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Anti photo-aging effect of conditioned-medium of dedifferentiated adipocytes on skin *in vivo* and *in vitro*: A mechanistic study

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This study aimed to investigate whether secretary factors from DAs could alleviate the ultraviolet B (UVB)-induced premature senescence and decreased collagen content in human dermal fibroblasts (HDFs) and the UVB-induced photo-aging of skin in mice, and to clarify the underlying mechanism. DAs were acquired by ceiling culture and verified based on cellular biomarkers and multilineage differentiation potential. The concentrations of cytokines in conditioned medium from DAs (DA-CM) were determined by enzyme-linked immunosorbent assay (ELISA). In vivo, pathological changes, collagen type I and III, and matrix metalloproteinase (MMP)-1 and -3 were evaluated following the injection of 10-fold concentrated DA-CM into photo-aged mice. In vitro, the effect of DA-CM on stress-induced premature senescence (SIPS) in HDFs was investigated by β-galactosidase staining. The influence of DA-CM and TGF-β1 on the secretion of collagen type I and III, MMP-1, and MMP-3, in HDFs was evaluated by ELISA. Different concentrations of several cytokines including TGF-β1 were detected in DA-CM. In vivo, subcutaneously injected ten-fold concentrated DA-CM increased the expression of collagen type I and III in the DA-CM-injected skin of UVB-induced photo-aged mice compared with control skin from the same mice($P<0.05$). In vitro, DA-CM clearly alleviated the senescence status of SIPS-HDFs ($P<0.05$). HDFs treated with DA-CM exhibited higher collagen type I and III secretion ($P<0.05$), and significantly lower MMP-1 and MMP-3 secretion($P<0.05$), than that of the control group cultured with DMEM. TGF-β1-neutralizing antibody could partially reduce the recovery effect. Our results suggest that DAs may be useful for aging skin, and their effects are mainly due to secreted factors, especially TGF-β1, which stimulate collagen synthesis and alleviate collagen degradation in HDFs.

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MiRNA-23a targets AMBRA1 to inhibit autophagy and plays a role in ultraviolet induced premature senescence

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Autophagy is a cellular catabolic mechanism activated in response to stress conditions including unltraviolet (UV), starvation and misfolded protein accumulation. Abnormalities in autophagy were associated with pathologies including aging and cancer. Hence, elucidation of the signaling pathways controlling autophagy is of utmost importance when studying photoaging. Recently, researchers described microRNAs (miRNAs) as novel and potent modulators of the autophagic activity. Here, we first describe autophagy levels were significantly decreased in UV induced premature model and MIR-23A (hsa-miR-23a-5p) as a new autophagy-regulating miRNA. We showed that antagomir-mediated inactivation of MIR-23A resulted in the stimulation of UVA- and UVB-induced autophagy and protects human keratioincytes and fibroblasts from photoaging. Moreover, overexpression of MIR-23A resulted in the attenuation of autophagy. We identified AMBRA1 as a MIR-23A target. Indeed, AMBRA1 cellular levels were decreased in cells upon MIR-23a overexpression and increased following the introduction of antagomirs. More importantly, overexpression of AMBRA1 rescued fibroblasts from UVB induced premature senescence in the presence of MIR181A. We also showed that the AMBRA1 3′ UTR contained functional MIR-23A responsive sequences sensitive to point mutations. Therefore, MIR23A regulated autophagy is a novel and important regulator of photoaging and AMBRA1 is a rate-limiting miRNA target in this effect.

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UVB wavelength dependency of HMGB1 production by human keratinocytes

