Expression and Function of FGF5 Isoform in Hair Growth
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Abstract

In mammals, hair cycle occurs continuously in three cycles: anagen, catagen, and telogen. One of the fibroblast growth factor (FGF) family, FGF5, has been known to act as an inhibitor of hair growth in the transition from anagen to catagen. The FGF5 gene is composed of a long form (FGF5) and a short form (FGF5s). We examined the effect of FGF5 isoform on hair growth in the cultured keratinocytes. To analyze the effect of FGF5 isoform, we examined the expression change of FGF5 gene. We observed that the FGF5 gene was initially reported as one of the oncogenes [13], but it was classified as the FGF family because it has high homology with FGF [14]. FGF5 consists of 3 exons, and the FGF5 gene is divided into long FGF5 (FGF5-longform, FGF5) and short FGF5 (FGF5-shortform, FGF5s) depending on the presence or absence of exon 2 [9, 15]. Long FGF5 (FGF5) is composed of three exons, short FGF5 (FGF5s) is composed of exon 1 and a part of exon 3 due to a frame shift and a stop codon initially appearing [15]. In addition, FGF5 gene has been reported to regulate the transition from growth phase to degenerative phase in the late growth phase of the mammalian hair cycle [10, 16].

It was observed that the FGF5 gene-deficient mice had abnormally long-lasting growth phase VI during the hair cycle [10]. In addition, FGF5s plays a role as an inhibitor of long FGF5 [17, 18], and it has been reported that it regulates the transition from growth phase to degenerative phase in the dermal papilla of cashmere goats. FGF5 gene was observed in the outer root sheath of human hair. FGF5 gene has recently been observed as a potentially important factor in alopecia [16].

The aim of this study is to find out how each of the FGF5 isoform in the hair works using over-expression of FGF5 isoforms in the cultured keratinocytes.

MATERIALS & METHODS

Cell Culture

Keratinocyte cells were cultured according to the previously reported method [19, 20]. SVKC, a keratinocyte cell line, was subcultured and maintained using Keratinocyte-SFM (Gibco, Gaithersburg, MD, USA). In addition, 293A cells were subcultured and...
maintained with 10% fatal bovine serum (Welgene, Korea) in DMEM (Welgene, Korea).

Viral vector amplification
After the 293A cells were subculture in DMEM culture media, the virus was infected with Keratinocyte-SFM media before 293A cells were infected with the virus. Thereafter, 293A cells were removed from the 95% cell culture dish, they were transferred to a tube and the freezing and thawing processes were repeated a total of 5 times. And after centrifugation at 3,000 rpm for 10 minutes, the supernatant was used.

Transfection of the genes associates with hair growth
Adenovirus/LacZ (Ad/LacZ), Ad/FGF5, Ad/FGF5s, green fluorescent protein/adenovirus (GFP-Ad), GFP-Ad/FGF5, and GFP-Ad/FGF5s were each transfected with SVKC for 48 hours. And virus transfection was observed at 24 hours and 48 hours using a fluorescence microscope.

RNA Extraction
After removing the culture medium of SVKC, washed it with 1 M PBS, added 300 µl of Tri-Reagent (MRC, Cincinnati, USA) and reacted on ice for 10 minutes, and then added 60 µl of chloroform (Sigma-Aldrich, Louis, MO, USA). After adding and sufficiently mixing, usual method was used for RNA extraction. After drying the pellet, diethyl pyrocarbonate was diluted to 0.1% using distilled water to dissolve the pellet, and then quantified using a UV spectrophotometer.

RT-PCR
RNA (2 µg) was made into cDNA using M-MLV reverse transcriptase. A polymerase chain reaction (PCR) was performed to prepare a primer for the gene to be examined (Table-1). Reverse Transcriptase (Promega, Madison, WI, USA) and an oligo (dT) primer was used in this experiment. Subsequently, 120 ng of the cDNA was used in PCR amplification to assess the expression of genes associated with hair growth. To normalize for differences in cDNA loading, primers designed to amplify glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used.

STATISTICAL ANALYSIS
One-way ANOVA and Newman-Keuls post-hoc test were performed to determine the difference in mRNA expression between the control group and the experimental group, and statistical significance was determined to be significant when the P value was less than 0.05.

RESULT
In order to analyze whether the virus is infected with SVKC into the cells, Ad/GFP-FFG5 and Ad/GFP-FFG5s viruses were infected for 48 hours, respectively, and observed under a microscope at 24 hours and 48 hours. As a result, it was confirmed that the virus was infected into SVKC. After infecting the cells with Ad/FGF5, Ad/FGF5s, Ad/GFP-FFG5, and Ad/GFP-FFG5s for 48 hours, the amount of FGF5 gene expression was confirmed through reverse transcription polymerase chain reaction. The expression pattern of FGF5 in the group infected with Ad/GFP-FFG5 and Ad/GFP-FFG5s was observed in a similar pattern in the group infected with Ad/FGF5 and Ad/FGF5s. Compared to the control group of each of the two groups, the amount of expression was increased in the group infected with FGF5 and FGF5s virus (Figure-1).

