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The New Era of microRNA Profiling and Its Impact on Modern Cosmetics

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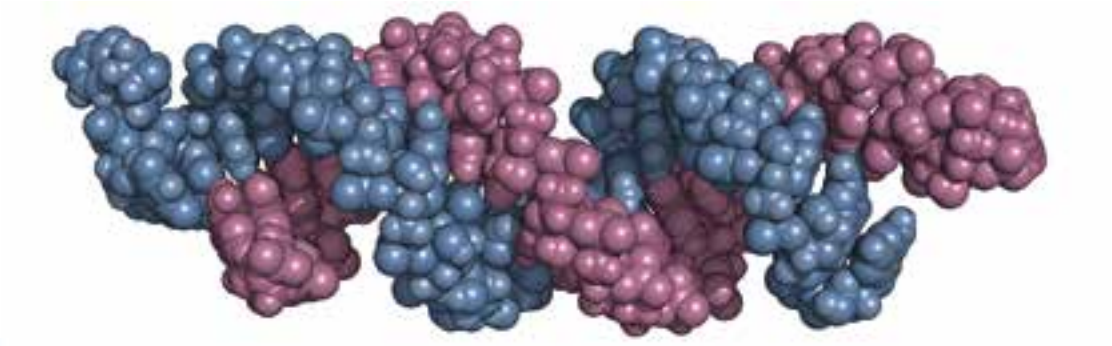

Abstract

The discovery of microRNAs and the gradual elucidation of their regulatory impact on protein expression has opened up a new arena of research into the development of therapeutics with cosmetic as well as pharmaceutical applications. It has been shown that disease states and environmental factors are capable of altering the expression of a person's microRNAs, thus causing an imbalance in their normal homeostatic levels that results in alterations in the cellular levels of certain proteins. As such, academic and industrial research groups have heavily investigated the dysregulation of specific microRNAs in response to extrinsic and intrinsic factors, culminating their findings into unique microRNA signatures; a process known as microRNA profiling. Here, we examine the microRNA profiles that have been developed for ultraviolet (UV) light-damaged skin cells and how a novel new bio-active ingredient for skin care products augments the activity of a DNA repair pathway and the microRNA regulatory pathways stimulated in response to UV light.

Keywords: microRNA, microRNA profiling, skin cells, UV damage, Sargassum vulgare

Introduction

The endogenous process of RNA interference (RNAi), where small non-coding RNAs (ncRNAs) anneal to the ends of messenger RNAs (mRNAs) and prevent their interaction with the protein synthesis machinery of the cell, was first reported in the late 1990s. This discovery subsequently led to an explosion of new research into how the effectors of this process, microRNAs (miRNAs or miRs) and short-interfering RNAs (siRNAs), impact various aspects of human health through their regulation of downstream protein expression[1]. Early in the century, biologists predicted that approximately 30% of all human genes were post-transcriptionally regulated by miRNAs[2], but as research into RNAi in mammalian systems has progressed, that number has rapidly doubled to more than 60% of all protein coding genes. This field of research has created the potential for various new diagnostic mechanisms as well as treatments and therapies to be developed for conditions and disorders that were previously "undruggable"[3].

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As miRNAs can diminish (RNAi) and sometimes augment gene expression (RNA activation or RNAa)[4], scientists have explored ways to manipulate this form of epigenetic regulation. One such approach is to increase the levels of particular miRNAs by introducing synthetic mimic molecules of an endogenous miRNA, which is called "miRNA replacement therapy"[5]. This is useful if the miRNA in question targets a deleterious gene product. In contrast, there are synthetic "antagomiR" molecules that are perfectly complementary to a natural miRNA and bind it irreversibly, so that it cannot interact with its target mRNA. This method would be employed if a host miRNA was directed toward the mRNA of a beneficial gene product.

