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"Cracking the longevity code with an innovative iris pallida root extract targeting humanin mitochondrial peptide"

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1. Introduction

Mitochondria have been extensively studied in aging because of their roles in cellular energy production, calcium homeostasis, and cell signaling. Recently, small open reading frames in circular mitochondria DNA have been discovered. They encode multiple microproteins, called mitochondria-derived peptides (MDPs). Currently, 8 MDPs have been published; the most studied is humanin [1]. Circulating humanin levels decreased with age in mice, monkey and human plasma. Intriguingly, it was not the case in long-living animal model showing negligible senescence and healthy aging. Human offspring of centenarians, who have a greater chance of living until 100 years old, even displayed elevated levels of circulating humanin compared with age-matched controls without family history of exceptional longevity. Likewise, using a Caenorhabditis elegans model, humanin overexpression was reported to increase lifespan via the regulation of Foxo factors involved in mammalian longevity [2,3].

In the present study, a peptidic sequence was specifically designed to target humanin (Humanin derived peptidic sequence). Preliminary testings have validated an increase in humanin expression in *ex vivo* human skin treated with this sequence. Then, artificial intelligence was used to predict natural extracts with homologous peptidic sequence. Several candidates were identified included Iridaceae family, Iris genus and more particularly Iris pallida species.
Based on this concept, a green Iris pallida extract was developed and studied on humanin, senescence and mitochondrial markers.

2. Materials and Methods

The effect of humanin derived peptidic sequence was first studied on humanin expression in *ex vivo* skin, treated for 2 days, by immuno-fluorescent staining.

The iris pallida extract was then obtained by a priorietary and patented PSR™ technology from dried roots. A conventional extraction was performed using same raw material to obtain a conventional extract to be compared to the iris pallida extract. The composition of the iris pallida extract was analyzed by UV spectrophotometer and HPLC-UV/MS analysis. A proteomic study was conducted by HPLC-MSMS and a gene enrichment analysis was performed using Database for Annotation, Visualization, and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/) [4,5]. The DAVID application provided functional annotation to understand the biological significance behind the identified predictive target genes. The DAVID database gives enriched biological themes, particularly Gene Ontology (GO) terms allowing to better understand the potential biological activities of the biofunctional.

The effects of PSR iris pallida root extract were finally studied at 0.1% on *in vitro* cells and 1% in *ex vivo* skin. Firstly, *ex vivo* skin biopsies were treated with 1 % of PSR iris pallida root extract, twice a day for 48 hours. A senescent-culture environment was made by mixing normal skin culture with senescent fibroblast culture medium (1:1). The control non-senescent was made by mixing normal skin culture with non-senescent fibroblast culture medium (1:1). Humanin and collagen I were then detected by immuno-fluorescent staning.

The impact of the PSR iris pallida root extract was then investigated on senescent-associated markers. Fibroblasts aged by replicative senescence were treated with 0.1% of PSR iris pallida root extract, once a day for 48 hours and analyzed *versus* senescent and non-senescent fibroblast control. Senescence-associated β galactosidase activity staining was detected by using X-gal as a substrate which is hydrolyzed to form a local blue precipitate product and mitochondrial mass was detected by using MitoTracker™ dye. Student's t-test, for independent samples with two-tailed direction of rejection was used for statistical analyses. $p \leq 0.05$ were considered as significant (*), $p \leq 0.01$ as very significant (**) and $p \leq 0.005$ as highly significant (***).

3. Results

3.1 Validation of the effect of humanin derived peptidic sequence on humanin expression

The effect of humanin derived peptidic sequence was evaluated on human *ex vivo* skin. After 2 days of application, a very significant increase in humanin expression was observed (Figure 1).