Gene expression associated with hair growth after overexpression of FGF5 isoform
The genes related to hair growth was identified with cDNA infected with Ad/FGF5 and Ad/FGF5s viruses in SVKC. It was observed that genes involved in hair growth, HGF was significantly increased in the group infected with Ad/FGF5s viruses than in the control and Ad/FGF5 groups. However, expression of the genes, stem cell factor (SCF), Dickkopf-related protein (DKK) 1 and FGF2 in the experimental groups were not changed significantly compared with the control group (Figure-2).

Genes directly associated with hair growth, keratin (KRT5), keratin 14 (KRT14), and bone morphogenic protein 6 (BMP6) which promoting the telogen-anagen transition in vivo, was evaluated in the SVKC infected with Ad/FGF5 and Ad/FGF5s viruses. BMP6 tend to be decreased (statistically not significant, p = 0.08) in the group infected with Ad/FGF5 virus, compared with the control and Ad/FGF5s groups. Expression of KRT5 and KRT14 genes in the experimental groups were not changed significantly compared with the control group (Figure-3).
Table-1: List of primer sequences used in RT-PCR

| Gene             | Primer sequence                      |
|------------------|--------------------------------------|
| FGF5 (upper band ; long form) (lower band : short form) | F 5’-AGTCAATGGATCCCATGGAAG-3’  
R 5’-TCATCTGTGAACCTTGG-3’  |
| HGF              | F 5’-TGATGAAGGACGAGCTACA-3’  
R 5’-GCCTACCTTGAGATTGCTTG-3’  |
| SCF              | F 5’-CGGAGATGGATGTTTGGCAA-3’  
R 5’-TGGTTCTGCTTTGCTTGAAT-3’  |
| FGF2             | F 5’-AGGAGTGTGTGCTAACCGTT-3’  
R 5’-CAGTTCTGCTTCAATGCAGCA-3’  |
| DKK1             | F 5’-ACCAGCTATCCAAATGATGTCG-3’  
R 5’-TGCAATACAGGGAGTGCTTC-3’  |
| KRT5             | F 5’-GTGCTACTGCGATGAATG-3’  
R 5’-TGATACCAGGACTCGGCTTC-3’  |
| KRT14            | F 5’-CTGTTCTACATCCCCTCCGCTTC-3’  
R 5’-AGGGGCTATTTGAGGCTTG-3’  |
| BMP6             | F 5’-CTCTACGACAAGCAGCCCTT-3’  
R 5’-TGGGTGCAATGAGTCCACTC-3’  |

Fig-1: The cells were infected with GFP-Ad/FGF5, GFP-Ad/FGF5s, Ad/FGF5 and Ad/FGF5s for 48 hours and then confirmed by RT-PCR.

Fig-2: Gene expression regulating hair growth after FGF5 isoform overexpression in the SVKC. The graph data are shown the means ± SD of three independent experiments. Statistically significant differences are indicated by **p<0.01, ***p<0.001
DISCUSSION
After overexpressing the FGF5 gene, the mRNA expression levels of the HGF, SCF, DKK1, and FGF2 were analyzed, respectively. HGF gene is a hormone secreted by mesenchymal cells and plays an important role in epithelial cells [21], it is known to regulate the interaction between dermal papilla and keratinocytes [22, 23], and promote DNA synthesis in hair bulbs. Through this study, it is estimated that FGF5s and HGF promote hair growth through interaction in gene or protein level [24].

FGF5 gene has been reported to regulate the degenerative phase in the late growth phase of the mammalian hair cycle [10, 16]. In our study, long form of FGF5 tend to suppress expression of BMP6 in the group infected with Ad/FGF5 virus than in the control and Ad/FGF5s group. However, no significant difference was found in other genes, KRT5 and KRT14, which are the differentiation genes of external root sheath in the hair follicle.

Additional study showed that a significant increase was observed in the group treated with siRNA1 (C-terminal knockdown of FGF5) in BMP6, compared to the control group (data not shown). BMP6 acts as an inhibitor in the inner bulge, and the hair cycle enters the growth phase from the degenerative phase [25]. It is presumed that BMP6 could affect hair growth by inhibiting FGF5 activity when the C-terminus of the FGF5 gene is knocked down.

Taken together, FGF5s might activate HGF in hair growth and FGF5 may inhibit BMP6 expression in hair growth suppression.

CONFLICT OF INTEREST: Author declares that there is no conflict of interest in this study.

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