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High-Mobility Group Box-1 (HMGB1) is a nuclear protein that acts as an architectural chromatin-binding factor to maintain nucleosome structure and regulate gene transcription. Extracellular HMGB1 contributes to the pathogenesis of chronic inflammatory and autoimmune diseases, including sepsis, rheumatoid arthritis, atherosclerosis, chronic kidney disease, and carcinogenesis. This protein also stimulates keratinocytes and recruits progenitor cells from the bone marrow for wound healing. Data regarding the use of ultraviolet (UV) light for wound healing are sparse. The effects of UV to enhance wound healing include increased epithelial cell turnover, followed by temporary epidermal hyperplasia, increased skin blood flow, and increased vascular permeability, which leads to the accumulation of cellular repair elements in the dermis. UVB was recently reported to induce HMGB1 from murine macrophages and human keratinocytes. Here we investigated the UVB wavelength that induces HMGB1 and whether the 310-nm wavelength that is used as a therapeutic wavelength can induce HMGB1 secretion without inducing apoptosis. Stratified keratinocytes were monochromatically irradiated at 280-320-nm using a multiwavelength irradiation spectral apparatus. HMGB1 mRNA expression was significantly increased with a peak response at 280-nm. HMGB1 protein was detected following irradiation with 280-310-nm UVB; 5 mJ of 280-nm irradiation was most effective for inducing HMGB1. We next used HaCaT cells to further investigate whether HMGB1 secretion was due to apoptosis. HaCaT cells were irradiated with 280-nm and 310-nm, with an irradiance level of 2 mJ, 5 mJ, or 10 mJ. After 24 h irradiation, significant levels of HMGB1 protein were detected in all groups. Although cells irradiated with 280-nm underwent apoptosis, irradiation with 310-nm induced no apoptosis. These findings indicate that 310-nm UVB, which is the peak of narrowband UVB, induces the secretion of HMGB1, and therefore UVB therapy could be used to repair skin tissue as well as to suppress the immune response.

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Dietary proanthocyanidins inhibit UV-induced immune suppression by decreasing the functional activation of suppressor T cells in mice

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Previously, we have shown that dietary grape seed proanthocyanidins (GSPs) inhibit UV-induced suppression of allergic contact hypersensitivity (CHS) response as well as inhibit photocarcinogenesis in mice. Studies have also shown that depletion of UV-induced suppressor T cells (Treg) can inhibit UV-induced skin carcinogenesis. To investigate the underlying mechanism, we have investigated whether dietary GSPs inhibit UV-induced immunosuppression by affecting the development and/or activity of UV-induced Treg cells in mice. For this purpose, C3H/HeN mice were exposed to UV (150 mJ/cm2) radiation on 4 consecutive days with and without treatment of mice with dietary GSPs (0.5%, w/w), mice were sensitized with 2,4-dinitrofluorobenzene (DNFB) 48 h after the last UV exposure, and then sacrificed 5 days after DNFB sensitization. The flow cytometry analysis of lymph node cells indicated that the numbers of Treg cells were not significantly reduced in the mice which were given GSPs in diet compared to non-GSPs-treated UV-exposed mice. We then analyzed effects of dietary GSPs on secretion of cytokines by Treg cells that were isolated from lymph nodes and spleens of mice that were exposed to UVB (150 mJ/cm2). Our ELISA data showed that in comparison to Treg cells isolated from control mice (GSPs-untreated), Treg cells isolated from mice that received GSPs in diet secreted a significantly higher level of IFNγ, while significantly lower levels of interleukin-10 and TGF-β, which are immunosuppressive in nature. Additionally, adoptive transfer experiments revealed that naïve recipients that received Treg cells from GSPs-treated, UVB-irradiated donors exhibited CHS response, whereas no significant CHS was observed in mice which were injected Treg cells from UVB alone-exposed mice compared to non-UVB-exposed control group. These data suggest that dietary GSPs inhibit UVB-induced immunosuppression by suppressing the functional activation, but not numbers, of Treg cells in UV-exposed mice.

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High-fat diet stimulates the growth of UV radiation-induced skin tumors by enhancing DNA hypermethylation and silencing of tumor suppressor genes in mouse skin and tumors

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Exposure of the skin to ultraviolet (UV) radiation results in elevation of inflammatory responses and the production of inflammatory mediators in the skin, which contribute to the development of various skin diseases including the risk of non-melanoma and melanoma skin cancers. We have found that administration of high-fat diet (HF-diet) to mice exacerbates UVB radiation-induced inflammation in the skin. Using a standard photocarcinogenesis protocol, administration of HF-diet to SKH-1 hairless mice enhanced the growth/development of UVB-induced skin tumors, and tumor multiplicity and tumor size were significantly higher ($P<0.01$-0.005) in the mice fed a HF-diet than the mice fed a control-diet (C-diet). As chronic inflammation alters the epigenetic regulators, we studied the effect of HF-diet on the epigenetic regulators in the UVB-exposed skin and UVB-induced skin tumors and data were compared with the C-diet-fed mice. It was observed that administration of HF-diet resulted in DNA hypermethylation in the UVB-exposed skin and UVB-induced skin tumors that was associated with elevated expression and activity of the DNA methyltransferases (Dnmt) 1, Dnmt3a and Dnmt3b compared to the mice which were given C-diet. To further explore the role of HF-diet on photocarcinogenesis, we determined its effect on the p16INK4a and RASSF1A tumor suppressor genes, which are transcriptionally silenced on methylation. We found that the levels of tumor suppressor genes were significantly lowered in the UVB-exposed skin and UVB-induced skin tumors in the mice which were fed HF-diet compared to UVB-exposed mice which were given C-diet. These data indicate that the regular intake of HF-diet stimulates the growth of UVB-induced skin tumors through enhancement of DNA hypermethylation, Dnmt activity and silencing of tumor suppressor genes.

