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Treatment with cyclohexyl salicylate, an olfactory receptor 2A4/7 agonist, promotes human hair follicle growth and bulge stem cell progeny expansion

J Edelkamp,[1] D Pinto,[2] H Erdmann,[3] T Purba,[4] F Jimenez,[5] R Paus,[1,6] M Bertolini,[1]

[1]Monasterium Laboratory, Münster, Germany; [2]Giuliani Spa, Milan, Italy; [3]Kosmed Klinik, Hamburg, Germany;[4]Centre for Dermatology Research, University of Manchester, UK;[5]Mediteknia Hair Transplant Clinic, Las Palmas de Gran Canaria, Spain; [6]University of Miami Miller School of Medicine, Miami, FL, USA

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Introduction:

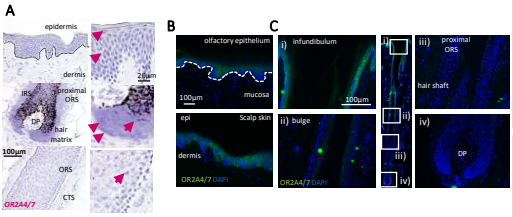
Hair Follicle (HF) disorders and aging result in hair thinning and loss. Affected individuals suffer from severe psychological stress. Therefore, developing topically applicable formulations that can serve as alternative or adjuvant to drugs to improve hair density, is of great importance. We recently demonstrated that stimulation of the olfactory receptor (OR) 2AT4 by the synthetic odorant Sandalore® prolongs anagen in human microdissected hair follicle *ex vivo* [1], and promotes human hair growth in telogen effluvium patients [2]. In a next step, we were interested whether other cosmetic OR ligands can unfold similar properties. For this purpose we focused on OR2A4/7, since its activation is known to promote epidermal keratinocyte proliferation and differentiation [3].

Materials & Methods:

- We used *in situ* hybridization and immunofluorescence staining on fresh human scalp skin, immediately frozen after extraction, to characterize OR2A4/7 mRNA and protein expression, respectively
- We used organ cultures of microdissected human HFs [4] in the presence of the cosmetic OR2A4/7 agonist, cyclohexyl salicylate (CHS), and investigated its effects on hair follicle stem cell and progeny number, by quantitative (immuno-)histomorphometry
- We used organ cultures of microdissected HFs in the presence of CHS and investigated its effect on hair cycle and hair matrix keratinocyte proliferation by quantitative (immuno-)histomorphometry and Masson Fontana staining

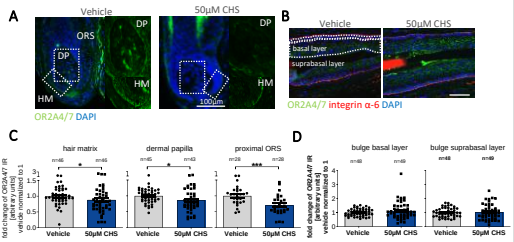
Results & Discussion:

OR2A4/7 mRNA and protein are expressed in the epidermis and hair follicle infundibulum of freshly embedded healthy human scalp skin



(A) Representative images of *OR2A4/7* mRNA expression (pink arrows) in olfactory epithelium (upper panel), hair follicle (HF) bulb (middle panel) and bulge (lower panel). **(B)** Representative images of OR2A4/7 protein expression in olfactory epithelium (positive control; upper panel), and fresh human scalp skin epidermis (lower panel). **(C)** Different HF compartments in fresh human scalp skin. n=3 independent donors. CTS: connective tissue sheath; DP: dermal papilla; IRS: inner root sheath; ORS: outer root sheath.

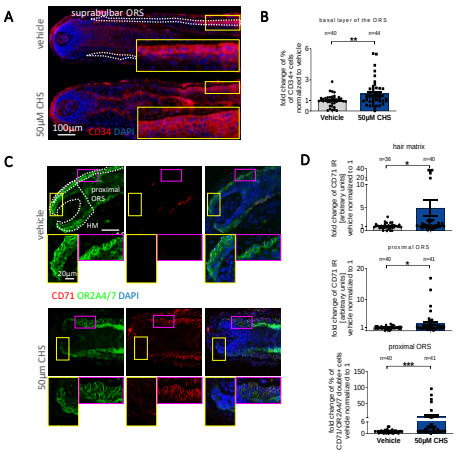
OR2A4/7 expression increases under organ culture conditions, and CHS treatment results in receptor downregulation in the HF bulb *ex vivo*



