Addressing dark spot through low redundant microRNA modulation and function

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Melanin is a skin chromophore produced by melanocyte within melanosomes. Melanin is transferred to adjacent keratinocytes in order to protect nuclear DNA and is responsible of the skin color. Melanin synthesis is catalyzed by at least 3 key enzymes: Tyrosinase (TYR), Tyrosinase-related protein 1 (TYRP1) and DOPAchrome tautomerase/Tyrosinase-related protein 2 (DCT/TYRP2).
Melanin synthesis is initiated by the rate-limiting enzyme called Tyrosinase (TYR) which converts tyrosine into dopaquinone. Then, dopaquinone undergoes several transformations until a final step of polymerization to produce the melanin pigment.

MicroRNAs (miRNA) are members of a class of small functional non-coding RNAs. They play important roles in targeted-gene regulation and are responsible for transient modulation of specific protein expression. Thus, miRNAs are key regulators of all biological processes and over 2000 miRNAs have been found in humans.

Because melanin synthesis could be controlled through epigenetic mechanisms, we investigated if specific miRNA modulation could be responsible for skin whitening. Members of miRNA families frequently target the same multigene set in a functionally redundant manner. Recent studies in microRNA functional analysis coupled with

bioinformatics have enabled us to identify the miR-490 suspected to regulate the melanogenesis process. In order to prove the direct link between this miR-490 and tyrosinase protein level, we used an innovative method combining the inhibition and over-expression of this miRNA in normal human melanocytes. Because these cells are delicate and difficult to transfect, we firstly optimized an efficient and non-toxic protocol approaching 90% of transfection rate. Further consistent bioinformatic analysis have been performed to study the miRNA-490 network and understand the functional link with other miRNAs.

In this study, we demonstrated that the low redundant miRNA, miR-490 is highly specific of the tyrosinase protein synthesis. Using gain- and loss-of-function approaches *in vitro*, we find that *miR-490* overexpression leads to a reduction of melanin production by human melanocytes through the repression of the tyrosinase protein synthesis by 50%. In addition, further consistent bioinformatic analysis have suggested that the studied microRNA might present low functional redundancy, meaning that its defect couldn't be counterbalanced by other miRNAs.
Moreover, we demonstrated the ability of a *Lansium domesticum* leaf extract to stimulate the miR-490 expression and to decrease the tyrosinase protein synthesis in Asian melanocytes. As a consequence, the extract inhibits the melanin production in a co-culture of melanocytes with keratinocytes.

In conclusion, the increasing bioinformatic tools for miRNA analysis produce a huge amount of data that generates a dramatic bottleneck. Indeed, the lack of experimental validation of these data may cause a misinterpretation of real relationship between the targeted proteins and microRNAs. Here, coupling innovative methods such as miRNA dedicated software and experimental modulation of miRNAs expression, we prove that the miR-490 may be an outstanding target to help reversing skin hyperpigmentation disorders such as dark spots. Finally, this helped us to discover a promising powerful cosmetic active ingredient able to finely control pigmentation and dark spot through a very precise action on tyrosinase.

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