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Research paper

Expression characteristics of BMP2, BMPR-IA and Noggin in different stages of hair follicle in yak skin

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ABSTRACT

Bone morphogenetic protein 2 (BMP2), BMP receptor-IA (BMPR-IA), and the BMP2 antagonist Noggin are important proteins involved in regulating the hair follicle (HF) cycle in skin. In order to explore the expression profiles of BMP2, BMPR-IA, and Noggin in the HF cycle of yak skin, we collected adult yak skin in the telogen, proanagen, and midanagen phases of HFs and evaluated gene and protein expression by real-time quantitative polymerase chain reaction (qRT-PCR), western blotting, and immunohistochemistry. qRT-PCR and western blotting results showed that BMP2 and BMPR-IA expression levels were highest in the telogen of HFs and higher than that of Noggin in the same phase. The expression of Noggin was significantly higher in proanagen and midanagen phases of HFs than in the telogen phase, with the highest expression observed in the proanagen phase. Moreover, the expression of Noggin in the proanagen phase was significantly higher than those of BMP2 and BMPR-IA during the same phase. Immunohistochemistry results showed that BMP2, BMPR-IA, and Noggin were expressed in the skin epidermis, sweat glands, sebaceous glands, HF outer root sheath, and hair matrix. In summary, the characteristic expression profiles of BMP2, BMPR-IA, and Noggin suggested that BMP2 and BMPR-IA had inhibitory effects on the growth of HFs in yaks, whereas Noggin promoted the growth of yak HFs, mainly by affecting skin epithelial cell activity. These results provide a basis for further studies of HF development and cycle transition in yak skin.

1. Introduction

Yaks (*Bos grunniens*) can adapt to the harsh plateau environment owing to its unique skin system (Gen, 2006; Lu et al., 2011). As a component of the skin, hair follicles (HFs) play an important role in the production of hair fibers, which help the animal to withstand the cold environment. HFs are regenerative mini-organs that show cyclic activity during postnatal development, with periods of relative resting (telogen), active growth and hair shaft production (anagen), and apoptosis-driven regression (catagen) (Cotsarelis and Millar, 2001). This cyclic activity of the HF is regulated by a tightly controlled balance between numerous growth stimulatory and inhibitory factors (Cotsarelis et al., 1990; Paus and Cotsarelis, 1999). Therefore, it is important to study the molecules involved in the regulation of the HF cycle of yaks, not only for animal husbandry but also to diversify breeding and improve regenerative medicine applications.

BMPs are secreted signaling molecules that belong to the transforming growth factor-beta (TGF-β) superfamily (Derynck and Zhang, 2003; Massagué and Chen, 2000; Miyazawa et al., 2002; Ten and Hill, 2004). Previous findings have indicated that BMPs are powerful regulators of cutaneous development, HF growth, and melanogenesis in normal postnatal skin and play important roles in the control of a variety of pathobiological processes in postnatal skin, including wound healing, psoriasis, and carcinogenesis (Botchkarev, 2003). As one of the most active members of the BMP family (Crowe et al., 1998), signaling of BMP2 is accomplished by binding to its receptor proteins termed BMPR-IA and BMPR-IB with high affinity (Kotzsch et al., 2008). Although the studies have showed that BMPR-IA and BMPR-IB bind BMP2 with similar

Abbreviations: HF, hair follicle; EP, epidermis; ORS, outer root sheath; HM, hair matrix.

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affinities, the expression of BMPR-IA is ubiquitously and is indispensable during development (Mishina et al., 1995), while the expression BMPR-IB seems spatially restricted (Chen et al., 2004). Furthermore, in a study of the mouse HF cycle transition, BMP activity was found to be modulated by the BMP antagonist Noggin (Botchkarev et al., 2001), which binds BMP2 with 10–15 times higher affinity than BMP receptors, indicating that Noggin can inhibit BMP function. (Groppe et al., 2002; Zimmerman et al., 1996).

The HF transition from telogen to anagen is a unique process of organ regeneration characterized by activation of cell proliferation in the proximal follicular epithelium (Cotsarelis et al., 1999; Cotsarelis et al., 1990; Kedzia, 2001). Studies have found that BMP2, BMPR-IA, and Noggin are involved in the mouse (Botchkarev et al., 2002) and human (Hwang et al., 2001) HF cycle transition. Experimental and genetic studies have suggested that the HF transition from telogen to anagen is associated with downregulation of inhibitory BMPs and upregulation of the BMP antagonist Noggin (Botchkarev et al., 2001). Moreover, long-term deletion of Noggin leads to strong upregulation of BMP2 in the mouse epidermis of Noggin-null skin transplants (Botchkarev et al., 2002), and absence of BMPR-IA in epithelial cells of HFs results in excessive proliferation of the hair matrix, eventually leading to the production of HF tumors (Ming et al., 2004). Studies on mice have shown that during postnatal life, neutralization of the inhibitory activity of BMP2 by the BMP antagonist Noggin is also essential for induction of new HFs (Botchkarev et al., 1999). Therefore, we hypothesized that BMP2, BMPR-IA and Noggin were also expressed in yak skin, in which BMP2 and BMPR-IA predominantly inhibited the growth of HFs while Noggin promoted HFs growth.

Based on the study of the changes of HF cycle in yak skin (Yang et al., 2017), in this study, we explored the expression profiles of BMP2, BMPR-IA, and Noggin in the HF cycle of yak skin using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR), western blotting, and immunohistochemistry in order to provide a basis for studying the molecular regulation of HF development and changes in yak skin.

