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“Unlocking Hair Growth: IRIS Rhizomes Extract Extends Anagen Phase via OR8D1 Olfactory Receptor”

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1. Introduction

The human hair follicle undergoes a dynamic cycle comprising three main phases: anagen (growth), catagen (regression), and telogen (resting). This cycle is crucial for maintaining hair density and quality. Disruptions in this cycle can lead to various forms of alopecia, with androgenetic alopecia (AGA) being the most prevalent. AGA is characterized by progressive hair thinning and loss, primarily influenced by genetic and hormonal factors. Despite extensive research, the molecular mechanisms underlying AGA remain incompletely understood.

Recent studies have begun to explore the role of sensory receptors, particularly olfactory receptors, in non-olfactory tissues, including the skin and hair follicles (HF) (1,2). These receptors, traditionally associated with smell, are now recognized for their involvement in various physiological processes beyond olfaction. The expression of olfactory receptors in hair follicles suggests a novel regulatory mechanism in hair growth and differentiation.

The objective of this study was to identify novel biological targets associated with androgenetic alopecia (AGA) through the analysis of transcriptomic profiles of hair follicles plucked from the scalp of men with and without AGA. Here, we demonstrated for the first time the expression and novel role of the olfactory receptor OR8D1 in promoting hair growth. We examined its modulation by an agonist and a botanical extract containing an antagonist. This research aimed to discover innovative and effective therapeutic strategies for the treatment of AGA.

2. Materials and Methods

Clinical study

This single center, parallel-group, comparative and open-label, clinical study was conducted at the Centre de Recherche sur la Peau Pierre Fabre [Skin Research Center] (Toulouse, France). It was carried out in accordance with the ethical principles stated in the Declaration

of Helsinki, in compliance with local legal requirements and after approval of the study protocol by the Ethics Committee.

Six subjects were included in two groups: a group with AGA and a control group. Subjects included in the AGA group (n=6) were male patients with a clinical diagnosis of AGA ranging from type III vertex (60%) to type IV (40%) according to the Norwood Hamilton classification, aged 18 to 45 years old (mean age: 36.7 ± 5.7 years).

At D0, 20 hair fibers with the bulb, were plucked from the vertex of control subjects, and along the bald area of patients with AGA, and stored at 80°C until analysis.

Expression profiling was performed using the Human Gene Expression 4x44K v2 Microarray from Agilent.

OR8D1 expression in scalp HFs

Normal human full-thickness occipital scalp skin reached in terminal HFs was collected from one caucasian woman aged 53 and two Caucasian men aged 54 and 67 and immediately embedded after extraction after informed consent of the donor and ethics committee approval

Ex vivo culture of microdissected HFs

Amputated HFs were microdissected either from follicular unit extraction or scalp skin collected after informed consent of the donor and ethics committee approval and cultured as previously described by Edelkamp et al. and Langan et al. (3,4) and cultivated in 48-well plate.

Sotolon: Amputated microdissected human HF ex vivo organ culture from follicular units (occipital) of a Caucasian man aged 54 were treated for four days, changing the medium every other day.

Treatment with IRE: female HFs (age 47 and 20yo) were treated for 5-11 days with IRE, changing the medium every other day.

Microscopic hair cycle staging was performed using Ki-67/TUNEL immunohistology and Masson Fontana histochemistry, as previously described (3,4).

Wnt/b catenin signaling pathways evaluation

HFDPC were transfected with a lentivirus expressing a luciferase gene under the control of Wnt/ β catenin promoter (TCF/LEF response element) (TCF/LEF Luciferase Reporter Lentivirus, Bioscience). Luciferase expression was measured using Bright-Glo™ substrate and luminescence was quantified by using a microplate reader (ClarioStar). Cells were incubated 24h with extract from Iris Rhizomes (IRE). Data were obtained from 3 independent donors.

3. Results/ Discussion

OR8D1 overexpression in plucked AGA hair follicles from men.

To enhance understanding of androgenetic alopecia (AGA) physiology, hair follicles were plucked from six men with and without AGA, followed by differential gene expression analysis using microarrays. Among the 354 genes identified as differentially expressed, Olfactory Receptor 8D1 (OR8D1) was significantly overexpressed in the AGA group (fold change of 1.726, p-value = 0.004). This overexpression suggests a potential association between OR8D1 activity and AGA progression.