Fluctuations in the levels of endogenous miRNAs often occur in response to exposure to environmental factors such as ultra-violet (UV) light, the development of congenital disorders, as well as bacterial and viral infections. Intriguingly, the specific miRNAs, whose expression levels are disrupted, tend to be unique to the stimulus catalyzing the change. As such, scientists have established unique "microRNA signatures" for certain conditions after conducting "microRNA profiling" studies. For example, hand-foot-and-mouth disease (HFMD) can be caused by either coxsackievirus-16 or enterovirus-71. MicroRNA profiling revealed that each virus produced a unique signature during the pathogenesis of HFMD, allowing doctors to develop it as a diagnostic test to distinguish the culprit viral agent[6].

An excellent example of how microRNA profiling was applied to a disease model is in the case of the foot-and-mouth disease virus (FMDV), the most contagious animal virus known that targets cattle, pigs, and other susceptible cloven-hooved mammals. In this instance, dysregulation of the normal homeostatic levels of miRNAs in blood circulation was examined in blood samples collected from uninfected cattle, acutely infected cattle, and cattle that had entered a "carrier state"[7]. MiRNAs were harvested and purified from the blood samples and subjected to real-time quantitative PCR (RT-qPCR) on array plates with hundreds of different miRNA species. These experiments generated miRNA dysregulation signatures for cattle in each of the aforementioned states, each with a unique series of miRNAs that were up- and down-regulated. The information gleaned from this study has helped guide future experiments attempting to find new approaches to combating and eventually eradicate FMDV globally.

Within the context of skin care, several scientific reports have been published identifying unique miRNA signatures in response to a variety of environmental and disease factors such as exposure of skin cells (keratinocytes, melanocytes, and fibroblasts) to UV-A and UV-B light[8]. Herein, the utility of miRNAs as a diagnostic tool and as a measure of a treatment's efficacy are detailed. In particular, a recently marketed, novel bio-active skin care ingredient is described along with its impact on miRNA levels in the skin and its protection of skin cell DNA from damage incurred from exposure to UV light.

MicroRNA profile of UV-damaged skin cells and a treatment

MicroRNA profile signatures for skin cells exposed to UV light

The UV-induced dysregulation of the normal homeostatic levels of miRNAs in various skin cells has been investigated in some detail. MicroRNA profiling has been conducted on keratinocytes, melanocytes, and fibroblasts that have been exposed to UV-A and UV-B light for different durations. The data generated from these studies elucidated unique miRNA expression signatures for each condition, cell type, and exposure time.

In the instance of fibroblasts exposed to UV-A light for 7 days, 12 miRNAs exhibited some form of dysregulation, whether up- or down-regulated[9]. Five fibroblast miRNAs saw their expression levels increase in response to the UV-A light, which included: miR-30b, miR-30c, miR-148a, miR-199a-3p, and miR-365. Collectively, these particular miRNAs contribute to the regulation of cell migration, cell invasion, and metastasis; all of which can become abnormally stimulated in response to UV exposure. Moreover, these miRNAs participate in modulating the immune response in skin cells as well as altering the methylation levels of regions of genomic DNA that can lead to gene silencing[10].

With regard to keratinocytes, the cells were exposed to either UV-A or UV-B light for no less than 6 hours[11]. Interestingly, different miRNA profiles were observed for keratinocytes exposed to UV-A and UV-B light, indicating that unique miRNA expression signatures could be established depending on the type of UV light. For keratinocytes exposed to UV-A, 14 miRNAs were down-regulated and 13 up-regulated. In contrast, UV-B light catalyzed decreased expression of 19 miRNAs and increased it for an additional 9 miRNAs. Between the two sets, 6 miRNAs were shared that were down-regulated and 2 that were up-regulated. Notable among this last set are miR-96 and miR-598. As will be further described in the subsequent section, these two miRNAs played key roles in modulating the DNA repair response to UV light as well as stimulating self-renewal and regeneration.