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Homeostasis of thymus-derived naturally occurring Foxp3+ regulatory T cells is controlled by ultraviolet B exposure in the skin

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Foxp3+ CD25+ CD4+ regulatory T cells (Treg cells), constitute about 5-10% of peripheral CD4+ T cells, mediate immunological-self tolerance. Treg cells suppress not only autoimmunity, but also anti-tumor immunity, transplant rejection, allergy, inflammation and responses to pathogens. To develop a new strategy to treat skin immunological disorders, it is important to identify how homeostasis of Treg cells is maintained in the skin. There are two origins of Treg cells, i.e., one is naturally occurring thymus-derived Treg cells (tTreg cells), and the other is adaptive, induced or peripherally derived Treg cells (pTreg cells). It has been shown that the homeostasis of pTreg cells is maintained mainly in the intestine by commensals or microbial metabolites. However, it is unclear how the homeostasis of tTreg cells is controlled in the periphery. In this work, using mice, we show that the homeostasis of tTreg cells is controlled by an exposure of ultraviolet B (UVB) in the skin. The UVB exposure induces the expansion of Treg cells in the skin, which lasts over two weeks. The proliferation of tTreg by UVB exposure is dependent on already existing tTreg cells in the periphery, rather than a new output of Treg cells from the thymus. An exposure of UVB to the skin, but not to the lymph nodes or thymus, is required to expand Foxp3+ Treg cells in situ. In the skin, the Foxp3+ Treg cells expanded by UVB irradiation closely locate to MHC class II+ dendritic cells (DCs), suggesting that DCs play a key role for expanding tTreg cells in the skin after UVB exposure. These results indicate that immunological self-tolerance in the skin may be mediated mainly by tTreg cells, and that the homeostasis of tTreg cells can be controlled by UVB exposure in the skin.

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Endogenous estrogen augments UV-induced sunburn and photoaging process in mice

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While the influence of estrogen on photosensitive dermatoses has been studied, the role of estrogen in the normal physiological response of skin to ultraviolet (UV) irradiation during sunburn and photoaging remains unclear. Thus, this study investigated the effects of endogenous estrogen on the response of skin to UV irradiation in vivo. Ovariectomized (OVX) hairless mice were used as a model of estrogen deficiency. In chronic photoaging experiment, OVX mice showed less degree of wrinkle formation after completion of the 8-week course of UV irradiation. In acute UV exposure experiment, UV-induced skin edema was decreased and minimal edema dose of mice dorsal skin was increased in OVX mice than in sham-operated mice. After UV exposure, OVX mice had a greater expression of proinflammatory mediators such as IL-1β, IL-6 and TNF-α compared with sham-operated mice. However, at baseline before UV exposure, OVX mice had a lower increase in expression of these proinflammatory mediators. Similarly, the expressions of MMP-13 and c-Jun were less induced after UV irradiation in OVX animals but were increased at baseline. Moreover, OVX mice showed a reduction of UV-induced apoptosis and a rapid clearance of DNA-damaged cells. Exogenous estrogen to OVX mice reversed these OVX-related changes and resulted in increased UV-induced inflammatory mediators and apoptosis compared with vehicle-treated OVX mice. Taken together, this study provides in vivo evidences that endogenous estrogen augments UV-induced skin inflammation and may exacerbate photoaging process.

