Involvement of olfactory receptor OR2AT4 in skin aging and the response to environmental pollution.

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Abstract

Background: Olfactory receptors (ORs) are 7-transmembrane G protein-coupled receptors expressed in the olfactory epithelium and mediate the odor perception. Among 400 ORs are known in human, their expression has been evidenced in various peripheral tissues such as skin. These ectopic receptors might regulate physiological functions beyond olfaction. OR2AT4 is expressed in the skin, especially in the epidermal keratinocytes, melanocytes, dendritic cells, and hair follicles. Its activation increases the proliferation and migration of keratinocytes, allowing faster re-epithelialization during wound healing. However, the roles of ORs in the skin are still incompletely described, especially, the possible link with intrinsic and extrinsic aging.

Methods: In this study, OR2AT4 expression was investigated in various senescent skin models *in vitro*. Besides, the consequences of ultrafine particle-induced skin damage were studied *via* the evaluation of OR2AT4 expression level and markers involved in skin senescence and differentiation.

Results: A significant decrease of OR2AT4 expression was observed in normal human keratinocytes cultured in a pro-aging environment, in senescent reconstructed human epidermis (RHEs) model and in RHEs from donors of various age. The level of OR2AT4, advanced glycosylation end products and ceramide synthase 3 expressions were decreased in *ex vivo* skin stressed by UFPs.

Conclusion: Our results showed that the expression of OR2AT4 was inversely correlated with aging and UFP-induced skin damage, suggesting that its modulation could be beneficial to limit the consequences of intrinsic and extrinsic aging into the skin.

Keywords: skin olfactory receptors; aging; urban pollution

Introduction.

Discovered by Axel and Buck in 1991, the olfactory receptors (ORs) belong to the 7-transmembrane G protein-coupled receptors family [1]. Mainly expressed in nasal tissue, their role is to detect thousands of odorant molecules. The activation of ORs induces adenyl cyclase, leading to cAMP production and so, triggers the influx of calcium, allowing the depolarization of membrane potentials in olfactory sensory neurons [2]. Moreover, the ORs are ectopically expressed in non-olfactory epithelium such as intestinal epithelium, prostate, spermatozoa, liver, and skin. Among the ORs described in the skin, OR2AT4 is detected in keratinocytes, melanocytes, dendritic cells, and hair follicles. The activation of OR2AT4 has been linked to downstream effects on cell proliferation, migration and re-epithelialization associated with wound healing [3]. Other studies mentioned the involvement of ORs in the chemosensory perception and the sensing of skin bacterial colonization [4]. However, the roles of ORs in the skin, and precisely of OR2AT4, are still incompletely described, in particular their possible involvement in the detection of pollution and in ageing.

Materials and Methods.

In this study, the expression of OR2AT4 was evaluated by immunocytochemistry in various senescent skin models *in vitro*: 1) RHEs obtained from donors of various age (3 years old to 62 years old); 2) senescent RHEs obtained thanks to FOXO3 siRNA and 3) normal human keratinocytes (NHKs) from young donor cultured in a pro-aging environment. In parallel, an extract of *Santalum album*, selected by bioinformatic studies for its ability to modulate the expression of OR2AT4, was evaluated. Secondly, the effect of urban pollution on the level of expression of OR2AT4 and related markers was studied by using ultrafine particles (UFPs) from diesel exhaust. After stress by pollution, the integrity of the skin barrier *via* ceramide synthase 3 expression study and the accumulation of advanced glycation end products (AGEs) were observed in *ex vivo* skin.

Results.

Modulation of OR2AT4 by an extract of *Santalum album*. In this study, we evaluate an extract of *Santalum album*, selected by bioinformatic studies for its ability to modulate the expression

of OR2AT4 and related markers. This extract was applied on *ex vivo* skin biopsies for 48 hours (Figure1).