So, can treatments be brought to market that can reverse the aberrant miRNA expression signatures induced by UV light exposure? An unrelated study looking into the involvement of miRNAs in the molecular pathogenesis of vitiligo would suggest that the answer is very much an affirmative. In 2015, a report was published describing four miRNAs whose expression is altered in peripheral blood mononuclear cells (PBMCs) during the development of vitiligo[12]. Specifically, miR-3940-5p was down-regulated and miR-224-3p, miR-2682-3p, and miR-4712-3p were up-regulated. When the same PBMCs were subsequently treated with thymosin, the aberrant expression pattern was completely reversed. These findings demonstrate that not only is a microRNA profile useful in identifying molecular pathways that are affected by an environmental stimulus or disease, but also for informing whether or not a particular treatment is effective in reversing the damage incurred.

It is important to note that the interpretation of such data is rarely as black and white as in the case of vitiligo described above. The alterations in the miRNA levels can be the direct result of the damage applied or they can be an aspect of the cellular repair response. Therefore, extrapolating the significance behind the expression changes must be done thoughtfully.

MicroRNA profile for a new skin care product aimed to shield against UV light

Recently, a new skin care product called DermalRx® FSE has been released by Biocogent, LLC (New York, USA). This material is marketed as a bio-active ingredient for skin care that is designed to protect against the accrual of DNA damage in skin cells exposed to UV-A and UV-B light.

This bio-active material is derived from a brown macroalgae called *Sargassum vulgare* (see Figure 1). This particular seaweed

Figure 1. *Sargassum vulgare.* Shown in the figure is a picture of *S. vulgare* brown seaweed in its natural environment. This particular seaweed grows in the Sargasso Sea of the North Atlantic Azores

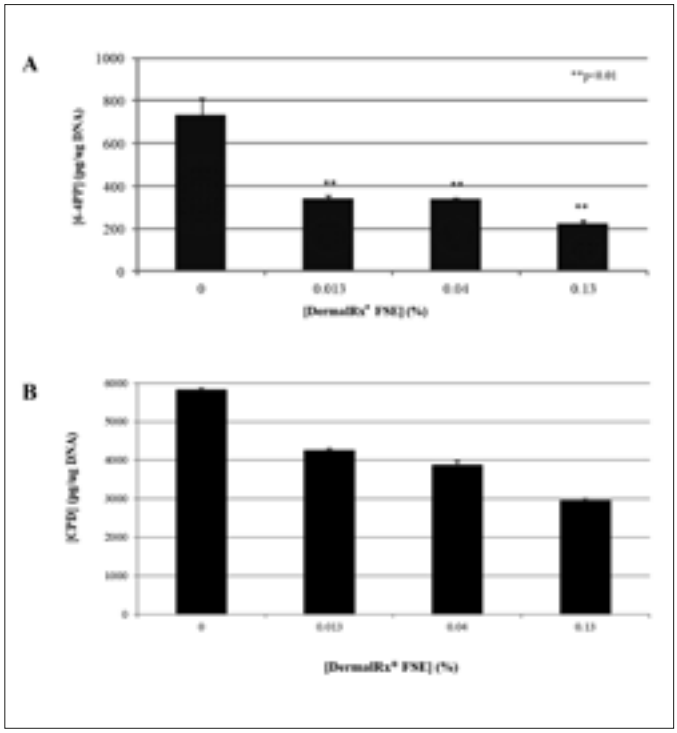

Figure 2. Enhanced DNA repair observed post-treatment with DermalRx® FSE and exposure to UV light. Multiple DNA repair pathways are activated in the skin after exposure to UV light to eliminate such types of mutations as A: 6-4 photoproducts (6,4-PP) and B: cyclo-pyrimidine dimers (CPD). Post-UV exposure, treatment with DermalRx® FSE enhances the nucleotide excision repair (NER) pathway that acts to remove these mutations.