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Cathepsin G as an important mediator of proteolytic ECM degradation in photoaging

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The term photoaging first emerged in the field of dermatology in the 80s, in link with the work of Dr. Kligman. Over sun exposure is known to induce various skin damages including accelerated aging characterized by solar elastose. For many years photoaging was attributed only to ultraviolet rays (UV); it has recently been described that infrared (IR) rays are also involved in this phenomenon. New research has evidenced that IR may affect the skin in two ways, either directly by interfering with cellular mitochondrial functions and generating reactive oxygen species (ROS) at the dermis level, or indirectly through the generation of heat and mobilisation of heat sensors at the surface of skin. Both pathways culminate in the activation of matrix-metalloproteinase-1 (MMP-1), making the protease a key element of UV- and IR-induced photoaging. As an inflammatory response, cathepsin G synthesis is stimulated by solar radiation (UV+IR). This enzyme digests fibronectin dimers into fragments that stimulate the production of MMP's involved in dermis fiber degradation. On another hand, the heat increase induced by IR absorption stimulates the production of heat shock induced MMP's, also involved in dermis fiber desorganization leading to thermal aging. Knotgrass (Polygonum aviculare) extract, a common wild plant belonging to polygonaceae family, is able to inhibit cathespsin G enzymatic activity and thus reduces the MMPs production. Cellular mechanisms, through free radicals, pro-inflammatory cytokines, as well as dermis fibers integrity were also studied. Finally, we demonstrated the clinical efficacy of this botanical extract formulated at 2% on sun over life-exposed volunteers. Knotgrass extract decreases wrinkles depth by 10.4% after one month and increases firmness and elasticity by 11.9% and 4.8% respectively. These results suggest new potential applications for cathepsin G inhibitors, as modulators of UV- and IR-induced skin damage and photoaging in general.

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UV-induced activation of the ATR-Chk1 pathway is mediated by 6-4PPs (not CPDs) via replication blockade

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Ultraviolet (UV) generates two major structurally distinct DNA lesions, cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6-4)pyrimidone photoproducts (6-4PPs). Because a moderate dose of UV induces tens of thousands of each of these lesions in a single cell, it has been difficult to determine their individual effects. Specifically, the roles of each lesion in DNA damage response induction and replication inhibition are not known. To address these questions, we used photolyases capable of selectively repairing either CPDs or 6-4PPs to create cells with only one type of lesion. Ataxia telangiectasia and Rad3 related (ATR) activation in cells with only a single type of lesion was monitored using four-color flow cytometry to track phosphorylation of checkpoint kinase 1 (Chk1) on serine 345 (a key ATR substrate), as well as cell cycle status and the presence of CPDs or 6-4PPs. Replication progression across CPDs or 6-4PPs was assessed by incorporation of BrdU or EdU-labeled thymidine analogs combined with microscopic analysis of "stretched" DNA fibers. Strikingly, we found that 6-4PPs (but not CPDs) are the major type of lesion to activate the ATR pathway, and that 6-4PPs are mostly un-bypassed and retain more single-stranded DNA binding protein (Replication protein A) on chromatin. Since ATR is required for survival of cells with mutagenic damage by avoiding stalled replication fork collapse, our findings provide a possible molecular basis by which ATR inhibition (such as via caffeine) suppresses UV carcinogenesis by promoting apoptosis of cells with forks stalled by 6-4PP DNA lesions.

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5-aminolevulinic acid-photodynamic therapy for multidrug resistant *Pseudomonas aeruginosa*

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Photodynamic therapy (PDT) is considered to have a bactericidal action due to the cytocidal effect of singlet oxygen and inhibition of thymidine synthesis in cellular DNA by the porphyrin derivative. PDT is known to inhibit the appearance of antibiotic-resistant bacteria, and therefore, has attracted attention as a new treatment for bacterial infections. Thus far, efficacy of PDT against gram-positive bacteria with various photo-sensitizers has been reported. However, gram-negative bacteria has been reported about the usefulness of PDT for gram-negative bacteria. Instead, it has been reported to respond poorly to PDT due to their double-membrane cell wall structure and biofilm, as well as the existence of drug efflux pumps. Furthermore, in case of multidrug resistant *Pseudomonas aeruginosa* (MDRP), various pigments in it hinder to assume effective wavelength for PDT. In this study, we investigated the effect of 5-aminolevulinic acid (ALA)-PDT for MDRP. First, we measured porphyrins in MDRP by high-pressure liquid chromatography. Addition of 5-ALA (1mg/mL) increased total porphyrins in 10^8CFU/mL MDRP by approximately 5-fold, and increased protoporphyrin IX (PpIX) by approximately 2-fold after 5 hours. After ethylenediaminetetraacetic acid (EDTA) was added simultaneously at 1 mg/mL to eliminate Fe^{2+}, production of PpIX showed a 10-fold increase. Next we performed irradiated to MDRP with 50 J/cm^2 of blue LED light (410 nm) at 5 hours after addition of 5-ALAwith or without EDTA. When the bacterial count was measured after 19 hours, proliferation of MDRP to 10^9 CFU/mL was found in cultures without EDTA, while that remained at 10^5 CFU/mL with EDTA. When MDRP was irradiated similarly using green (517 nm), red (642 nm) and white LED lights, no antiproliferative effect was detected. These results suggest that ALA-PDT in the presence of EDTA using blue LED light is a possible option for the treatment of MDRP infection.