(A) Representative images showing OR2A4/7 expression (green) in HFs after culture for 6 days in the presence of 50 µM CHS or 0.1% DMSO as vehicle control. High magnifications of the DP and HM are shown on the right. **(B)** Quantification of the effect of CHS treatment on OR2A4/7 expression in compartments of the HF bulb (HM, DP, ORS) by immunohistomorphometry **(C)** Representative images showing OR2A4/7 expression (green) and Integrin α6 (red) expression in HFs after culture for 6 days in the presence of 50 µM CHS or 0.1% DMSO as vehicle control in bulge basal and suprabasal layers **(D)** Quantification of the effect of CHS treatment on OR2A4/7 expression in the bulge by immunohistomorphometry. Data are presented as mean±SEM of (D) n=28–49 HFs from N=5–8 independent donors and (F) n=48–49 HFs from N=8 independent donors. Data were quantified using ImageJ. Representative images from (A) the bulb and (B) bulge of N=8 independent donors. The data were tested for normality with the D'Agostino & Pearson omnibus normality test and further compared with an unpaired student's t-test when the datasets followed a normal distribution or a Mann-Whitney test when they did not. *p<0.05, ***p<0.001. DP: dermal papilla; HM: hair matrix; ORS: outer root sheath.

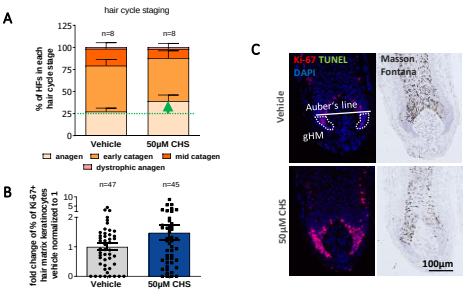
Results & Discussion:

OR2A4/7 stimulation significantly increases HF stem cell progeny *ex vivo*



(A-D) HFs were cultured for 6 days in the presence of 50 µM CHS or 0.1% DMSO as vehicle control. **(A)** Representative images showing CD34 exspression in the HF **(B)** Quantification of % of CD34+ cells in the basal layer of the suprabulbar ORS. **(C)** Representative images showing CD71 (red) and OR2A4/7 (green) in the HF bulb. Yellow inserts show zoom in on the HM and pink inserts show zoom in on the proximal ORS. **(D)** Quantification of CD71 expression and CD71/OR2A4/7 double positive cells in the hair matrix and proximal outer root sheath by immunohistomorphometry. Bar graphs show the individual data points as well as the mean±SEM of (B) n=40–44 and (D) n=36–41 HFs from N=8 individual donors. Data were quantified using ImageJ and tested for normality with the D'Agostino & Pearson omnibus normality test and further compared with an unpaired student's t-test when the datasets followed a normal distribution or a Mann-Whitney test if not. *p<0.05, **p<0.01, ***p<0.001. Nuclei are counterstained with DAPI. HM: hair matrix; gHM: germinative hair matrix; ORS: outer root sheath.

OR2A4/7 stimulation prolongs anagen and tendentially induces hair matrix keratinocyte proliferation in HFs *ex vivo*



(A-C) HFs were cultured for 6 days in the presence of 50 µM CHS or 0.1% DMSO as vehicle control. **(A)** Microscopic hair cycle staging was determined by standardized quantitative (immune-)histomorphometry using Ki-67/TUNEL immunohistology and Masson Fontana histochemistry. **(B)** Hair matrix proliferation was determined by quantifying the percentage of Ki-67+ cells within this region. **(C)** Representative images of Ki-67/TUNEL immunohistology and Masson Fontana histochemistry. Bar graphs show the individual data points as well as the mean±SEM of (G) n=45–47 HFs from N=8 individual donors. Data were quantified using ImageJ and tested for normality with the D'Agostino & Pearson omnibus normality test and further compared with an unpaired student's t-test when the datasets followed a normal distribution or a Mann-Whitney test if not. Nuclei are counterstained with DAPI. HM: hair matrix; gHM: germinative hair matrix; ORS: outer root sheath.

Conclusions:

Thus, stimulating OR2A4/7 via the non-drug CHS promotes hair growth and expands the progeny of K15+ stem cells, inviting the use of CHS as a novel cosmetic adjuvant treatment for hair loss disorders characterized by premature catagen development and a reduced capacity of K15+ stem cells to generate progeny, such as androgenetic alopecia.

For more information contact: m.bertolini@monasteriumlab.com or j.edelkamp@monasteriumlab.com

References

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