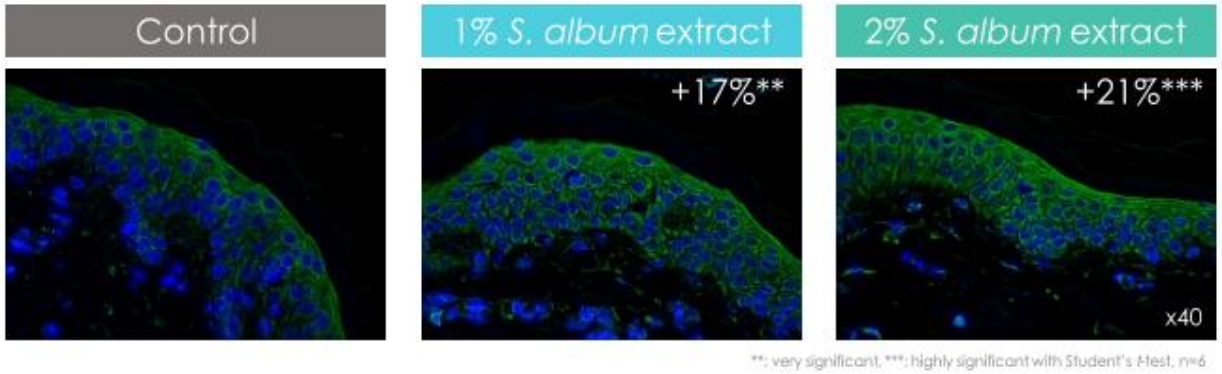
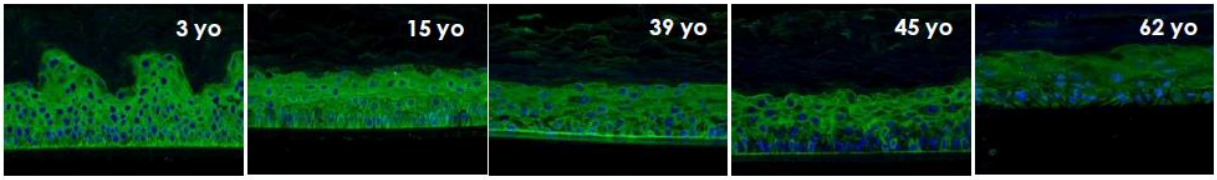


Figure 1: Expression of OR2AT4 on *ex vivo* skin after 48 hours of treatment. x40 objective. Green: OR2AT4 staining, Blue: Nucleus staining using DAPI. *Ex vivo* skin biopsies were treated with placebo (control), or 1% or 2% *S. album* extract twice a day for 2 days.

A significant increase of OR2AT4 protein level was associated with 48h application of 1% or 2% *S. album* extract, on *ex vivo* skin biopsies.

Expression of OR2AT4 in skin aging

The modulation of OR2AT4 was observed in RHEs obtained from donors of various age (Figure 2).



Immunodetection of OR2AT4 appears in green. Nuclei are stained with DAPI blue. 20x objective lens.

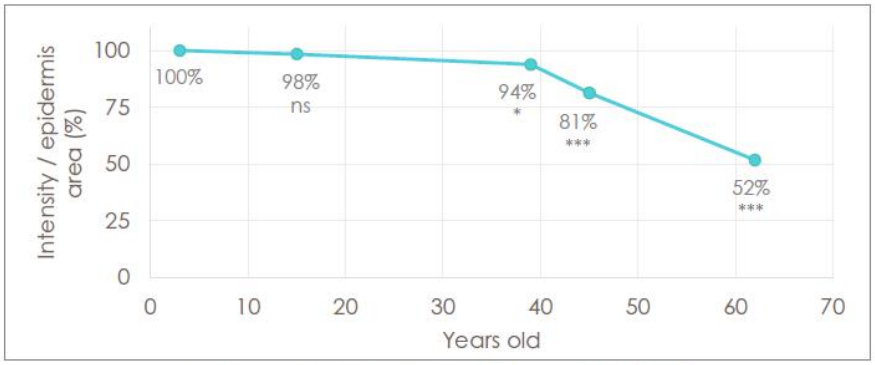


Image analysis was performed with Volocity* software.
Statistical analyses were expressed *versus* RHE from 3 yo donor.
n=6; ***: highly significant, *: significant, ns: not significant with Student's *t*-test.

Figure 2: Expression of OR2AT4 in RHEs obtained from donors of various age. x20 objective. Green: OR2AT4 staining, Blue: Nucleus staining using DAPI.

In the described experimental conditions, a significant decrease of OR2AT4 protein level was observed in RHEs when the age of the donor increased.

To confirm this result, a senescent RHE model, induced by FOXO3 downregulation, was used. In addition, the senescent RHEs were treated with *S. album* extract and the level of OR2AT4 was observed by immunostaining (Figure 3).

