New insights into exosome-autophagy pathway interactions in skin pigmentation modulation

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Maurin, Véronique[1] ; Xu, Yicheng[1] ; Saint-Auret, Gaëlle[2] ; Chevalier, Pascal[1] ;

[1] Research and Development Department, Biocosmethic, Bonnelles, France
[2] Genel Laboratoire, Grenoble, France

Introduction:

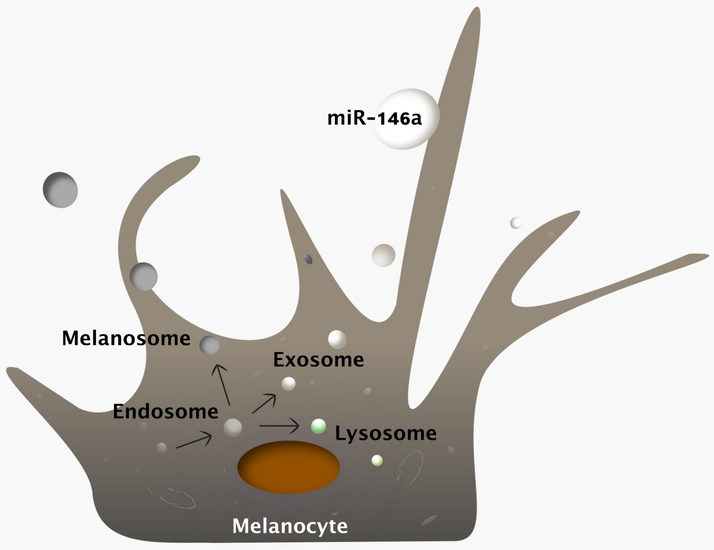

Figure 1: The heterogeneity of endosome pathways in melanocyte

Autophagy is defined as a strictly regulated program that promotes cell survival[1]. Several regulators related to autophagy have been described to be involved in the formation, maturation and degradation of melanosomes in melanocytes[2]. Exosomes are nano-sized lipid vesicles, which are currently being considered to be crucial mediators of cell-to-cell communication. Recent studies indicate that exosomes can regulate the autophagic processes through various signaling pathways. Interestingly, autophagy may also modulate biogenesis and release of exosomes. Exosome biogenesis has several links with endocytosis, lysosomal degradation as well as autophagocytosis[3].

The coordination between exosomes and autophagy pathways is critical for maintaining intracellular homeostasis[4,5]. Previously we showed an epidermal paracrine microRNA 146a (miR-146a) network from melanocyte-derived exosomes to target keratinocytes in hyperpigmentation prevention. The crosstalk between autophagy and endogenous miR-146a as well as exosome-mediated autocrine miR-146a network remains unclear. The purpose of this study is to understand more the exosome-autophagy pathway interactions in the modulation of skin pigmentation.

Materials & Methods:

Preparation of the multifunctional lightening agent
A Chinese medicinal plant traditionally used for skin conditioning has been identified, then an optimized version of traditional drying process and an extraction method similar to local decoction process have been developed in order to obtain the natural multifunctional lightening agent AMR.

Exosome purification and quantification
Primary melanocytes (NHEMs) were pre-treated with AMR at 0.05% during 24h. The exosomes were purified from the treated and untreated culture media and the exosomal protein was quantified using a quantification kit.

Target genes Measurement and LC3B immunostaining
8 days after the UV irradiation, the total RNA from NHEMS and NHEM-derived exosomes were extracted and purified. The modulation of the targeted mRNA and miRNA was measured by RT-qPCR. The immunostaining of an important autophagy actor LC3B was performed by adding the primary and secondary antibody. After staining, the fluorescence intensity was measured using HCS platform.

Statistical analysis
The results presented are means ± SEM. Differences between groups were assessed by means of Student's unpaired T-test. Significance level was set at *p<0.05; **p<0.01; ***p<0.001.

Conclusions:

The effects of UV radiation on the melanogenic genes and autophagy-related factors tested do not seems to be synchronized. Under the treatment of AMR, UVRAG and BNIP3 induce BECN1/LC3B-associated autophagy. The up-regulation of miR-146a in melanocytes and melanocyte-derived exosomes seems to be positively correlated with this AMR-induced BECN1/LC3B-related autophagy. The synergy of BECN1/LC3B-associated autophagy pathway and exosome-mediated autocrine/paracrine miR-146a network may have potential applications in skin pigmentation modulation.

Aknowledgments:

The authors thank Dr. Jean-Eric Branka for his support.
This work was financially supported by Biocosmethic SAS.

Results & Discussion:

MODULATION OF ENDOGENOUS MIR-146A IN NHEMS & NHEM-DERIVED EXOSOMES

As a key microRNA involved in cell proliferation, inflammation and pigmentation, miR-146a in NHEMs was statistically repressed by UV and significantly induced by AMR. After UV exposure, miR-146a level significantly increased in NHEM-derived exosomes. Treatment by AMR induced 12 times more than UV the expression of this miRNA in NHEM-derived exosomes. miR-146a has been described to promote autophagy by depressing its target B-cell lymphoma-2 (BCL-2)[6]. The latter is an inhibitor of BECN1, miR-146a causes BECN1 up-regulation and increases autophagy[6]. On the other hand, the overexpression of miR-146a has also been shown to inhibit autophagy[7]. The effects of miR-146a on autophagy seem to be context-specific.

