Yonsei Medical Journal
Vol. 42, No. 6, pp. 581 - 586, 2001

Original Article

Comparison of Bone Morphogenetic Protein Receptors Expression in the Fetal and Adult Skin

Eun A Hwang[1], Hoon-Bum Lee[2], and Kwan Chul Tark[2]

[1]Department of Plastic and Reconstructive Surgery, National Health Insurance Corporation Ilsan Hospital, Koyang, Korea; [2]Department of Plastic and Reconstructive Surgery and Institute for Human Tissue Restoration, Yonsei University College of Medicine, Seoul, Korea.

Wounds on fetal skin can be repaired without leaving scars until the second trimester, but after this period, skin wounds leave scars as in adults. It's known that certain growth factors such as TGF-β, and bFGF are present at a very low levels during wound repair in fetal skin. These low levels of growth factors minimize inflammatory response and fibroblast proliferation at the wound site, which in turn inhibit collagen synthesis, and thus, allows scarless wound healing. Recently bone morphogenetic proteins (BMPs), one of the TGF-β superfamily members, have been studied in the wound healing process. According to several studies, BMPs are related to the differentiation and growth of epithelial and mesenchymal cells, but the precise functions of BMPs and of BMP receptors on skin wound healing have not been elucidated.

In this study, we investigated the expression pattern of BMP receptors in fetal skin during the second trimester and in adult skin by immunohistochemical staining and RT-PCR. BMP receptors were detected on the suprabasal epithelial cells and in the hair follicles in adult skin, but were not defected in the fetal skin except for the hair follicles. This was confirmed by confirming mRNA levels of BMP receptors by RT-PCR in both adult and fetal skins.

In conclusion, BMPs and BMP receptors seem to be related to fetal and adult wound healing, and low levels of BMPs and BMP receptors during the second trimester seem to contribute to scarless wound healing in the fetus, as is TGF-β during the second trimester.

Key Words: Fetal wound healing, BMP, BMP receptor, immunohistochemical stain, growth factors

Received October 31, 2001

This study was supported by Brain Korea 21 Project for Medical Science, Yonsei University.

Reprint address: requests to Dr. Kwan Chul Tark, Department of Plastic and Reconstructive Surgery, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul 120-752, Korea. Tel: 82-2-361-5694, Fax: 82-2-393-6947, E-mail: kctark@yumc.yonsei.ac.kr

INTRODUCTION

Wounds on fetal skin can be repaired without scarring until the second trimester, but subsequently skin wounds leave scars as in adults.[1] The mechanism of scarless wound healing during the fetal period is not clearly known, though several studies have been conducted. One of more intriguing observations is that various growth factors, such as transforming growth factor-β (TGF-β), which is known to induce fibrosis in adult, is present at very low levels during wound healing in fetal skin.[2] This low level of growth factors minimizes inflammatory response and fibroblast proliferation at the wound site, which in turn inhibits collagen synthesis, and thus, allows scarless wound healing.[3]

Recently, the functions of bone morphogenetic proteins (BMPs), members of the TGF-β superfamily, have been studied in the wound healing process.

Bone morphogenetic proteins are multi-functional growth factors that were originally isolated from the bone as proteins that induce bone and cartilage formation.[4-6] BMPs are 30-38 kDa dimeric proteins and are classified into ten BMP subfamilies according to their aminoacid sequences.[7] Since their initial isolation, it has been demonstrated that BMPs are critical during mammalian development,[8] have an important role in cellular chemotaxis, differentiation and cellular apoptosis,[9-11] and are also known to affect the synthesis of collagen and proteoglycans. The BMPs are, therefore, a family of growth and differentiation factors that have different functions on different cells.[12,13]

BMP signaling follows the paradigm established by TGF-β signaling, and occurs through an interaction with a heteromeric complex of BMP receptors types I and II. Specifically, ligand binding results in the cross-phosphorylation of type I receptor by type II; type I, in turn, propagates BMP signaling.[14] Currently, three type I receptors (ActR-I, BMPR-IA, and BMPR-IB) and three type II receptors (ActR-II, ActR-IIB, and BMPR-II) have been identified. *In vitro* experiments have shown that all members of the BMP family that belong to the TGF-β superfamily bind to BMPR-II in combination with BMPR-IA or -IB. In contrast, ActR-II/-IIB and ActR-I do not bind BMP-4. Therefore, BMPR-II, -IA, and -IB may be considered BMP-specific receptors.[14]