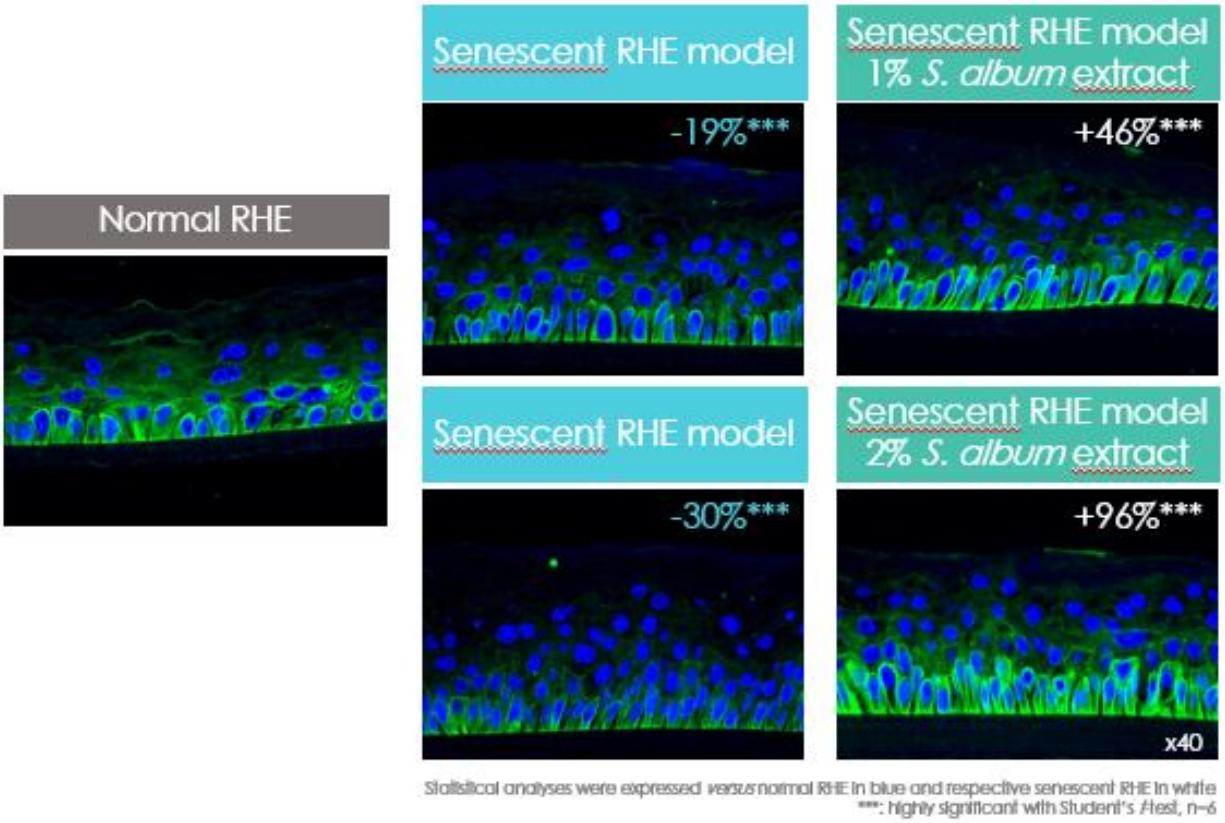


Figure 3: Expression of OR2AT4 in senescent RHEs. x40 objective. Green: OR2AT4 staining, Blue: Nucleus staining using DAPI. Senescent RHEs were treated with placebo or 1% or 2% *S. album* extract during 6 hours per day for 2 days.

A significant decrease of OR2AT4 protein level was observed in this senescent RHEs model obtained by FOXO3 downregulation. When senescent RHEs were treated with 1% or 2% *S. album* extract, the decrease of the OR2AT4 protein was limited.

In parallel, the expression of OR2AT4 was studied in NHKs from young donor cultured in a pro-aging environment *i.e.*, conditioned medium from aged NHKs. The treatment with *S. album* extract was performed on aged NHKs: *S. album* extract at 0.001% was applied in cell culture medium for 2 days. This medium was eliminated and fresh medium without extract was added for 24 hours. This supernatant was collected and applied on young NHKs (Figure 4).