Effect of OR8D1 modulation in Hair Follicle Biology

To date, no studies have described OR8D1 expression and its role in hair follicles (HF) or AGA. Immunofluorescence analysis in healthy scalp skin revealed that OR8D1 is highly expressed

in the lower portion of the HFs, specifically in the precortical hair matrix, inner root sheath, and terminal keratinization zone of the hair shaft, indicating a potential role in hair keratinocyte differentiation (Fig.1).

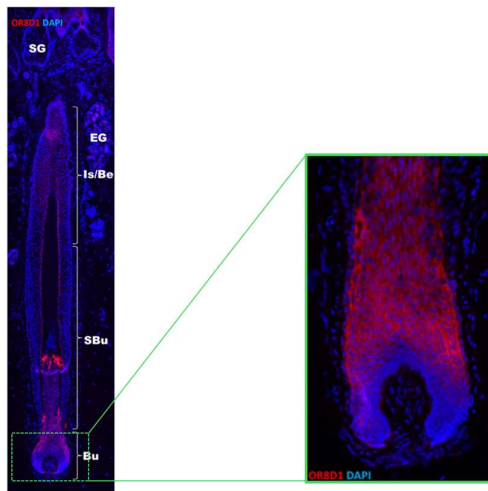


Figure 1 : OR8D1 expression in human hair follicles. Image representative for 3 donors.

Sotolon, identified as an OR8D1 agonist (5), reduced the number of microdissected HFs in the anagen phase and decreased Ki-67+ cell numbers in the matrix region upon incubation (Fig. 2). Thus, OR8D1 activation by Sotolon (3 μ M) induces premature transition to the catagen phase, suggesting its involvement in hair follicle regression.

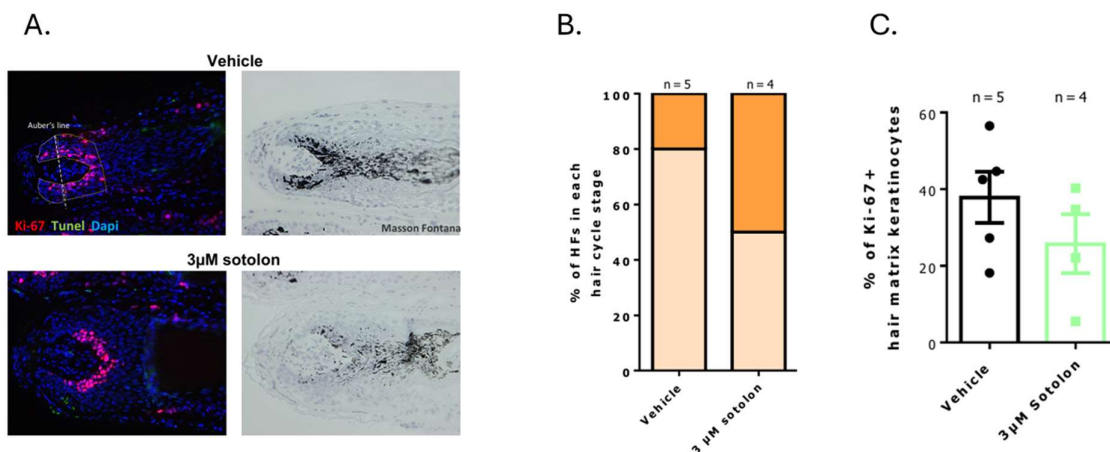


Figure 2 : Effect of Sotolon, an OR8D1 agonist, on the hair cycle. A. Representative images for hair cycle staging, evaluation of proliferating and apoptotic hair matrix keratinocytes. B. Quantification of HF in each hair cycle stage (n= 4-5 HF/group from one healthy donor). C. Percentage of Ki-67+ positive nuclei in hair matrix keratinocytes (4-5 HF/group from one healthy donor)

Hair Growth stimulation by a botanical extract containing an OR8D1 antagonist

Given that our data suggest that higher OR8D1 expression may characterize AGA HFs, and that its activation may lead to premature catagen development, it is conceivable that an OR8D1 antagonist may be beneficial for inhibiting AGA progression. Thus, we have identified an

extract from Iris Rhizomes (IRE) that is enriched in irones, mainly alpha isomethyl ionone, which is a known competitive inhibitor of the OR8D1 receptor. This extract was evaluated for its potential to stimulate hair growth *ex vivo*. Hair cycle staging analysis, following treatment of HFs in organ culture with IRE at a concentration of 10 $\mu\text{g/mL}$ demonstrated efficacy in prolonging the anagen phase (Fig. 3). These findings further support the interest of targeting OR8D1 in HFs to stimulate hair growth.

