Cyclosporin A inhibits TGF-β2 expression via suppression of NFAT activation in human dermal papilla cells

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1. Introduction

Hair growth is a highly regulated cyclical process. Three distinct phases have been defined for the mammalian cycle: anagen (growing phase), catagen (regressing phase) and telogen (resting phase) (Fig. 1). Hypertrichosis is a well-known side effect in patients receiving an immunosuppressant, cyclosporin A (CsA) [1]. CsA also elongates anagen phase in an organ culture system of human hair follicle [2], therefore, CsA is thought to cause hypertrichosis by anagen elongation. However, the mechanism is unclear. In immunosuppression, CsA and cyclophilin (CyP) form a complex, and this complex inhibits an activity of a phosphatase, calcineurin (CaN), which stimulates the translocation of nuclear factor of activated T-cells (NFAT) into the nucleus (Fig. 2) [3].

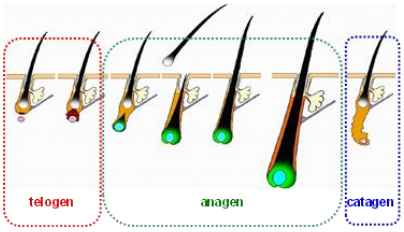

Fig. 1 Hair cycle

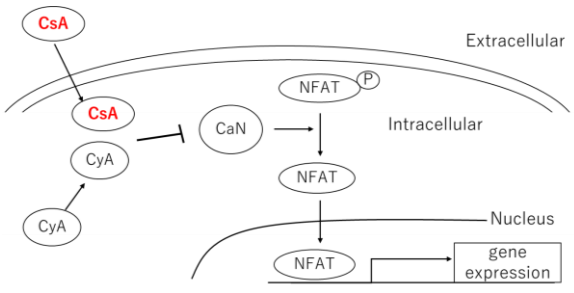

Fig. 2 Mechanism of immunosuppression

The aim of this study is to elucidate whether CsA-mediated anagen elongation follows a similar mechanism to immunosuppression or a different mechanism.

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).

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 or a NFAT inhibitor, VIVIT, 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.

3. Results

3-1. Expression of CyP, CaN and NFAT in DPCs

The seven isoforms of CyPs were expressed DPCs. All

five isoforms of NFATs were expressed DPCs. NFAT5 was the most highly expressed in DPCs. Both CaN-A and CaN-B were also expressed in DPCs. These results suggested that DPCs are capable of accepting CsA and VIVT.

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

TGF-β2, a catagen-inducing factor, is expressed when the hair cycle shifts from the anagen phase to the catagen phase [4]. Expression of TGF-β2 was suppressed by CsA and VIVIT in DPCs. These results suggested that CsA and VIVIT inhibits expression of TGF-β2 [5].

4. Discussion

CyP, CaN and NFAT which involved in the immunosuppressive mechanism of CsA were expressed in DPCs. In addition, it was suggested that CaN and NFAT were involved in anagen extension by CsA. The expression of a catagen-inducing factor, TGF-β2 was suppressed by CsA and a NFAT inhibitor, VIVIT. This suggesting that CsA might inhibit TGF-β2 expression via suppression of NFAT activation in DPC.

References

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