Targeting Small Non-coding RNAs to Diminish the Epigenetic Ravages of Aged Skin

Poster ID #53

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Introduction:

There has been considerable research in the study of epigenetics, whereby expression of specific genes is modulated in absence of any alteration to the genetic code. Given that one aspect of chronological aging is the gradual reduction in activity of critical genes in skin cells, the application of epigenetic mechanisms to skin care regimens could be of substantial benefit. Although there are several characterized epigenetic mechanisms, post-transcriptional gene silencing (PTGS) through RNA interference (RNAi) has received much attention. Here, short non-coding RNA molecules such as microRNAs (miRNAs) intercept messenger RNAs (mRNAs) from expressed genes before they can interact with ribosomes and initiate protein synthesis.

Many endogenous miRNAs have been investigated to determine the downstream gene products that they target, so as to decipher their silencing pathway. This has led to so-called "miRNA profiling" studies to delineate "signatures" of miRNA activation and deactivation with respect to specific skin conditions. The outcomes of these endeavors have inevitably led to efforts to devise strategies to intervene in the toggling of gene expression up and down.

Natural "mimics" of specific miRNAs are easily generated that could bolster normal endogenous levels of a particular miRNA, thereby diminishing expression of certain target genes. Alternatively, "antagomiRs" of miRNAs have also been synthesized that prevent their interception of mRNAs, resulting in augmentation in the expression of targeted genes. The current limitation of the deployment of these non-coding RNA molecules in skin care products is the development of a suitable delivery vehicle.

Herein, we describe a novel formulation that is capable of transfecting miRNAs in cell culture as well as allowing their penetration into the skin upon topical application. The formulation further affords protection against thermolytic and enzymatic degradation of the miRNA cargo. This represents a significant advancement in the field of epigenetic driven skin care.

Materials & Methods:

Materials: Human miRNA mimics, negative control mimics, and antagomiRs were purchased from Thermo Fisher Scientific. RNEasy purification kit, RT2 first strand synthesis kit, miRCURY SYBR green reagent, and miRCURY LNA focus panel array were purchased from Qiagen. MIRNAC™ transfection/delivery reagent produced by Biocogent, LLC (patent pending).

Cell Culture: S91 cells were cultured in RPMI media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) at 37° C in 5% CO_2. NHDF cells were grown in DMEM with 10% FBS, 1% P/S, and 1% HEPES at 37° C/5% CO_2.

miRNA Profiling: Changes in miRNA levels were detected via RT-qPCR (QuantStudio3) using the miRCURY LNA focus panels. Briefly, cellular RNA was purified from skin cells treated with specific materials, followed by conversion of the purified miRNAs to cDNA using reverse transcriptase (RT) in the RT kit. The resulting cDNA was mixed with miRCURY SYBR green reagent and analyzed on the aforementioned focus panels

Transfection Assay: Cell cultures were treated with miRNA mimics or antagomiRs at working concentration of 50 nM mixed with ~3% MIRNAC™. The mixture was allowed to incubate on cell monolayers for at least 72 hours before harvesting.

MelanoDerm™ Assay: Three-dimensional melanocyte-infused skin model (MelanoDerm) was treated with a miRNA mimic or a control for 4 weeks. At the conclusion of the study, the tissues were thoroughly rinsed, imaged, and processed.

Western Blot Analysis: Cells and tissues were lysed and boiled using a commercially available version of Laemmli lysis buffer. Proteins were separated by molecular weight via sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE). The gel was electro-transferred to a nitrocellulose membrane that was blocked and probed with antibodies specific to skin proteins (depending on the indicated experiment). The protein gel was stained with Coomassie blue post-transfer to normalize relative protein levels between lanes when blots were subsequently evaluated by densitometry scanning using ImageJ software.

Immunofluorescent Microscopy Analysis: Cells cultured on glass coverslips were treated as indicated. Afterwards, the coverslips were rinsed in phosphate-buffered saline (PBS), fixed with 1% paraformaldehyde, permeabilized with 0.1% Triton-X100 in PBS, and blocked with 3% bovine serum albumin (BSA). Subsequently, the cells were probed with rabbit-anti-collagen-1 diluted in 1% BSA followed by goat-anti-rabbit secondary antibodies conjugated to AlexaFluor™-555 (red fluorophore). Finally, the coverslips were mounted onto glass microscope slides using anti-fade mounting medium infused with 4,6-diamidino-2-phenylindole (DAPI) that fluorescently stains nuclei (blue).

RNAse A Protection Assay: miRNA mimics were mixed with MIRNAC™ at various percentages and initial concentrations were determined using the Qubit™ miRNA concentration assay. Samples were subsequently incubated with RNAse A at 25° and 37° C in parallel for 0, 60, 120, and 180 minutes. Afterwards, the relative miRNA concentration remaining in each sample was evaluated using the Qubit™ assay.

