Environmental stress protection and inflammaging prevention: A novel synergistic 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), mitigating intracellular ROS and reducing ROS-induced inflammaging markers. Using a Design of Experiment approach, a novel, non-phototoxic synergistic 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 inflammaging (photoaging and hyperpigmentation) caused by environmental oxidative stress (PGES-1, IL-8, MAPK-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-inflammaging benefit.

The clock 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 of c-myc, a pro-oncogene with the potential to drive keratinocyte transformation. 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.

The clock protein BMAL1 maintains the diploid status of human keratinocytes via a functional interaction with c-myc

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 supplemented 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 permeability 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 phospholipids of the skin model. Therefore, the supplementation with DHA caused successful incorporation of DHA into the skin substitutes. Furthermore, retroconversion of DHA was registered in the skin substitutes as increased levels of eicosapentaenoic acid were measured in the epidermis after DHA supplementation. Taken all together, these results showed that the addition of DHA into the culture media modulates the lipid profile of the skin models, leading to an improved skin barrier function.

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 retroviruses (ERVs), largely distributed in the constitutive and facultative heterochromatin domains. ERVs in mammalian 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 immunological disorders in many organs. H3K9me1/methyltransferase SETDB1 and SWI/SNF chromatin-remodeling protein LSH regulating heterochromatin maintenance are both expressed in the epidermal 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 results underscore the importance of repetitive sequences, increased expression of ERV-specific dsRNAs and, as a result, induction of interferon-mediated activation of innate immune response 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 MDA5, helicases LGP2, RIG-1 and proinflammatory cytokines. In vivo, 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. Thus, these data reveal distinct mechanisms of heterochromatin maintenance and retrotransposition in the epidermis mediated by Setdb1 and Lsh and suggest their role in the control of epidermal homeostasis and inflammatory skin conditions.

Translation and growth pathways are directly influenced by autoinflammatory regulator (Aire) in skin keratinocytes

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Autoinflammatory regulator, Aire, is commonly known to function as a transcriptional regulator in medullary thymic epithelia. However, we and others have identified Aire to express and function in non-thymic tissues, including the skin, where it greatly influences inflammation and tumorigenesis. We report here that within skin keratinocytes, Aire protein localize in a variety of cellular processes outside of its classically defined role in the nucleus. We employed a proximity based biotinylation screen (BioID) to examine the localization of Aire and its binding partners within keratinocytes. Aire is observed by structured illumination microscopy to localize in distinct subcellular compartments within the nucleus, cytoplasm, and along cytoskeletal tracks. Disease causing and function blocking mutations in Aire substantially alter these distribution patterns in keratinocytes, suggesting a link between Aire subcellular compartmentalization and pathogenesis. Using an isobaric labeling method (Traq) coupled to tandem mass spectrometry, we identified 295 common Aire binding partners (99.0% probability), most of them novel, and quantitatively assessed the impact of Aire mutations on binding partner associations. Specifically, Aire binding partners associated with protein translation and cell growth (e.g. multiple 40S and 60S ribosomal protein subunits, cell growth-regulating nuclear protein, eukaryotic translation initiation factors 4 and 6, elongation factor 1 alpha 1) were significantly altered in cells expressing mutant Aire compared to wild-type Aire. Pulse-sequence assays confirmed a positive correlation between loss of wild-type Aire expression in keratinocytes and increased translation associated signaling (P-4E-BP1, S6K1, S6K1(S473), P-4EBP1, P-4E-BP1(S37) and P-4E-BP1(S65)). These newly identified partners for Aire expand our understanding of Aire function in the skin and may provide a basis for the pro-tumorigenic role for Aire in skin cancers.

Skin barrier dysfunction initiates psoriasis inflammation via activating FPR1-ER stress-NLR4 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 defect caused by tape stripping or topical use of acetate exacerbates the psoriasis-like inflammation, such as increasing the expressions of inflammasome NLR4, its downstream cytokines, and other pro-inflammatory mediators, in IMQ-induced mouse model. And FLG deficiency mice also exhibit severe psoriasis-like inflammation when treated with IMQ. In turn, topical application of emollients rescues the epidermal barrier injury and skin inflammation. Moreover, silencing NLR4 also markedly reduces the psoriasis-like inflammation in vivo. Through next-generation transcriptome analyses we show that the epidermis from FLG deficiency mice over-expresses the pattern recognition receptor FPR1, activating which will up-regulate NLR4, IL1B, IL1B, and other immune-related genes. In vi tro experiments, we further show that UVA regulates the PERK-eIF2α (ER stress) pathway to modulate NLR4 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 psoriatic skin. Our findings suggest that deleterious features of psoriasis aggravates inflammation by activating FPR1-ER stress-NLR4 in keratinocytes, which is responsible for the feed-forward amplification of inflammatory responses in psoriasis. This work identifies FPR1 or NLR4 as a novel potential therapeutic target for psoriasis and other inflammatory skin diseases involving the skin-barrier homeostasis.