Several studies upon the role of BMPs in wound healing have been reported. The skin wounds of fetal sheep at 70 days of gestation, treated with BMP-2, healed without scar formation.[15] Moreover, in the wound healing process of the adult mouse, BMP-7 and BMP type II receptor were found to increase on day eight.[16]

These data suggest that BMPs influence the regeneration of epithelium and wound healing, but the mechanism by which BMPs affect the wound healing process is unknown. In this study, we investigated the expressions of BMP receptors in human fetal and adult skins, which are known to utilize different wound healing process.

MATERIALS AND METHODS

Tissue Harvest and Preparation

We obtained fetal skins from backs of five fetuses aborted in the second trimester. The adult skin samples were obtained from five normal adults undergoing reconstructive surgery or aesthetic surgery. The harvested tissues were snap frozen in liquid nitrogen and stored at -70℃.

Immunohistochemistry

The four-micrometer-thick sections were prepared from paraffinized skin tissues, and incubated 12 hours. The slides were deparaffinized with xylene and rehydrated using an ethyl alcohol/water gradient. After antigen retrieval, skin tissue slides were incubated with 2% normal goat serum for 1 hour. The skin tissues were then reacted with primary antibody (anti BMP receptor-IA, IB/ anti BMP receptor-II rabbit serum, R&D Systems, Minneapolis, MN, USA) and secondary antibody (anti-rabbit goat IgG, Vector laboratory, Burlingganme, CA, USA). After preparing with avidin-biotin-horseradish peroxidase complex (Vector laboratory, Burlingganme, CA, USA), the skin tissues were counterstained with diaminobenzidine tetrahydrochloride (Research Genetics, Elmundo, AL, USA).

If the epidermis of the skin samples was unstained, the sample was considered negative and if the epidermis was stained, the sample was considered positive. Differences between the fetal and adult groups were determined by using the t-test.

RT-PCR (Reverse Transcription-Polymerase Chain Reaction)

Snap frozen skin tissues were ground using a homogenizer and the RNA was extracted using an RNA purification kit (RNeasy Mini Kit, QIAGEN). The extracted mRNA was converted to single strand cDNA using random hexamers and Moloney murine leukemia virus reverse transcriptase (BRL, Gaithersburg, MD, USA). 1 μl of cDNA was used in each RCR; the sequence of primers used for PCR are described in Table 1. A 30 cycle PCR process, including denaturation, annealing, extension, were performed in thermal processor. To visualize the PCR products, the samples were subjected to electrophoresis in 1% agarose gel and stained with ethidium bromide. Product authenticity was confirmed by sequencing.

RESULTS

Immunohistochemistry

BMPR-I and BMPR-II were not found in fetal skin except in the hair follicles of the dermis, while BMPR-IA, BMPR-IB, and BMPR-II were expressed on the suprabasal layer of the epidermis and in the hair follicles of the dermis in adult skin (Fig. 1 and 2).

Table 1. Primers for RT-PCR

	Primer	Size(bp)
B-ACTIN	GACTACCTCATGAAGATCCT GCGGATGTCCACGRCACACT	313
BMPR-IA	GCATAACTAATGGACATTGCT TAGAGTTTCTCCTCCGATGG	1401
BMPR-IB	GCAGCACAGACGGATATTGT TTTCATGCCTCATCAACACT	634
BMPR-II	ACGGGAGAGAAGACGAGCCT CTAGATCAAGAGAGGGTTCG	694

