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Effect of DNA Aptamer (WD-Aptamer) on Hair Regrowth by Inhibiting Wnt Signaling Negative Regulation

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Abstract

CXXC5 negatively regulates the Wnt signaling pathway by binding to the Wnt signaling component protein disheveled (Dvl). The Dvl protein comprises three domains: the DIX domain, the DEP domain, and the PDZ domain. The PDZ domain binds to the CXXC5 zinc finger domain. We prevented the CXXC5 effect by using a DNA aptamer (WD-Aptamer) that binds to the Dvl1_PDZ domain. The inhibitory effect of CXXC5 was confirmed through tests of cell proliferation in HaCaT cells and in vivo experiments using mice.

Keywords: DNA aptamer • Androgenetic alopecia • Minoxidil • Hair growth

Introduction

Hair loss, which is an issue of concern for people worldwide, is caused by various factors, such as aging, hormones, and stress [1]. The most common form of hair loss is Androgenetic Alopecia (AGA), which happens when the level of Dihydrotestosterone (DHT) increases through excessive activity of the 5α-reductase enzyme [2,3]. The FDA has approved minoxidil as the only treatment for AGA that inhibits the 5α-reductase enzyme; however, the efficacy of minoxidil is low and it has side effects [4,5].

The Wnt/β-catenin signaling pathway has an important role in the AGA system. The Wnt signaling pathway affects hair follicle development, hair regeneration in adults, and DHT–induced suppression [6-8]. CXXC5 is a protein containing the zinc finger domain and is known to negatively regulate the Wnt signaling pathway by binding to the Wnt pathway component protein Disheveled (Dvl) [9-11].

Previously, we reported our development of the DNA aptamer (WD-Aptamer) that binds to the Dvl protein and confirmed, by means of in vitro testing, which the WD-Aptamer blocks the negative regulation of the Wnt signaling pathway by CXXC5 [12]. In this report, through a cell viability test and in vivo experiments using mice, we confirmed that the WD-Aptamer affects activation of the Wnt signaling pathway.

Materials and Methods

Cell viability test in HaCaT cells

We cultured HaCaT cells (human keratinocytes) in Dulbecco's Modified Eagle Medium, supplemented with heat-inactivated FBS (10%), penicillin (100 units/mL), and streptomycin (100 μg/mL) (Gibco BRL, USA). We maintained the cells at 37 °C in a CO2 incubator.

To ascertain the effect of the WD-Aptamer on HaCaT cell viability, we carried out a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma-Aldrich, USA). Cells were seeded in 96-well plates at a density of 5 × 104 cells/well and incubated for 24, 48, and 72 h at 37°C. After 24, 48, and 72 h treatment with different concentrations (10 and 100 μM) of WD-Aptamer, we added MTT (final concentration: 0.5 mg/mL) to each well and incubated the cells for 2 h at 37°C. When the cell-free supernatant was removed and the insoluble purple formazan crystals were solubilized in 100 μL of DMSO (Daejung, Korea), we measured absorbance (570 nm) using a microplate reader (Synergy HT, USA).

Mice

Male C57BL/6 J mice (6 weeks) were purchased from OrientBio (Seongnam, Gyeonggi, and Republic of Korea). All mice were kept in a controlled environment with a temperature of 22°C ± 2°C, a humidity of 50% ± 10%, a 12-h light/dark cycle, and ad libitum access to food and water. Our study was approved by the Kyung Hee University Institutional Animal Care and Use Committee (KHUASP (SE)-18-134). All experiments were conducted according to the

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approved animal protocols and guidelines established by Kyung Hee University.

We injected sodium pentobarbital (30 mg/kg) intraperitoneally to induce anesthesia before using an animal clipper (Babion SBC-6630) to remove hair from the backs of the animals. Then, we applied Nair hair removal cream (Church and Dwight UK Ltd., Folkestone, Kent, UK) and left this on for one minute before washing with water to cleanly remove hair follicles. We applied either 0.001% WD-Aptamer solution, 1% minoxidil solution, or vehicle as a spray to the dorsal skin of the mice twice per day for 21 days. At 7, 15, and 21 days after the start of the experiment, we administered a small amount of ether to induce mild anesthesia in the animals, and then we visually inspected the hair growth and took pictures of it.