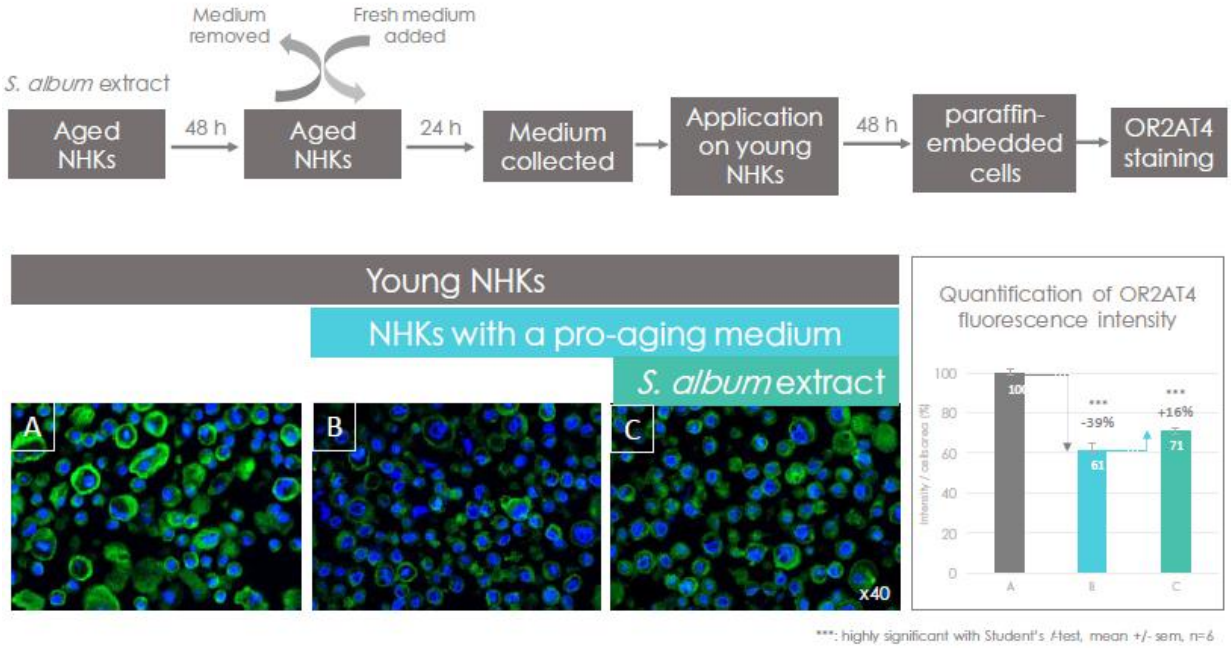


Figure 4: Expression of OR2AT4 in paraffin-embedded young NHKs cultured in pro-aging environment. x40 objective. Green: OR2AT4 staining, Blue: Nucleus staining using DAPI. A: young NHKs; B: young NHKs treated with pro-aging medium from aged NHKs treated with placebo; C: young NHKs treated with pro-aging medium from aged NHKs treated with *S. album* extract.

A significant decrease of OR2AT4 protein level was observed when young keratinocytes were treated with conditioned medium from aged keratinocytes, compared to classical medium. When aged keratinocytes were treated with *S. album* extract, the decrease of OR2AT4 protein expression observed in young keratinocytes treated with conditioned medium from adult keratinocytes, was limited.

Effect of the environmental pollution on OR2AT4 level

In this part, we studied the impact of urban pollution on OR2AT4 expression in skin. In addition, two other markers were observed: AGEs compounds, which accumulate in extrinsically aged

skin, and ceramide synthase 3 (CERS3) involved in the integrity of barrier function. To mimic the urban pollution, *ex vivo* skin biopsies were exposed to UFPs from diesel exhaust overnight. Then, *S. album* extract was applied on *ex vivo* skin biopsies for 48 hours (Figure 5).