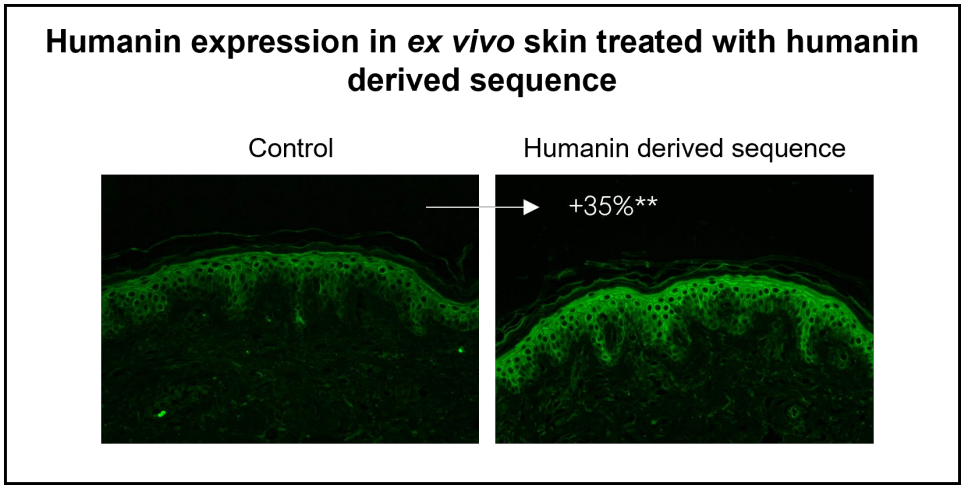

Figure 1. Humanin expression in *ex vivo* skin treated or not with the humanin derived peptidic sequence. **: very significant with Student's *t*-test.

Then, artificial intelligence was used to predict natural extracts with homologous peptidic sequence. Several candidates were identified including Iridaceae family, Iris genus and more particularly Iris pallida species. Based on this concept, a green Iris pallida extract was developed.

3.2 Characterization of the PSR iris pallida root extract

Analytical studies demonstrated that iris pallida extract has an interesting phytocompounds' signature, including phenolics coumponds, proteins, vitamins and sugars (Table 1).

Table 1. Phytochemical composition of iris pallida extract using HPLC (*) and UV spectrophotometry (**).

Phytocompounds	Total phenolic coumponds** (g/kg)	Total organic acids* (mg/kg)	Total sugars** (g/kg)	Total vitamins* B3 + B5 (mg/kg)	Total proteins** (g/kg)
PSR iris pallida extract (n=3, average)	541	92.7	4	0.46	3.1
conventional extract (n=1)	367	46.4	2.1	0.23	1.5

A comparative proteomic study was conducted on both extracts of iris pallida (PSR™ and conventional extraction). Analysis allowed the identification of 1566 proteins in the PSR iris

pallida root extract *versus* 599 proteins in the conventional extract, supporting the evidence of a more complex and diverse analytical signature brought by the PSR™ technology (Figure 2).

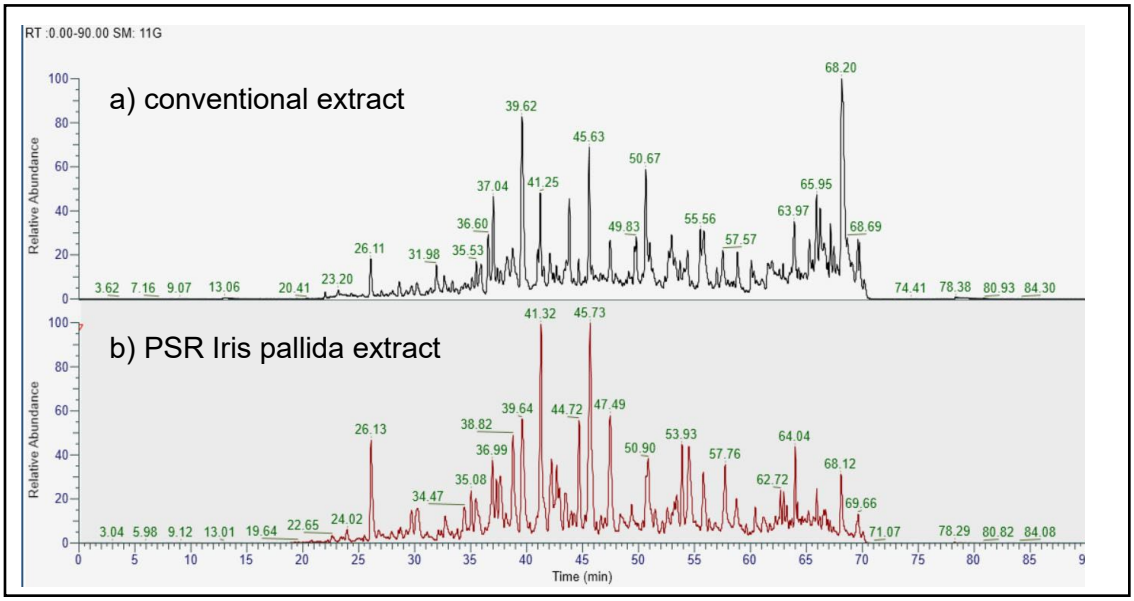

Figure 2. Proteomic study of iris pallida extracts, total ion chromatograms by HPLC-MSMS

Gene enrichment evaluation highlighted predicted effects of the PSR iris pallida root extract in several cellular functions: protein homeostasis, cytoskeleton, keratin, NAD & ATP synthesis and mitochondrial functions.

