Environmental stress protection and inflamedgng prevention: A novel synergetic antioxidant blend
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We present an efficient approach to slowing the progression of skin damage using a multi-mechanistic antioxidant blend suitable for cosmetic formulations and validate its performance in vitro. Three different mechanisms were targeted simultaneously: scavenging reactive oxygen species (ROS), inhibiting intracellular ROS and reducing ROS-induced inflamming markers. Using a Design of Experiment approach, a novel, non-phototoxic synergetic blend of antioxidants was identified and characterized in vitro for its ability to quench free radicals (DPPH assay) and intracellular ROS generated by UVA (DCFH assay). Further, we demonstrated that use of the blend results in a mitigation of markers correlated with inflamming (photaging and hyperpigmentation) caused by environmental oxidative stress (PGES, IL-8, MMP-1). These results support the notion that a multi-mechanistic antioxidant blend may effectively alleviate environmentally induced skin damage and be easily incorporated into skin care formulations providing an anti-inflamming benefit. Brewer, M.S. “Natural Antioxidants: Sources, Compounds, Mechanisms of Action and Potential Applications.” Comprehensive Reviews in Food Science and Food Safety, vol. 10, 2011; McMullen, Roger L. Antioxidants and the Skin. Allured Books, 2013; Oswald, T., Crane, C.M., Dueva-Koganov, O., Bianchini, R. Design of Experiments to Optimize a Novel Antioxidant Blend [conference presentation]. Innovations in Dermatological Sciences, Rutgers, NJ 2018.

The cell protein BMAL1 maintains the diploid status of human keratinocytes via a functional interaction with c-myc
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A diverse array of biological processes is under the control of the circadian clock composed of four core proteins, including BMAL1, CLOCK, PERs, and CRYs. These circadian oscillators are present in major cell types within different skin compartments and regulate diverse aspects of skin homeostasis at the local level. We found that loss of BMAL1 promoted differentiation in human keratinocytes. This effect was accompanied by a significant increase in the cell population with polyploidy and strong induction of r-h2AX, a marker for DNA damage. These results indicate that BMAL1 is crucial for maintaining genome stability and proliferation potential in human keratinocytes. Mechanistic studies showed that loss of BMAL1 enhanced the expression c-myc, a pro-oncogene with the pro-differentiation function in keratinocytes. More importantly, co-depletion of c-myc with BMAL1 genes could reverse premature differentiation and polyploidy caused by the loss of BMAL1. These data suggest that the clock protein BMAL1 plays an essential role in maintaining the diploid stem cell potential by suppressing the expression and activity of c-myc in human keratinocytes.

Optimization of the barrier function of a tissue-engineered skin model through supplementation of cell culture media with docosahexaenoic acid
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Percutaneous absorption studies showed that tissue-engineered skin models are more permeable than normal human skin. These observations were partly explained due to the lower levels of polyunsaturated fatty acids, such as docosahexaenoic acid (DHA), found in the epidermal phospholipids of the skin model. In this study, we investigated the impact of a supplementation of the culture media with DHA on the barrier function of a reconstructed skin model. To this end, tissue-engineered human skin substitutes were produced according to the self-assembly method using culture media supplemented with 10 mM DHA and compared with their respective counterparts. The skin substitutes produced with or without DHA presented similar skin morphology, as they both displayed a differentiated epidermis. Moreover, the supplementation with DHA did not influence the skin substitute thickness. Percutaneous absorption of testosterone assayed using a Franz cell diffusion system was significantly decreased in skin substitutes produced with DHA, showing that addition of DHA into the culture media can affect skin impermeability in vitro. The incorporation of DHA into the phospholipid fraction of the epidermis was evaluated using gas chromatography analyses. According to these analyses, higher levels of DHA were measured in the epidermal phospholipid fraction of skin models supplemented with DHA. This successful incorporation of DHA may contribute to the improvement of barrier function of tissue-engineered skin models.

Heterochromatin maintenance is crucial for terminal keratinocyte differentiation and inhibition of inflammatory responses in the epidermis
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Mammalian genome is largely populated with transposable elements (TEs) including endogenous retroviroes (ERVs), largely distributed in the constitutive and facultative heterochromatin. ERV retrotransposition in mouse and human genome include DNA methylation and repressive post-translational histone modifications. Aberrant reactivation of transposable elements has significant impact on normal mammalian development and pathobiology of multiple inflammatory disorders in many organs. H3K9me1/methyltranslace SETD81 and SWI/SNF chromatin-remodeling protein LSH regulating heterochromatin maintenance are both expressed in the keratinocytes (KCs). Conditional Krt14-driven Setdb1 and Lsh gene ablation leads to marked alterations in the epidermal structure, development of skin lesions and premature death. These data implicate the expression of repetitive sequences, increased expression of ERV-specific dsRNAs and, as a result, induction of interferon-mediated activation of innate immune responses in skin. ATAC-seq and Chip-seq analyses of primary KCs isolated from LshKO and Setdb1KO mice showed alterations in distribution of heterochromatin domains compared to controls. RNA-seq analysis showed upregulation of multiple antiviral response pathways activated by ERVs including cytoplasmic RNA sensor MD25, helicases LGP2, RIG1 and predominantly antiviral response in KCs. Loss of SETDb1 in KCs leads to significant upregulation of proteins interacting with short chain non-coding regulatory RNAs, such as PWWL2, TDRD1, TDRD12, RNF17 and RNF165. These data reveal distinct mechanisms of heterochromatin maintenance and retrotransposition silencing in the epidermis mediated by Setdb1 and Lsh and suggest their role in the control of epidermal homeostasis and inflammatory skin conditions.

Skin barrier dysfunction initiates psoriasis inflammation via activating FPR1-ER stress-NLRC4 axis in keratinocytes
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Decreased skin barrier function may aggravate or even initiate psoriasis, and aiding barrier reestablishment using emollients helps improve the psoriatic symptoms, but the mechanisms for such changes have remained unclear. Here we show that epidermal barrier disruption caused by tape stripping or topical use of acetate exacerbates the psoriasis-like inflammation, such as increasing the expressions of inflammasome NLRC4, its downstream cytokines, and other pro-inflammatory mediators, in IMQ-induced mouse model. And FLG deficiency mice also exhibited severe psoriasis-like inflammation when treated with IMQ. In turn, topical application of emollients rescues the epidermal barrier injury and skin inflammation. Moreover, silencing NLRC4 also markedly reduces the psoriasis-like inflammation in vivo. Through RNA-seq and ChIP-seq analyses we note that the epidermis from FLG deficiency mice over-expresses the pattern recognition receptor FPR1, activating which will up-regulate NLRC4, IL1B, IL1B, and other immune-related genes. In in vitro experiments, we further show that UCA regulates the PERK-eIF2α (ER stress) pathway to modulate NLRC4 expression and activation, thus contributing to the immune responses of keratinocytes. Importantly, FPR1 antagonist also attenuates the skin symptoms and normalizes the barrier dysfunction in IMQ-induced mice. Taken together, our findings suggest that FPR1 contributes to psoriasis aggravation by activating FPR1-ER stress-NLRC4 in keratinocytes, which is responsible for the feed-forward amplification of inflammatory responses in psoriasis. This work identifies FPR1 or NLRC4 as a novel potential therapeutic target for psoriasis and other inflammatory skin diseases involving the skin barrier homeostasis.