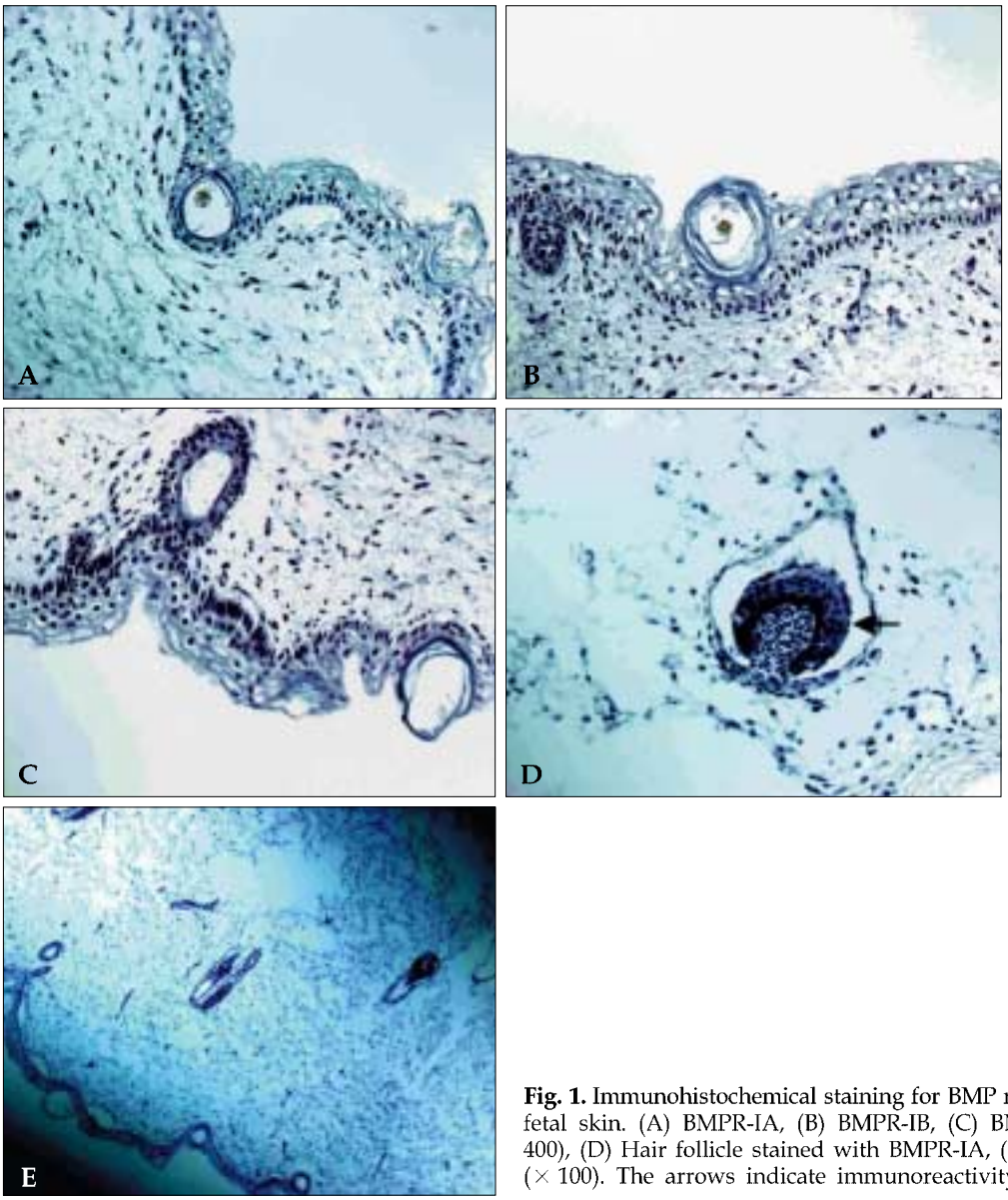

Fig. 1. Immunohistochemical staining for BMP receptors in fetal skin. (A) BMPR-IA, (B) BMPR-IB, (C) BMPR-II (× 400), (D) Hair follicle stained with BMPR-IA, (E) BMPR-II (× 100). The arrows indicate immunoreactivity.