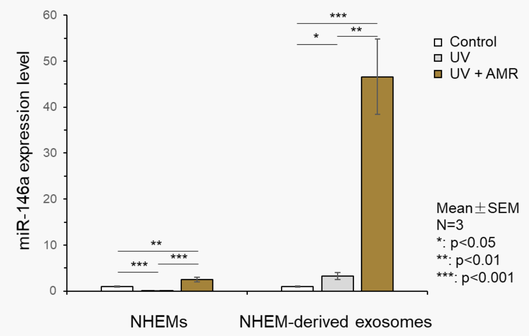

Figure 2: miR-146a expression level in NHEMs & NHEM-derived exosomes

INHIBITION OF MELANOSOME BIOGENESIS IN NHEMS

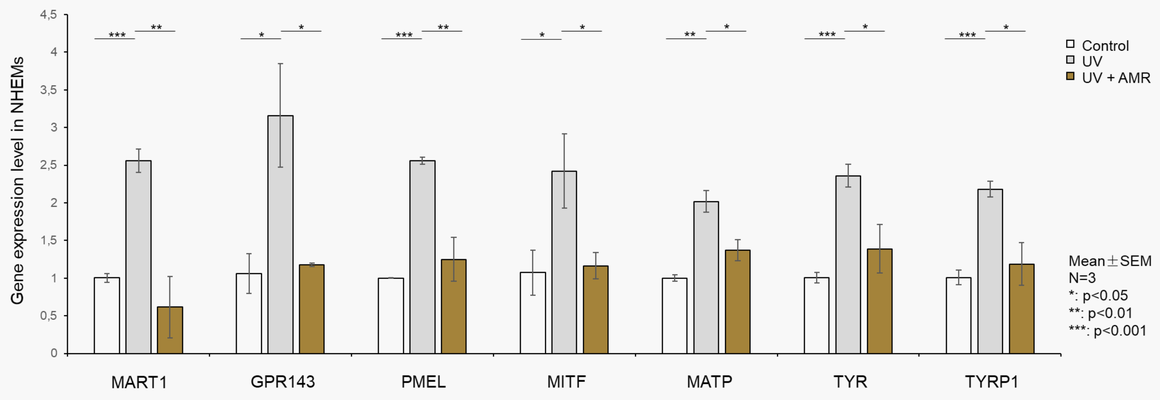

Figure 3: Effect of AMR on melanosome biogenesis-related genes expression in NHEMs

The expression levels of key genes implicated in all stages (I to IV) of melanosome biogenesis (MART1, GPR143, PMEL, MITF, MATP, TYR and TYRP1) increased significantly after UV irradiation. Parallelly, AMR at 0.05% statistically down-regulated the UV-depend induction of all these genes. Several autophagy-related regulators may play an indispensable role in melanogenesis and melanosome biogenesis. BECN1 is involved in melanogenesis by changing the subcellular distribution of MITF[8]. UVRAG is believed to be required for the dynamic integrity and maturation of melanosomes[9]. BNIP3 may be a potential regulatory target for preventing inflammatory skin color disorders[10].

MODULATION OF AUTOPHAGY-RELATED GENES & LC3B PROTEIN EXPRESSION IN NHEMS

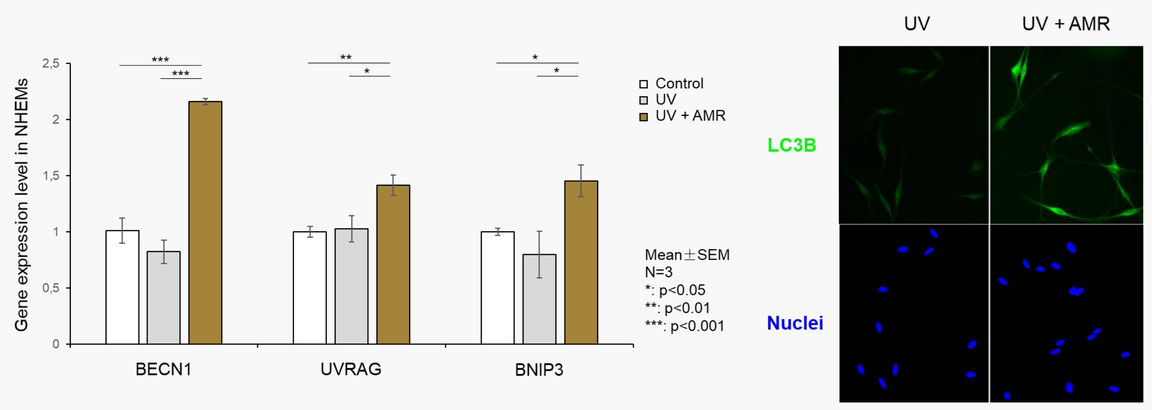

Figure 4: Effect of AMR on autophagy-related gene expression (left) & LC3B protein level (right) in NHEMs

The expression level of BECN1, UVRAG and BNIP3 genes did not change statistically after UV irradiation. In the meantime, AMR at 0.05% seems to induce autophagy process in UV-irradiated NHEMs by significantly over-expressing these 3 autophagy-related genes as well as LC3B protein. In spite of several autophagy-related factors that have been reported to be involved in melanogenesis, it seems that the effects of UV radiation on the tested melanogenic genes and autophagy-related factors are not synchronized. Moreover, UV-induced miR-146a attenuation does not appear to impact BECN1-related autophagy in melanocytes. Under the treatment of the natural lightening agent AMR, UVRAG and BNIP3 induce BECN1/LC3B-associated autophagy in melanocytes. The up-regulation of miR-146a in melanocytes and melanocyte-derived exosomes seems to be positively correlated with AMR-induced BECN1/LC3B-related autophagy.

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