ³⁹ Gleason score가 I II

TGF- , ³⁷

가 가 TGF- I II

^{38,39} 가

TGF- I 가

apoptosis 가 , ⁴⁰ TGF- II

가 ⁴¹ 가

TGF- TGF-

Kim ⁴² BMP Gleason

score가 , BMP

TGF- 가

, BMP

가 BMP

BMP-6

112 pSBE4 transient transfection

luciferase activity BMP-6 4
 luciferase activity가 가 , BMP-6 BMP
 Smad .
 BMP-6 가
 Smad luciferase activity 가가 .
 BMPR-II pSBE4 co-transfection
 luciferase activity , BMP-6
 117 2 luciferase activity가 가 ,
 181 4 가 luciferase activity가 가 .
 112 BMPR-IA, BMPR-IB BMPR-II가
 BMP-6-BMPR ligation Smad
 cascade pathway가 가 ,
 112 BMP-6 .
 117 181 BMPR-II
 Smad cascade pathway 가
 .
 Northern blot analysis 112 BMPR-II
 mRNA , 117 181
 BMPR-II mRNA . Western blot analysis
 112 BMPR-II ,
 117 181 BMPR-II
 . 117 181 BMPR-II가
 .
 117 181 BMP-6

BMPR-II

,

Smad cascade

BMP-6

.

BMPR-II

가

,

가

.

V.

bone morphogenetic proteins

bone

morphogenetic protein

bone

morphogenetic protein

1. Bone morphogenetic protein-6

가

2. bone morphogenetic protein-6

Smad

3. Bone morphogenetic protein-6

117 181 II bone morphogenetic protein

receptor

bone morphogenetic protein-6

Smad

가

4. Bone morphogenetic protein-6

117 181 II bone morphogenetic protein

receptor가

bone morphogenetic protein-6가

BMPR-II

가

가

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Abstract

Response of human renal cell carcinoma cells to bone morphogenetic proteins and the expression of bone morphogenetic protein receptors

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Bone morphogenetic protein is a pleiotropic growth factor that has been suggested to play critical role during the development and homeostasis of kidney. In the present study, we evaluated the response of bone morphogenetic protein-4, -6, and -7 (BMP-4, -6, and -7) to the human renal cell carcinoma cell lines, 112, 117, and 181. There were no growth inhibitory effect of BMP-4 and -7 on these three cell lines. However, the effect of BMP-6 was correlated with the expression of BMP receptors (BMPRs) in the human renal cell carcinoma cell lines, 112, 117, and 181. Of these three cell lines, BMP-6 inhibited the proliferation of 112 cells but not 117 and 181 cells in a dose-dependent manner. By Northern blot and immunoblot analysis, it was demonstrated that 117 and 181 cells have undetectable levels of expression of BMP receptor type II (BMPR-II).

To demonstrate that the level of defect in BMP-6 signaling is at the receptor level, the two BMP-6 resistant cell lines were infected with adenovirus containing constitutively active form of BMP receptor types IA and IB (BMPR-IA-CA and IB-CA, respectively). After the infection, the cells were transfected with pSBE4, a BMP-6-responsive luciferase reporter construct. The results demonstrated that the level of luciferase activity following adenovirus infections was elevated in both 117 and 181 cells, suggesting that the down-stream signaling molecules of BMP-6 is intact in these cell lines. Finally to demonstrate that the insensitivity to BMP-6 is due to the decreased levels of expression of BMPR-II, these cells were transiently co-transfected with BMPR-II and pSBE4. The results demonstrated that BMP-6 signaling was restored following transfection with BMPR-II. Taken together, these results demonstrate that the human RCC cell lines 117 and 181 are resistant to the growth inhibitory effect of BMP-6 because they have decreased levels of expression of BMPR-II.

Key Words: renal cell carcinoma, bone morphogenetic protein, bone morphogenetic protein receptor