Hypoxic response of the hair cycle regulating-factors in human dermal papilla cells

Student: Shogo Ueno, Marin Sugano
Supervisor: Tokuro Iwabuchi

Affiliation: Tokyo University of Technology, Faculty of Bioscience and Biotechnology

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1. Introduction

Dermal papilla cells (DPC) existing in the center of hair-bulb secrete hair cycle-modulating factors. Hair growth-stimulating factors promote differentiation and proliferation of hair follicle epithelial cells, surrounding DPCs to grow hair shaft (Fig 1a). DPCs play a pivotal role as a control tower in hair cycle. However, between at the end of the anagen phase and the catagen phase, TGF-β is produced by DPCs. TGF-β affects hair follicle epithelial cells and induces apoptosis in the bottom part of hair follicle. It destroys the structure of hair follicle and causes the hair follicle to shift to the telogen phase. In the anagen phase, hair follicles are surrounded by capillary blood vessels (Fig 1b) and be supplied with oxygen or nutrition. On the other hand, in the catagen phase, capillaries will disappear and oxygen concentration would be extremely decreased.

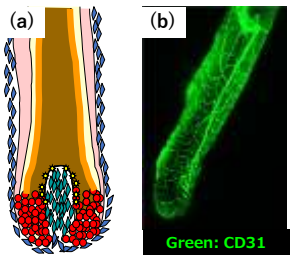

Figure1
(a) structure of hair follicle in the anagen phase
(b) capillaries surrounding hair follicle

In the catagen phase, hair follicle will be under hypoxic condition. There is only a few information about hypoxic response of DPCs. In this research, we evaluated hypoxic response and its influence of the hair cycle regulating-factors in DPCs.

2. Materials and methods

2-1. Cells and culture conditions：

Cell lines of hDPCs immortalized with large T antigen were used. DMEM (ThermoFisher Scientific) containing 10% fetal bovine serum (FBS) and antibiotics [3]. Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2. Cell culture was carried out under atmospheric oxygen for a basic culture (normoxia; oxygen concentration of 21%). Hypoxic cell culture was performed under 5% and 1% oxygen concentration conditions. Cobalt(II) chloride ($CoCl_2$) which is an inhibitor for prolyl hydroxylase (PHD) was added to the cells to prepare a pseudo-hypoxic condition in which hypoxia-inducible factor (HIF) is not degraded.

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

Subconfluent cells were cultured in culture dish. 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, Osaka) accordingto the respective manufacturer’s instructions.

3. Results

3-1. Expression of HIF-1α and HIF-2α under pseudo-

hypoxic conditions in DPC:

Under normoxic conditions, HIF-1α and HIF-2α are degraded by PHD, but under hypoxic conditions, PHD is inactivated and thus HIFs are not degraded. Because $CoCl_2$ inhibits PHD, HIF is not degraded even in normoxic conditions. In other words, the addition of $CoCl_2$ is equivalent to the same condition as hypoxic conditions. One of the target genes of HIF-1α is phosphoinositide-dependent protein kinase 1 (PDK1), and one of the target genes of HIF-2α is L-type amino acid transporter 1 (LAT1). Thus, to confirm the activity of HIF-1α and HIF-2α, we also examined the expression of PDK1 and LAT1 [3]. When DPCs were incubated with $CoCl_2$, the expressions of HIF-1α, HIF-2α, PDK1, and LAT1 were increased. These indicate that DPCs can response to hypoxic condition via HIF expression.

3-2. Expression of catagen-inducing factors under hypoxic conditions in DPC:

TGF-β2 is one of the catagen inducers in the hair cycle regulation [4]. TGF-β2 expression in DPCs at 21, 5, and 1% of oxygen concentrations was evaluated by a real time PCR. Expression of TGF-β2 was increased in a hypoxia-dependent manner.

3-3. Expression of telogen maintenance factor under hypoxic conditions in DPC:

BMP-4 is one of the telogen maintenance factors in the hair cycle regulation [5]. BMP-4 expression in DPCs at 21, 5, and 1% of oxygen concentrations was evaluated by a real time PCR. Expression of BMP-4 was increased in a hypoxia-dependent manner.

3-4. Expression of hair growth-promoting factor under hypoxic conditions in DPC:

FGF7 is one of the hair growth-promoting factors in the hair cycle regulation [6]. FGF7 expression in DPCs at 21, 5, and 1% of oxygen concentrations was not changed by a real time PCR. Expression of FGF7 might be not affected by an oxygen concentration.

4. Discussion

The expression of HIF-1α and its target gene, PDK1, was increased in DPCs under hypoxia. In addition, the expression of HIF-2α and its target gene, LAT1, was also upregulated under hypoxia. In DPCs, the expression of hypoxia-related genes such as HIFs was increased in response to changes in oxygen concentration status caused by hair cycle changes.

A catagen inducer, TGF-β2, is up-regulated in hypoxic conditions. This suggesting that the catagen phase might be further accelerated when hair follicles are subjected to hypoxia. This suggesting that exposure of hair follicles to hypoxia might further accelerate the progression to the telogen phase. A telogen maintenance factor, BMP-4, was up-regulated in hypoxic conditions. The results suggest that hair follicles might respond to hypoxia by remaining in telogen phase for a longer period of time. The expression of an anagen-activating factor, FGF7, was almost unaffected by hypoxia. It was thought that hypoxia condition does not cause a response that actively promotes hair growth.

Considering the overall response of the three hair cycle regulators (catagen-inducing factor, telogen maintenance factor, hair growth-promoting factor) to hypoxia, DPCs seemed to show a response to hypoxia, to maintain a telogen phase. In the future, the response of anagen-inducing factor to hypoxic conditions will be of much interest.

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