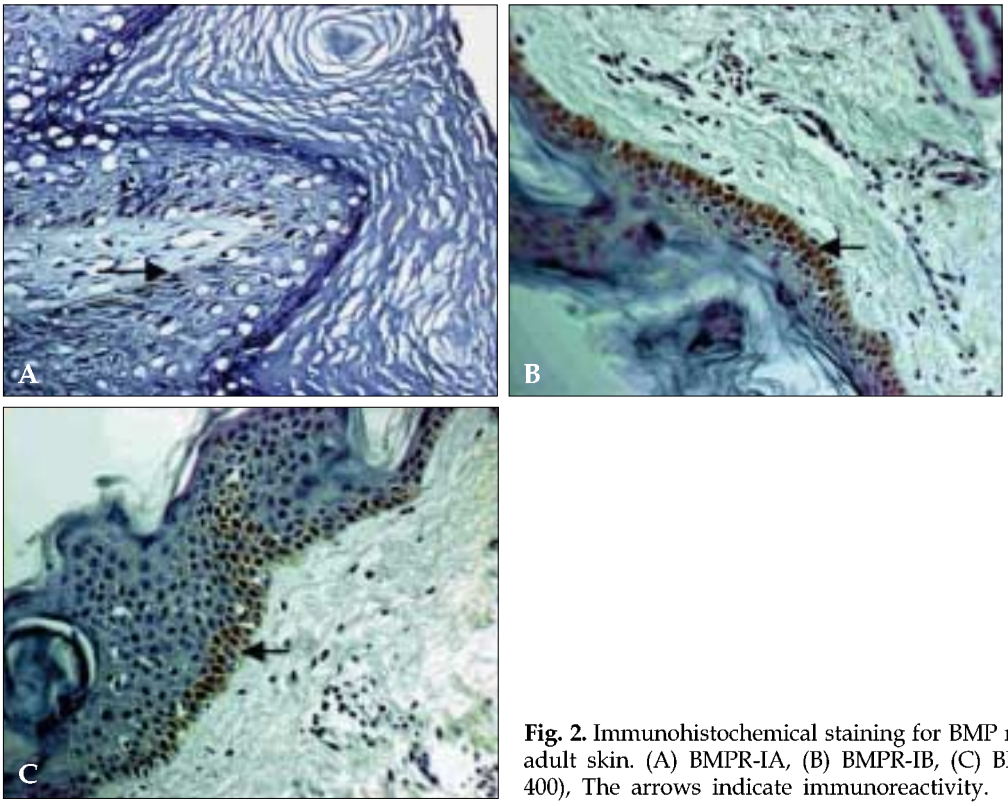

Fig. 2. Immunohistochemical staining for BMP receptors in adult skin. (A) BMPR-IA, (B) BMPR-IB, (C) BMPR-II (× 400), The arrows indicate immunoreactivity.

Table 2. Expression of BMP Receptors in Fetal and Adult Skin

Fetal skin*				Adult skin			
No.	BMPR-IA	BMPR-IB	BMPR-II	No.	BMPR-IA	BMPR-IB	BMPR-II
F1-1	-	-	-	A1-1	+	+	+
F1-2	-	-	-	A1-2	+	+	+
F1-3	-	-	-	A1-3	+	+	+
F2-1	-	-	-	A2-1	+	+	+
F2-2	-	-	+	A2-2	+	+	+
F2-3	+	+	-	A2-3	+	+	+
F3-1	-	-	-	A3-1	-	+	+
F3-2	-	-	-	A3-2	+	-	+
F3-3	-	-	-	A3-3	+	+	+
F4-1	+	-	-	A4-1	+	+	+
F4-2	-	+	+	A4-2	+	-	+
F4-3	-	+	-	A4-3	-	-	-
F5-1	-	-	-	A5-1	+	+	+
F5-2	-	-	-	A5-2	+	+	+
F5-3	-	-	-	A5-3	+	+	+

* F1; 19 weeks' fetus, F2; 20 weeks' fetus, F3, F4; 24 weeks' fetus, F5; 25 weeks' fetus.

Fifteen skin samples from five individual of each group, were analyzed. In the fetal group, BMPR-IA was expressed on two slides (13%), BMPR-IB on three slides (20%), and BMPR-II on two slides (13%). In the adult group, BMPR-1A was expressed on thirteen slides (87%), BMPR-IB on 12 (80%) slides, and BMPR-II on 14 (93%) slides (Table 2 and 3).

