TGF-β2 and FGF5 which are inhibited by cyclosporin A mutually stimulate their expression in dermal papilla cells

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1. Introduction

There are three phases in hair cycle: anagen (growing phase), catagen (regressing phase) and telogen (resting phase). Immunosuppressive immunophilin ligands, cyclosporin A (CsA), is known as a powerful hair growth modulator in human [1]. One of side effects of CsA is hyper trichosis in patients and extending hair growth in hair follicle organ culture [1, 2]. However, a molecular mechanism of prolongation of anagen by CsA is unclear. It is known that transforming growth factor-beta 2 (TGF-β2) produced by dermal papilla cells (DPCs) cause apoptosis by acting to the outer root sheath cells (ORSCs) [3]. TGF-β2, a catagen-inducing factor, is expressed when the hair cycle shifts from the anagen phase to the catagen phase. Since the hairs of fibroblast growth factor5 (FGF5)-knock out mice showed long hair, FGF5 is also considered to be a catagen-inducing factor [4].

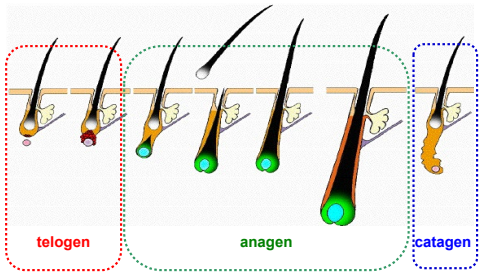

Fig. 1 Hair cycle

It has been known that CsA inhibits TGF-β2 expression in DPCs. The aim of this study was to determine the effect of CsA on the expression of FGF5, another catagen-inducing factor. Furthermore, the interaction between the two catagen-inducing factors is also investigated.

2. Materials and methods

2-1. Cells and culture conditions：

Cell line of hDPCs immortalized with large T-antigen was used. DMEM (ThermoFisher Scientific) containing 10% fetal bovine serum (FBS) and antibiotics. Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2. When testing the effects of factors and chemicals on gene expression, the cells were cultured in DMEM without FBS (basal DMEM).

Another cell line of hORSCs immortalized with large T-antigen was also used. Keratinocyte-SFM (K-SFM; ThermoFisher Scientific) containing the supplement supplied with the medium and antibiotics.

2-2. mRNA extract、cDNA synthesis、real time PCR：

After culturing to subconfluent cells in 10%-FBS DMEM, the cells were changed to the basal DMEM containing CsA and cultured for another 4 hours. The mRNA was extracted using ISOGEN II (Nippon Gene) in accordance with the manufacturer’s instructions. The mRNA was reverse-transcribed using SuperScript III (ThermoFisher Scientific), then real-time quantitative PCR was performed (QuantStudio 5 Real-time PCR System; ThermoFisher Scientific) using Thunderbird Next SYBR qPCR Mix (Toyobo) according to the respective manufacturer’s instructions.

2-3. Cell proliferation assay of ORSCs

Cells were seeded into 96-well culture plates (Iwaki

Glass). After pre-incubation, the medium was replaced with the basal medium containing FGF5. The cells were then cultured for 1 days, and proliferation was assayed (AlamarBlue; Bio-Rad).

3. Results

3-1. Expression of TGF-βRI、TGF-βRII and FGFR1 in DPCs

Expression of TGF-βRI and TGF-βRII in DPCs was detected by PCR. Expression of FGFR1 was also detected in DPCs. These results suggest that DPCs are capable of accepting TGF-β2 and FGF factor signals.

3-2. Effect of CsA on TGF-β2 and FGF5 expression

Expression of TGF-β2 and FGF5 was suppressed by CsA in DPCs, respectively. These results suggested that CsA inhibits expressions of the two catagen-inducing factors, TGF-β2 and FGF5.

3-2. Effect of FGF5 on TGF-β2 expression

The expression of TGF-β2 was repressed by FGF5 in DPCs. Furthermore, when FGF5 receptor (FGFR1) inhibitor was added, the inhibitory effect of FGF5 on TGF-β2 expression was lost in DPCs. These results suggest that the regulation of TGF-β2 expression is downstream of FGFR1 signaling.

3-3. Effect of FGF5 expression by TGF-β2

The expression of FGF5 was repressed by TGF-β2 in DPCs. Furthermore, when TGF-βRs inhibitor was added, the inhibitory effect of TGF-β2 on FGF5 expression was lost in DPCs. These results suggest that the regulation of FGF5 expression is downstream of TGF-βRs signaling.

3-4. Proliferation of ORSCs affected by FGF5

The effect of FGF5 on the proliferation of ORSCs was evaluated by the AlamarBlue assay. FGF5 did not affect the proliferation of ORSCs.

4. Discussion

The prolonged effect of CsA on anagen was thought to be due to the inhibition of TGF-β2 and FGF5 expressions. In addition, TGF-β2 and FGF5 might mutually stimulate their expression in DPCs. It is known that TGF-β2 induce catagen by apoptosis. However, the mechanism of FGF5 induction of the degenerative phase is not fully understood. As a possibility of catagen induction mechanism by FGF5, it is considered that expression of TGF-β2 might be enhanced.

Since FGF5 does not affect the viability of ORSCs, it is possible that TGF-β2 directly induces catagen in hair cycle. It is possible that FGF5 does not directly induce catagen by apoptosis, but induces catagen by indirect action.

References

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