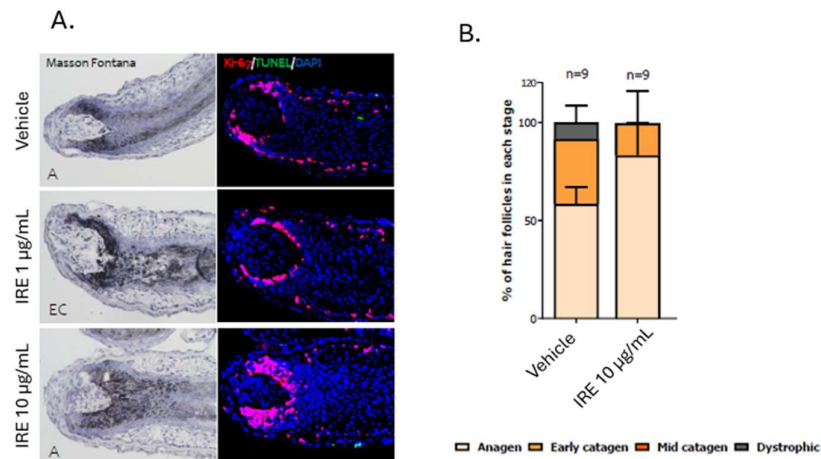


Figure 3 : IRE-induced anagen phase extension in human HFs. A. Representative images for hair cycle staging, evaluation proliferating and apoptotic hair matrix keratinocytes after treatment with 10 $\mu\text{g/mL}$ IRE. B. Quantification of HF in each hair cycle stage (n= 7-9 HF/group from two donors).

Wnt/Bcatenin pathway activation in HFDPC by Iris Extract

The Wnt/ β -catenin pathway plays a crucial role in anagen initiation and maintenance. Activation of this pathway in hair follicle dermal papilla cells (HFDPC) triggers the production of secreted factors essential for anagen onset and hair growth, promoting proliferation and differentiation of epithelial stem cells in the bulge and matrix cells (6,7). This study aimed to explore whether IRE possesses hair growth-promoting properties independent of OR8D1, specifically through the activation of the Wnt/ β -catenin pathway in HFDPC. IRE extract significantly induced Wnt/ β -catenin activation in HFDPC in a dose-dependent manner, ranging from 1 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$, supporting its potential to stimulate hair growth (Fig.4).

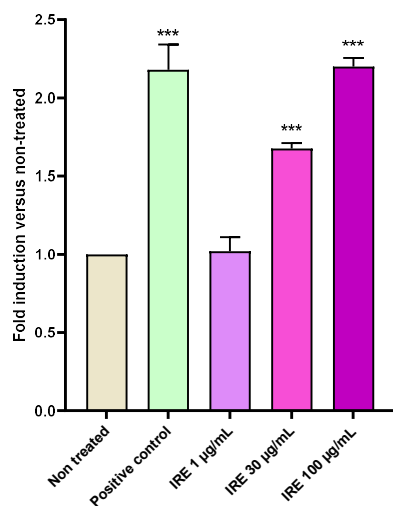


Figure 4 : Dose-dependant activation of the Wnt/b-catenin pathway by IRE in HFDPC. Fold induction of Wnt-b-catenin pathway compared to non-treated control (n= 9 from three donors).

4. Conclusion

In summary, the overexpression of OR8D1 in AGA hair follicles suggests a significant role in the progression of AGA. Immunofluorescence analysis indicates OR8D1 involvement in hair keratinocyte differentiation, particularly in the pre-cortical hair matrix and inner root sheath. Activation of OR8D1 by Sotolon promotes premature transition to the catagen phase, highlighting its potential contribution to HF regression. Conversely, targeting OR8D1 with an antagonist, such as alpha isomethyl ionone found in IRE, effectively prolongs the anagen phase and stimulates hair growth. Furthermore, IRE ability to activate the Wnt/b-catenin pathway in HFDPC underscores its potential as a therapeutic agent for hair growth stimulation. These findings collectively support the therapeutic targeting of OR8D1 in HFs as a promising strategy for the treatment of AGA. The studies underscore the significance of OR8D1 in HF biology and its potential as a therapeutic target for AGA.

The identification of IRE as an OR8D1 antagonist offers a promising natural treatment option to extend the anagen phase and combat hair loss.

These findings pave the way for further research into olfactory receptors roles in non-olfactory tissues and their therapeutic applications.

6. References

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