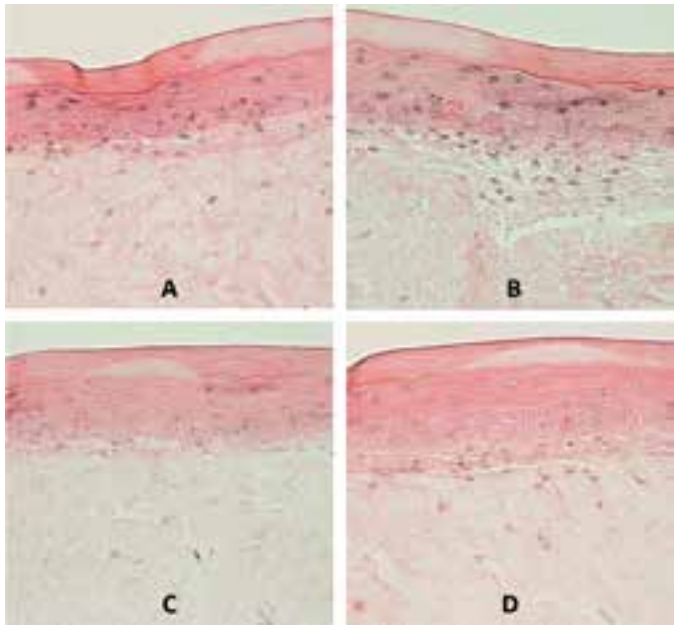

Figure 3. Stimulation of DNA repair in three-dimensional organotypic skin models. Duplicate untreated tissues were irradiated with 80 mJ/cm^2 resulting in strong CPD signaling (dark blue spots) detected by immunohistochemistry with anti-CPD antibodies (Panels A and B). Treatment of tissues with DermalRx® FSE stimulated DNA repair and reduced CPD levels resulting from UV-B exposure after 24 hours (Panels C and D).

produces multiple biochemical products that are of interest to human health. Among these products are sulfated fucans, phlorotannins, alginic acid, and diterpenes. Phlorotannins have an excellent profile of activities that include: anti-cancer, anti-diabetic, anti-viral (notably against the human immunodeficiency virus and multiple herpes viruses), and anti-oxidation activities [13]. Moreover, they protected against UV-induced DNA damage.

Extensive research indicated that a ferment of *Sargassum vulgare* (DermalRx® FSE) stimulated the nucleotide excision repair (NER) pathway upon exposure to UV light. To that end, two different forms of DNA damage incurred from UV exposure: 6,4-photoproducts (6,4-PP) and cyclo-pyrimidine dimers (CPD) were substantially reduced post-treatment with DermalRx® FSE (see Figure 2A and 2B). This was substantiated further with an immunohistochemistry experiment showing that after exposure to UV-B light, tissues treated with DermalRx® FSE exhibited considerably fewer CPD lesions (see Figure 3).

To further examine the impact of DermalRx® FSE on skin health, a microRNA profiling study was conducted on a MatTek three-dimensional skin model. Relative to a negative control substance, DermalRx® FSE altered the expression patterns of four skin-relevant miRNAs: miR-96, miR-429, miR-598, and miR-720 (see Table 1). All four of these miRNAs were down-regulated in response to the application of DermalRx® FSE to the skin model. Notably, miR-598 exhibited reduced levels in response to both UV-A and UV-B exposure in keratinocytes, while miR-96 was increased after UV-A exposure and decreased following UV-B exposure [11].

A cursory search of curated online microRNA databases describes miR-96 playing a regulatory role in wound healing and the UV response. Interestingly, miR-96 is also up-regulated in diabetic skin. Some of the notable genes that are post-transcriptionally

microRNA	Gene Targets	Predicted Functions
miR-96	AQP5, CELSR2, IGF-1R, MITF, RAD51, REV1, TIMP1	• UV-B DNA damage response • double-stranded break repair • DNA repair scaffold • extracellular matrix integrity • miR-96 is up-regulated in diabetic skin
miR-429	BAP1, BCL2, EZH2, FN1, MYC, PKP1, SOX2	• regulation of keratinocyte proliferation • self-renewal and regeneration • maintenance of tight junction and extracellular matrix integrity
miR-598	SIRT1, VEGF-A	• miR-598 down-regulated post-UVA/B expsoure • miR-598 down-regulated post-ionizing radiation exposure
miR-720	P63, TWIST1	• miR-720 increase is a melanoma bio-marker • miR-720 keratinocyte differentiation and proliferation toggle