3.3 Evaluation of the PSR iris pallida root extract

First, humanin and collagen I were analyzed in *ex vivo* skin biopsies cultured in a senescent environment. Results showed a decrease of both markers compared to a “non-senescent” skin control. The application of the PSR iris pallida extract allowed to reverse the effect of the senescence (Figure 3).

Then, the effects of the PSR iris pallida extract were observed on senescent markers evaluating the SA-β-gal activity and the mitochondrial mass. Application of 0.1% of the extract on *in vitro* cells allowed to reverse the effect of the senescence (Figure 4)

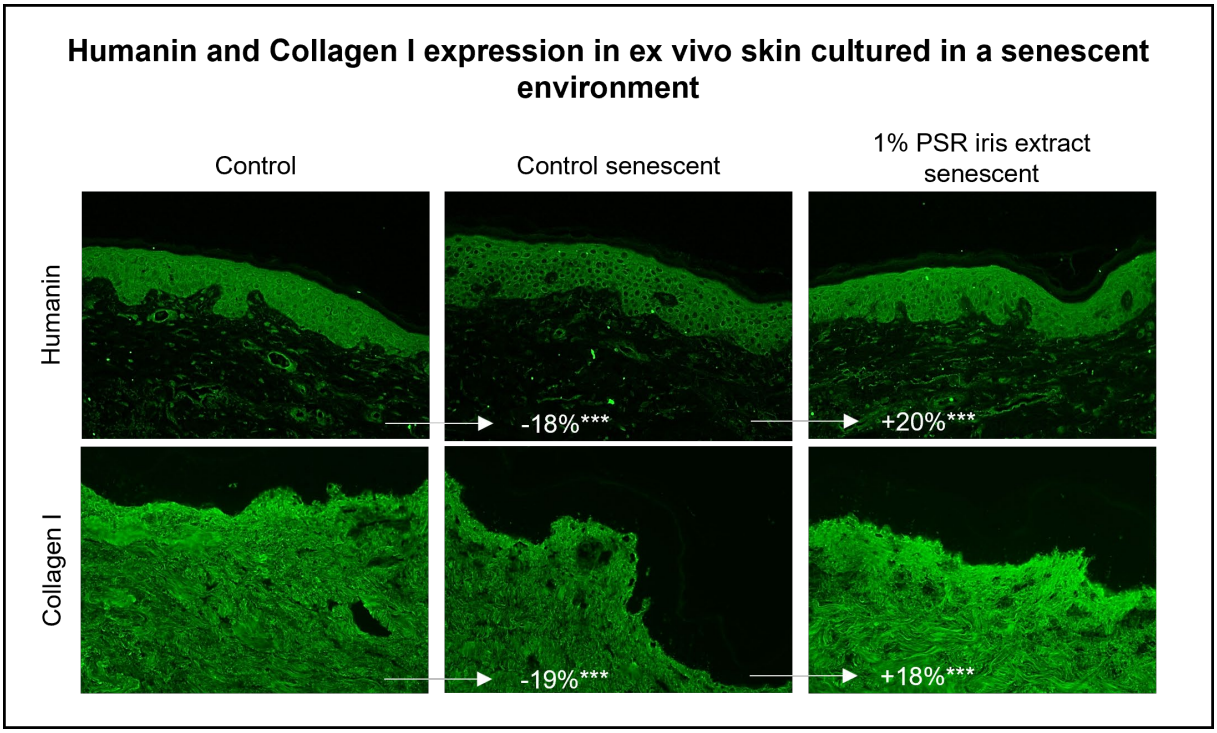

Figure 3. Humanin and collagen I expression in *ex vivo* skin cultured in a senescent environment and treated or not with the PSR iris pallida extract at 1%. ***: highly significant with Student's *t*-test.