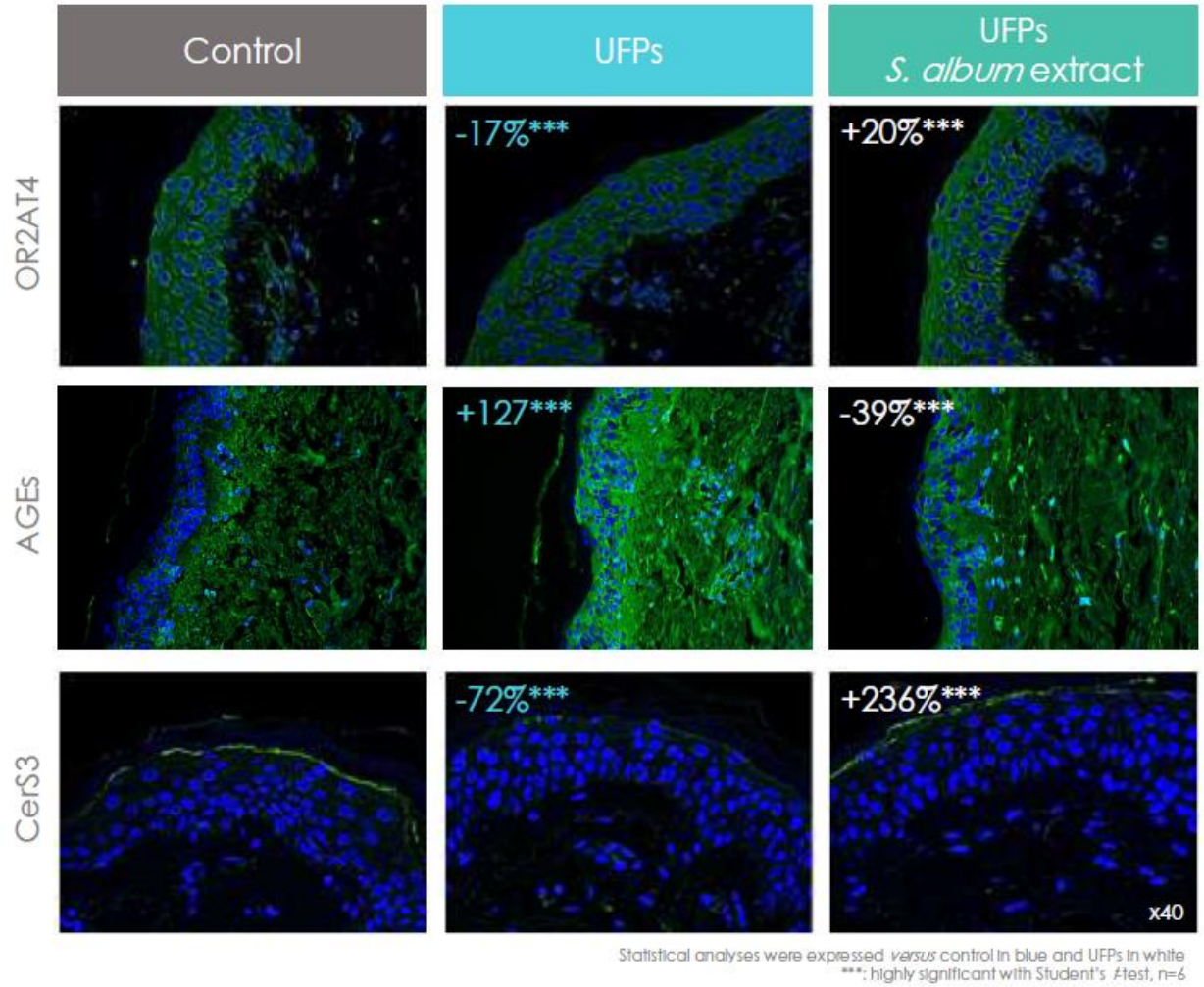


Figure 5: Expression of OR2AT4, AGEs and CerS3 in *ex vivo* skin stressed by UFPs overnight and treated with *S. album* extract for 48 hours. x40 objective. Green: OR2AT4, AGEs or CerS3 staining, Blue: Nucleus staining using DAPI. *Ex vivo* skin biopsies were stressed by UFPs overnight and then treated with *S. album* extract for 48 hours.

A significant decrease of OR2AT4 and CerS3 protein levels and an increase of AGEs compound level were observed when *ex vivo* skin was exposed to UFPs. When *ex vivo* skin exposed to UFP was treated with *S. album* extract, the expression of OR2AT4 and CerS3 came back to initial basal level and AGEs content was limited.

Conclusion.

Our results showed that the expression of OR2AT4 was inversely correlated with aging and UFP-induced skin damage, suggesting that its modulation could be beneficial to limit the consequences of intrinsic and extrinsic aging into the skin.

Conflict of Interest Statement. NONE.

References.

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