Table 1. Tabulated are the microRNAs that were observed to be dysregulated in response to the application of DermalRx® FSE to a 3D MatTek skin model. Included in the table are some of the proposed genes that are post-transcriptionally regulated by the microRNAs indicated as well as the phenotypes exerted by them.

regulated by miR-96 include: IGF-1R, RAD51, REV1, and TIMP1. TIMP1 contributes to the maintenance of the extracellular matrix (ECM) integrity. Relevant to DNA damage, RAD51 is involved in the double-stranded DNA break repair pathway, REV1 contributes to the formation of scaffolds for DNA polymerase to function in DNA repair, and IGF-1R is a key promoter of DNA repair pathways activated in response to UV-B light exposure.

miR-429 has been reported to contribute to the regulation of keratinocyte proliferation by controlling the expression of key genes such as BCL2, EZH2, KLF11, MYB, and MYC. Perhaps of greater relevance to the skin, miR-429 down-modulates the expression of PKP1 and FN1, both of which assist in maintaining the integrity of tight junctions and the extracellular matrix. Moreover, SOX2 is also a target of miR-429, which is responsible for self-renewal and regeneration.

As described in the Kraemer study, miR-598 is decreased post-exposure to UV-A and UV-B light [11]. Its levels are also reduced in response to ionizing radiation. Therefore, many scientists have concluded that miR-598 is a key regulator in the response pathways to various forms of radiation. Notably, SIRT1 is a target of the post-transcriptional activity of miR-598. The sirtuin protein encoded by this gene facilitates multiple DNA repair pathways and certain anti-aging mechanisms.

The fourth miRNA whose expression levels were found to be reduced in response to DermalRx® FSE administration was miR-720. Scientists have established this miRNA as a bio-marker for melanoma development when its levels are increased. Moreover, it has been implicated in keratinocyte proliferation. The notable genes found to be regulated via miR-720 include: the tumor suppressor p63, TWIST1 that links the autophagy pathway to DNA repair mechanisms and also impacts cellular proliferation, and NANOG that triggers increased expression of DNA methyl transferases (DNMTs). Of note, maintenance of DNA methylation in certain regions of genomic DNA has been found to be key in skin aging and sustaining self-renewal and the identity of skin progenitor cells [14].

Conclusions

The impact of epigenetics on modern cosmetics and skin care cannot be understated, and the unique contribution of microRNAs will guide many efforts to develop and market more efficacious products. One of the most studied aspects of skin health is with regard to the impact of exposure to UV-A and UV-B light. Several scientific studies have detailed the dysregulation of various endogenous microRNAs in direct response to UV exposure, such that unique microRNA profiles have been established. These signatures have now been utilized to evaluate the efficacy of new skin care products aimed at ameliorating the long-term genetic damage that is acquired from prolonged exposure to such forms of light radiation. Of note in this article, the newly marketed DermalRx® FSE has been demonstrated to exert a positive effect in the repair of two types of DNA damage that are incurred as a result of UV exposure. The application of DermalRx® FSE augmented the activity of the nucleotide excision repair pathway, leading to a reduction in two forms of DNA mutation: 6,4-PPs and CPDs. Moreover, this bio-active material also favorably modulated the levels of four different microRNAs that have been implicated as key regulators of gene pathways affiliated with the cellular response to UV-induced DNA damage. These findings highlight the importance of the post-transcriptional gene silencing activity of microRNAs to curtailing the negative impact of UV exposure and point to the exciting potential for novel bio-active ingredients to ameliorate the resulting damage to the skin.

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