TABLE 1: BIO-MATERIAL #1

Algorithm	miRDB			miRmap			miRTarBase		
microRNA:	150-5p -3.03 p<0.05	92a-3p -1.77 p<0.03	let7c-5p -1.53 p<0.03	150-5p -3.03 p<0.05	92a-3p -1.77 p<0.03	let7c-5p -1.53 p<0.03	150-5p -3.03 p<0.05	92a-3p -1.77 p<0.03	let7c-5p -1.53 p<0.03
Target Genes:	ADIPOR2	CDH10	ADIPOR2	ADIPOR2	CDH10	CLDN1	ADIPOR2	CDH10	ADIPOR2
	CERS3	COL1A2	CLDN1	CERS3	COL1A2	CLDN16	HAS2	ITGA5	CDH10
	COL19A1	COL11A1	CLDN16	COL19A1	COL11A1	COL1A2		LPIN1	ITGB3
	FNDC5	COL12A1	COL1A2	FNDC5	COL12A1	COL3A1			SIRT1
	GJC1	COL19A1	COL3A1	GJC1	COL27A1	COL4A6			
	GJD4	COL27A1	COL4A6	GJD4	DSC2	COL14A1			
	ITGAX	DSC2	COL14A1	ITGAX	FBN1	FNDC3A			
	ITGB3	FBN2	FNDC3A	ITGB3	HAS2	GJC1			
	RAD23B	HAS2	GJC1	RAD23B	HAS3	IL10			
		HAS3	HAS2	SIRT1	ITGA5	ITGB3			
		ITGA5	IL10		LPIN1	KRT5			
		LPIN1	ITGB3		SIRT6	PPARA			
		SIRT6	KRT5			SERPINB9			
			PPARA						
			SERPINB9						

Results & Discussion:

TABLE 2: BIO-MATERIAL #2

Algorithm	miRDB		miRmap		miRTarBase	
microRNA:	150-5p -5.60 p<0.02	320a +2.01 p<0.01	150-5p -5.60 p<0.02	320a +2.01 p<0.01	150-5p -5.60 p<0.02	320a +2.01 p<0.01
Target Genes:	ADIPOR2	ADAM28	ADIPOR2	ADAM28	ADIPOR2	CD2
	CERS3	CEMIP2	CERS3	CD2	HAS2	CTNNAL1
	COL19A1	IGF1R	COL19A1	JMY		IGF1R
	FNDC5	JMY	FNDC5	MCM4		MED23
	GJC1	MCM4	GJC1	NPR1		NPR1
	GJD4	MED21	GJD4			
	ITGAX	MED23	ITGAX			
	ITGB3		IOTGB3			
	RAD23B		RAD23B			
			SIRT1			

Tables 1 and 2. Two separate miRNA profiling studies of normal human keratinocytes treated with different skin care ingredients.

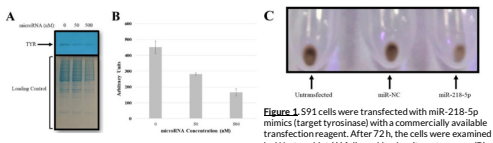

Figure 1. S91 cells were transfected with miR-218-5p mimics (target tyrosinase) with a commercially available transfection reagent. After 72 h, the cells were examined by Western blot (A) followed by densitometry scans (B). The cell pellets were also visually assessed for pigmentation changes (C).

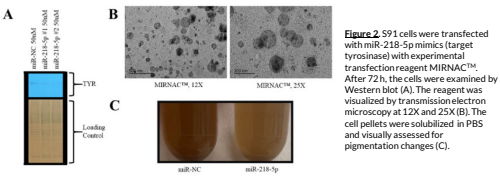

Figure 2. S91 cells were transfected with miR-218-5p mimics (target tyrosinase) with experimental transfection reagent MIRNAC™. After 72 h, the cells were examined by Western blot (A). The reagent was visualized by transmission electron microscopy at 12X and 25X (B). The cell pellets were solubilized in PBS and visually assessed for pigmentation changes (C).

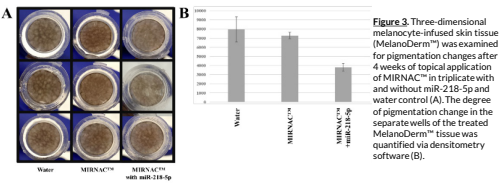

Figure 3. Three-dimensional melanocyte-infused skin tissue (MelanoDerm™) was examined for pigmentation changes after 4 weeks of topical application of MIRNAC™ in triplicate with and without miR-218-5p and water control (A). The degree of pigmentation change in the separate wells of the treated MelanoDerm™ tissue was quantified via densitometry software (B).

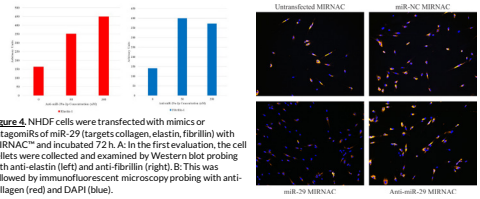

Figure 4. NHDF cells were transfected with mimics or antagomiRs of miR-29 (targets collagen, elastin, fibrillin) with MIRNAC™ and incubated 72 h. A: In the first evaluation, the cell pellets were collected and examined by Western blot probing with anti-elastin (left) and anti-fibrillin (right). B: This was followed by immunofluorescent microscopy probing with anti-collagen (red) and DAPI (blue).

Conclusions:

The field of epigenetic research continues to accrue new data at an astonishing rate. Understandably, the cosmetic care industry has investigated the utility of this technology as a means to improve skin health by driving increases in the expression of beneficial gene products and down-modulating deleterious ones. The experiments presented here and elsewhere reinforce the argument that current skin care regimens stimulate naturally occurring PTGS mechanisms. Furthermore, the effectors of these pathways can be carefully targeted and harnessed to promote a therapeutic balance in critical skin proteins. However, the success of such efforts hinges on finding qualified delivery vehicles that will allow these molecules to safely penetrate the skin barrier.

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