Table 3. Statistical Analysis of Expression of BMP Receptors Between Fetus and Adult Skin

		Fetus	Adult	t-test
BMPR-IA	positive (%)	13	87	$p<0.01$
	negative (%)	87	13	
BMPR-IB	positive (%)	20	80	$p<0.01$
	negative (%)	80	20	
BMPR-II	positive (%)	13	93	$p<0.01$
	negative (%)	87	7	

Statistical analysis, using the t-test, showed significant differences in the expressions of BMP receptors for the fetal and adult groups.

RT-PCR (Reverse Transcription-Polymerase Chain Reaction)

By RT-PCR, the mRNA of each of the BMP receptors (BMPR-IA;1401bp, BMPR-IB;634bp, and BMPR-II; 694bp) was detected both in fetal and adult skin (Fig. 3).

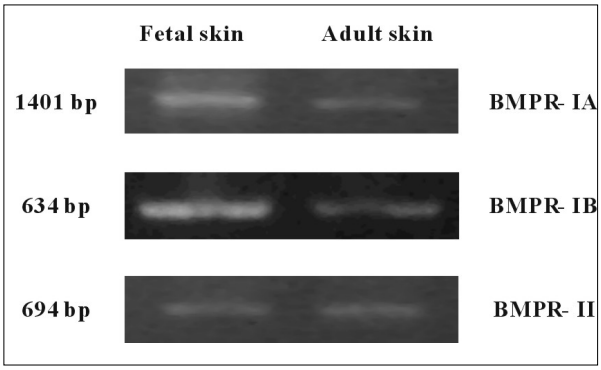

Fig. 3. RT-PCR for BMP receptors.

DISCUSSION

Since it was first revealed that skin wounds through the second trimester of fetal development heal without leaving scars in mammals,[1] efforts have been made to determine the nature of the fetal wound healing process. However, the mechanisms underlying scarless wound healing remain unknown, though several important differences between fetal and adult wound healing have been found. Fetal wounds heal with minimal inflammation and neovascularization. This minimal inflammation is attributed to the low levels growth factors, such as, TGF-b (known as the tissue fibrosis factor) and bFGF.[17]

Recently, growth factors, known as BMPs, were characterized, and these have since been studied during mammalian development.

BMPs have various functions on the differentiation and proliferation of epithelial cells and mesenchymal cells, as well as during the development of the kidney,[18] eye[12] and the teeth.[19]

More specifically, BMPs have also been studied with regard to wound healing. For example, BMP-2 was found to be expressed at low levels in the developing hair follicle and in the epidermis of normal human fetal skin. Exogenously treated BMP-2 induced massive dermal and epidermal thickening, and fetal wounds treated with BMP-2 healed with an adult-like pattern and left scarring.[15]

In BMP-6 overexpressing transgenic mice, re-epithelization of skin wounds was significantly delayed, suggesting that the strict spatial and temporal regulation of BMP expression is necessary.[20]

In an wound healing model using adult mice, BMP-2 and -4 levels were unchanged during the healing process; however, BMP-7 rose to high levels on day 8, and this was associated with the highest expression of BMP receptor (BMPR-II).[21]

These studies suggest that BMPs and BMP receptors are involved in wound healing. Therefore, in this study, we investigated the different expressions of BMP receptors in fetal and adult skin by immunohistochemical staining.

Our results show that BMP receptors are to be founded in the epithelial cells of the suprabasal layer of adult skin, but not in the fetal skin, except in the developing hair follicles.

In a study into the effect of TGF-β receptors on the fetal fibroblasts of the mouse, the effect of TGF-β was found to be inhibited when the levels of TGF-β receptors were low.[22]

These findings imply that different expressions of BMP receptors may induce different level of BMPs during wound healing, and that this may explain the different modes of wound healing in the fetus and adult.