Results and Discussion

Cell penetration and proliferation

To examine the potential effect of WD-Aptamer on HaCaT cells over 24, 48, and 72 h, we treated HaCaT cells with various concentrations of WD-Aptamer. Treatment with various concentrations of WD-Aptamer over 24, 48, and 72 h significantly increased proliferation of HaCaT cells in a dose-dependent manner (Figure 1).

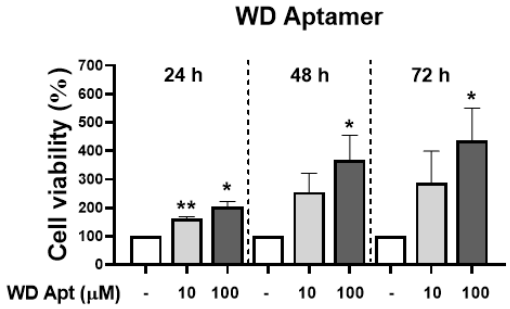

Figure 1. The effect of WD-aptamer in HaCaT cells. Viability in HaCaT cells was measured via the MTT assay after 24, 48, and 72 h treatment with WD-Aptamer.

Accelerated hair regrowth induced by WD-Aptamer

To examine the effect of WD-Aptamer on hair regrowth activation, we divided mice into three groups: vehicle, WD-Aptamer, and minoxidil). Darkening of the dorsal skin first appeared on day seven in the group treated with WD-Aptamer. Furthermore, on day 15, hair regrowth in the group treated with WD-Aptamer appeared faster than did that in group treated with minoxidil (Figure 2). The findings show WD-Aptamer plays a role in accelerating hair growth.

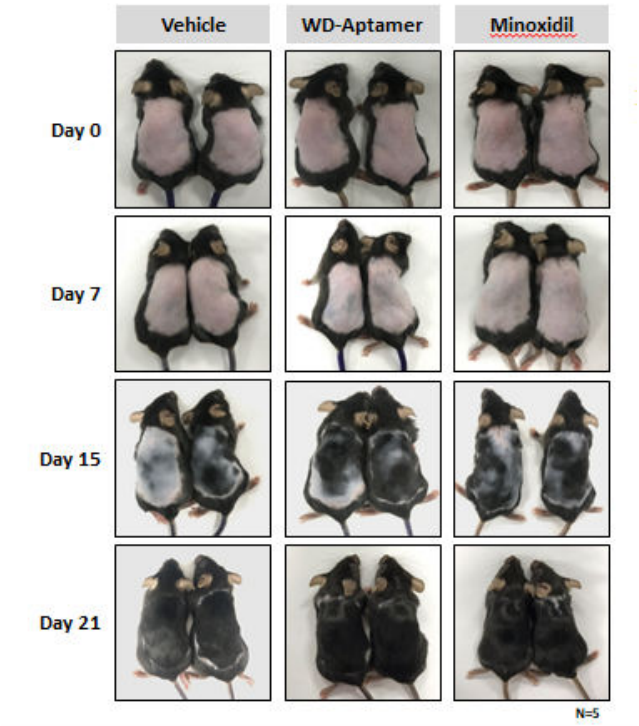

Figure 2. Hair growth–promoting effect of WD-Aptamer. Either vehicle, WD-Aptamer solution, or minoxidil were applied topically to the shaved dorsal skin of telogen-matched seven-week-old C57BL6 mice. Photographs were taken on the day of application of each solution and at 7, 15, and 21 days after application.

Conclusion

WD-Aptamer was developed to inhibit the negative regulation of the Wnt signaling pathway by CXXC5. The efficacy of WD-Aptamer was confirmed through cell proliferation tests and in vivo experiments using mice. These results showed that WD-Aptamer has potential therapeutic value in the treatment of AGA. Despite the effect of minoxidil on hair loss, its use has been restricted due to some side effects such as irritant contact dermatitis with the typical symptoms of itching and scaling. The results of this study suggest that WD-Aptamer will be a desirable substance that can replace restrictions of minoxidil.

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