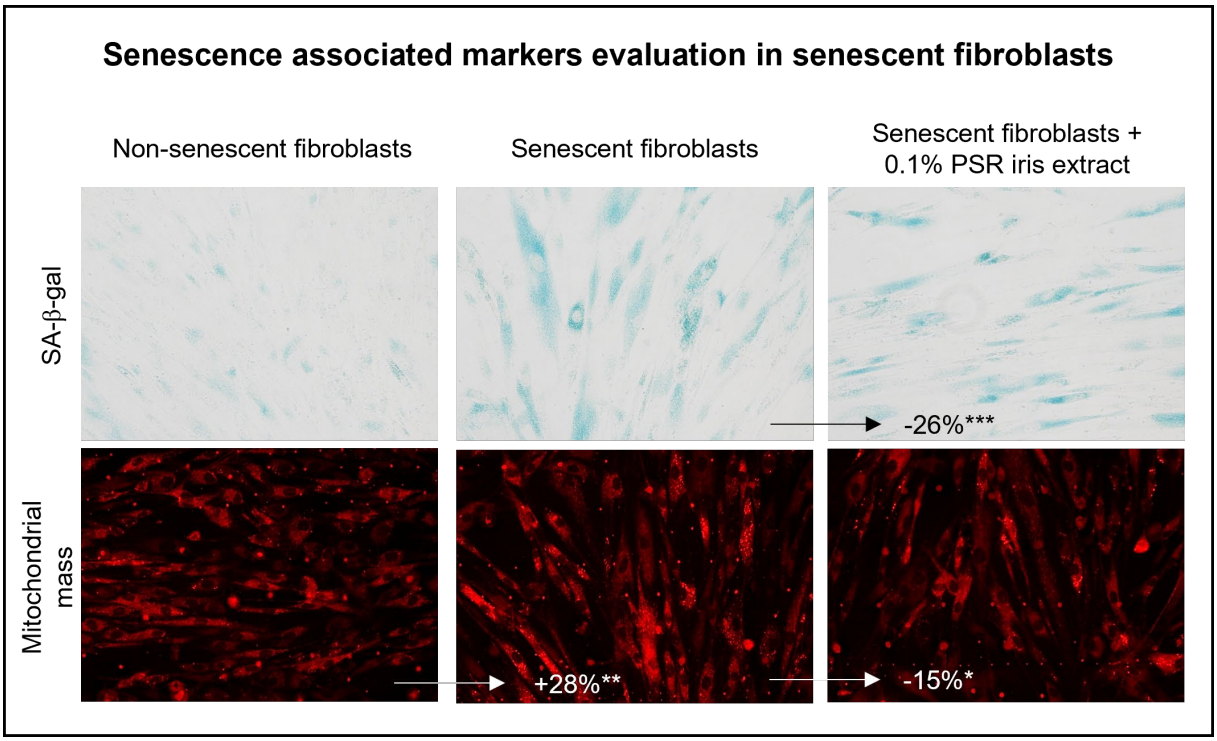

Figure 4. SA-β-gal and mitochondrial mass in fibroblasts in replicative senescence and treated or not with the PSR iris pallida root extract at 0.1%. *: significant, **: very significant, ***: highly significant with Student's *t*-test.

4. Discussion and conclusion

Humanin is the first mitochondrial derived peptides identified. It was initially cloned from the resilient occipital lobe of an Alzheimer's disease patient's brain and found to protect against amyloid-β toxicity in neuronal cells. Secreted in response to cellular stress, humanin has broad cytoprotective and neuroprotective effects [1].

In line with these data, we were interested in studying the impact of humanin expression on human skin. We have first designed a peptide derived from humanin sequence and validated its ability to increase the expression of humanin protein in human skin.

Then, we have selected the iris pallida as species that potentially comprises humanin derived sequence in its genome, and thus that can potentially increase humanin expression.

Thus, we have developed an innovative Iris pallida root extract using the patented PSR™ technology. Analytical evaluation allowed to highlight an interesting phytocompounds' signature, with higher level of phenolics compounds, proteins, vitamins and sugars compared to a conventional extract of iris pallida. A comparative proteomic study showed twice much more proteins in the extract processed with the PSR™ technology. A predicted gene enrichment study highlighted a potential impact of the extract on mitochondrial functions. Hence, biological evaluations were oriented towards humanin, aging and mitochondria.

On a senescent model of *ex vivo* skin, humanin and collagen I expressions were decreased. The application of 1% PSR iris pallida root extract allowed to counteract the impact of the senescence. Finally, the positive impacts of the PSR extract on senescence and mitochondria were showed by a decrease of the SA-β-gal activity and the mitochondrial mass in senescent cells.

This study emphasizes the role of humanin mitochondrial peptide as powerful longevity molecules through the testing of an innovative Iris pallida root extract, comprising humanin sequence homology.

5. References

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