Further studies upon the roles of BMP receptors

on wound healing are necessary, and these studies could be conducted by using BMP receptor agonist or antagonist. such as noggin or chordin.[23]

REFERENCES

1. Lorenz HP, Longaker MT, Perkocha LA, Jennings RW, Harrison MR, Adzick NS. Scarless wound repair. A human fetal skin model. Development 1992;114:253-9.
2. Whitby DJ, Ferguson MWJ. Immunohistochemical localization of growth factors in fetal wound healing. Dev Biol 1991;147:207-15.
3. Nath RK, LaRegina M, Markham H, Ksander GA, Weeks PM. The expression of transforming growth factor - beta in fetal and adult rabbit skin wounds. J Pediatr Surg 1994;29:416-21.
4. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, et al. Novel regulators of bone formation molecular clones and activities. Science 1988;242:1528-34.
5. Wozney JM. The bone morphogenetic protein family and osteogenesis. Mol Reprod Dev 1992;32:160-7.
6. Sampath TK, Maliakal JC, Hauschka PV, Jones WK, Sasak H, Tucker RF, et al. Recombinant human osteogenic protein-1(hOP-1) induces new bone formation *in vivo* with a specific activity compatible with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation *in vitro*. J Biol Chem 1998;267:20352-62.
7. Massague J, Weis-Garcia F. Serine/threonine kinase receptors: mediators of transforming growth factor β family signals. Cancer Surg 1996;27:41-64.
8. Hogan BL. Bone morphogenetic proteins: Multifunctional regulators of vertebrate development. Genes Dev 1994;10:1580-94.
9. Lind M, Eriksen EF, Bunger C. Bone morphogenetic protein-2 but not bone morphogenetic protein-4 and -6 stimulates chemotactic migration of human osteoblasts, human marrow osteoblasts, and U2-OS cells. Bone 1996;18:53-7.
10. Paralkar VM, Weeks BS, Yu YM, Kleinman HK, Reddi AH. Recombinant human bone morphogenetic protein 2B stimulates PC 12 cell differentiation, potentiation and binding to type IV collagen. J Cell Biol 1992;119:1721-8.
11. Zou H, Niswander L. Requirement for BMP signaling in interdigital apoptosis and scale formation. Science 1996;272:738-41.
12. Luo G, Hofmann C, Bronckers AL, Sohocki M, Bradley A, Karsentry G. BMP-7 is an inducer of nephrogenesis and is also required for eye development and skeletal patterning. Genes Dev 1995;9:2808-20.
13. Winnier G, Blessing M, Labosky PA, Hogan BL. Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. Genes Dev 1995;9:2105-16.
14. Yamashita H, Ten-Dijke P, Heldin CH, Miyazono K. Bone morphogenetic protein receptors. Bone 1996;19:569-74.
15. Stelnicki EJ, Longaker MT, Holmes D, Vanderwall K, Harrison MR, Largman C, et al. Bone morphogenetic protein-2 induces scar formation and skin maturation in the second trimester fetus. Plast Reconstr Surg 1998;101:12-9.
16. Blessing M, Schirmacher P, Kaiser S. Overexpression of bone morphogenetic protein-6 in epidermis of transgenic mice: inhibition or stimulation of proliferation depending on the pattern of transgene expression and formation of psoriatic lesions. J Cell Biol 1996;135:227-39.
17. Hsu M, Peled ZM, Chin GS, LIu W, Longaker MT. Ontogeny of expression of transforming growth factor-(beta)1, TGF-(beta)3 and TGF-(beta) receptors I and II in fetal rat fibroblast and skin. Plast Reconstr Surg 2001;107:1787-94.
18. Hogan BL. Bone morphogenetic proteins: multifunctional regulators of vertebral development. Genes Dev 1994;10:1580-94.
19. Thesleff I, Nieminen P. Tooth morphogenesis and cell differentiation. Curr Opin Cell Biol 1996;8:844-50.
20. Kaiser S, Schirmacher P, Philipp A, Protschka M, Moll I, Nicol K, et al. Induction of bone morphogenetic protein-6 in skin wounds. Delayed reepithelization and scar formation in BMP-6 overexpressing transgenic mice. J Invest Dermatol 1998;111:1145-52.
21. Shephard P, Grunewald M, Hafner M, Krieg T, Smola H. Bone morphogenetic proteins in skin wound healing. J Invest Dermatol 2000;115:561.
22. Moban RR, Kim WJ, Moban RR, Chen L, Wilson SE. Bone morphogenetic protein 2 and 4 and their receptors in the adult human cornea. Invest Ophthalmol Vis Sci 1998;39:2626-36.
23. Zimmerman LB, De Jesus-Escobar JM, Harland RM. The spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. Cell 1996;86:599-606.