2. Materials and methods

2.1. Animals and tissue collection

A total of 15 male yaks were obtained from the Xi Ning slaughterhouse. The yaks were intravenously anesthetized with sodium pentobarbital (20 mg/kg) and then euthanized via exsanguination, after which experimental samples were collected. The experimental animals were all handled according to the Animal Ethics Procedures and Guidelines of the People's Republic of China, and the study was approved by the Animal Ethics Committee of Gansu Agricultural University.

Experimental samples of the skin in telogen, proanagen, and midanagen (five adult yaks, 3–4 years old) were taken from the neck region. Skin specimens used for immunohistochemistry were stored in 4% neutral paraformaldehyde phosphate buffer (pH 7.3) for 2 weeks at room temperature, and skin specimens used for qRT-PCR and western blotting were stored at −80 °C.

2.2. Primary antibodies selection

Rabbit polyclonal antibodies against human BMP2 (Bioss, Beijing, China; rabbit bs-10696R), BMPR-IA (Abcam, Cambridge, UK; rabbit ab174815) Noggin (Bioss; rabbit bs-2975R), and β-actin (Bioss, Beijing, China; rabbit bs-0061R) were used.

Some studies have reported that the BMP2 (Reddi, 1998), BMPR-IA (Shenasa, 2008), and Noggin (Xu and Thomson, 2009) are highly conserved. Thus, the epitopes of BMP2, BMPR-IA and Noggin are similar among different species. Moreover, the sources of the anti-BMP2, anti-BMPR-IA and anti-Noggin antibodies are human, and by analyzing the phylogenetic relatedness of the extracellular domains of these proteins, we demonstrated that the similarities of BMP2, BMPR-IA and Noggin between yaks and humans were 90.68%, 96.26% and 98.17%, respectively.

2.3. Relative qRT-PCR

Total skin tissue RNA was isolated using TRIzol reagent (Invitrogen, CA, USA). RNA was reverse transcribed to single-stranded cDNA with a Reverse Transcription kit (MBI Fermentas, Canada) according to the manufacturer's instructions. The qRT-PCR primers were designed according to the *Bos taurus BMP2*, *BMPR-IA*, and *Noggin* gene sequences (GenBank accession numbers: NM_001099141.1, NM_001076800.1, and XM_582573.9, respectively) using Primer 5 software and synthesized by the Beijing Genomics Institute BGI company (China). The qRT-PCR primer sequences are shown in Table 1. qPCR was conducted with a LightCycler480 thermocycler (Roche, Germany) and a 20 μL reaction volume consisting of 1 μL cDNA, 1 μL forward primer, 1 μL reverse primer, 10 μL 2 × SYBR Green II PCR mix (TaKaRa, Shiga, Japan), 0.4 μL Rox, and 6.4 μL nuclease-free H_2O. The PCR conditions were as follows: 95 °C for 30 s, 95 °C for 5 s, and 60 °C for 34 s for a total of 40 cycles; 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. Four replicates were set for each sample to ensure the accuracy of the relative expression of the target gene in the sample. After amplification, according to the system-generated Ct value, the $2^{-\triangle\triangle Ct}$ method was used with *β-actin* as an internal standard to obtain the relative expression of *BMP2*, *BMPR-IA*, and *Noggin* mRNA.

2.4. Western blotting

Total protein of skin tissues with HFs of different stages was extracted by adding 1 mL RIPA buffer with 10 μL phenylmethylsulfonyl fluoride (Solarbio, Shanghai) for 30 min, followed by centrifugation at 12000 × *g* and 4 °C for 5 min. The supernatant was then collected and quantified using an Enhanced BCA protein assay kit (Bio Tek, VT, USA). Next, 4 × sodium dodecyl sulfate (SDS) loading buffer was added to equal amounts (40 μg) of protein, and proteins were denatured at 100 °C for 10 min. Proteins were separated by SDS-polyacrylamide gel electrophoresis. Proteins were transferred onto polyvinylidene difluoride (Solarbio) membranes, and the membranes were blocked with 5% skim milk powder in Tris-buffered saline containing 0.1% Tween 20 at room temperature for 2 h. The membranes were then incubated with polyclonal anti-BMP2 antibodies (1:200 dilution), polyclonal anti-BMPR-IA antibodies (1:1000 dilution), polyclonal anti-Noggin antibodies (1:1000 dilution) and polyclonal anti-β-actin antibodies (1:1000 dilution) at 4 °C overnight. The membranes were then incubated with a goat anti-rabbit IgG antibody (Bioss; bs-0295G-HRP, anti-rabbit, 1:1000 dilution) after washing with phosphate-buffered

Table 1
Primers used in real-time RT-PCR.

Genes	Primer sequences (5′-3′)	Length (bp)	Annealing (°C)
BMP2	F: GCTCTTTATGCTCAGAAGGACTCAA R: ACAAGCTGATGGAACAGAGAACTG	96	60
BMPR- IA	F: TAAGCAGGGCTCTGGTGTCTA R: AGGTGGCAGTCAAAGTGTTCT	228	60
Noggin	F: CGAGCGAGATCAAAGCG R: CTGCGACCACAGCCACA	107	60
β-actin	F: AGGCTGTGCTGTCCCTGTATG R: GCTCGGCTGTGGTGGTAAA	207	60