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Ultraviolet-induced decrease of leptin and adiponectin in subcutaneous fat may contribute to exacerbation of photoaging process

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Ultraviolet (UV) exposure to the human skin significantly reduces triglycerides contents and lipid synthesis in the subcutaneous (SC) fat tissues. Leptin and adiponectin are the most abundantly expressed adipokines in the SC fat tissues. To elucidate their potential roles in photoaging, we examined the expression of leptin and adiponectin in acute UV-irradiated skin as well as photoaged skin. The expressions of leptin, adiponectin, and their corresponding receptors were significantly decreased in sun-exposed forearm skin, compared with sun-protected buttock skin of the same elderly individuals, indicating that chronic UV exposure decreases both adipokines in SC fat tissues. Acute UV irradiation also decreased the expression of leptin and adiponectin in SC fat tissue of human skin *in vivo*. Moreover, while exogenous leptin administration prevented UV- and TNF-α induced MMP-1 expression, it also increased TNF-α-induced decrease of type I procollagen synthesis. Adiponectin and its receptor silencing by siRNA led to an increased MMP-1 expression, which was reversed by treatment with recombinant human adiponectin. In conclusion, UV exposure decreases the expression of leptin and adiponectin, leading to the exacerbation of photoaging by stimulating MMP-1 expression and inhibiting procollagen synthesis.

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Suppression of epidermal growth factor receptor activation by receptor protein tyrosine phosphatase-kappa reduces ultraviolet irradiation-induced epidermal hyperplasia and inflammation in a transgenic mouse model

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Solar ultraviolet (UV) irradiation is the major predisposing environmental factor for premature skin aging and skin cancer. Activation of epidermal growth factor receptor (EGFR) plays a critical role in initiating epidermal responses to UV exposure. EGFR activation results from auto-phosphorylation of C-terminal tyrosines. UV enhances this tyrosine phosphorylation by oxidative inhibition of EGFR phosphatase, receptor protein tyrosine phosphatase-kappa (RPTP-κ). Reduction of RPTP-κ activity results in elevated EGFR tyrosine phosphorylation and EGFR activation. In order to better understand the function of RPTP-κ in skin biology, we generated transgenic mice that express human RPTP-κ in the epidermis, under control of the keratin 5 (K5) promoter. UV irradiation-induced EGFR tyrosine phosphorylation was reduced 4-fold in *K5-RPTP-κ* transgenic mice, compared to wild type littermates ($p<0.05$). In addition, downstream activation of MAP Kinase pathway MEK1/2 and ERK1/2 were markedly suppressed (75% and 60%, respectively) in *K5-RPTP-κ* transgenic mice. Consistent with this reduction, UV-induced up-regulation of transcription factor AP-1 components c-Fos and c-Jun were significantly decreased by 80% and 70% ($p<0.05$), respectively. Similarly, induction of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α by UV irradiation was inhibited by approximately 70%, 90%, 80% ($p<0.05$), respectively in *K5-RPTP-κ* transgenic mice. Importantly, UV-induced epidermal hyperplasia and inflammation were significantly reduced in *K5-RPTP-κ* transgenic mice, compared to wild type littermates. These genetic studies support the concept that RPTP-κ is a critical regulator of EGFR-dependent responses of the epidermis to UV irradiation. Enhancing RPTP-κ function via protection against UV irradiation-induced oxidation may be an effective strategy to lessen detrimental effects of sun exposure and thereby reduce risk of skin cancer and premature skin aging.

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Downregulation of miR-15b is associated with increased sirtuin 4 (SIRT4) expression in photoaging of human skin

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Since (i) SIRT4 is known to negatively impact mitochondrial oxidative capacity and (ii) disturbed mitochondrial function of human dermal fibroblasts (HDF) is thought to be of major relevance for photoaging, we tested the hypothesis that dysregulation of SIRT4 expression may be linked to photoaging of human skin. We found that SIRT4 mRNA levels were significantly increased in vitro in HDF (i) as a function of increasing passage number, i.e. replicative senescence, or (ii) after repetitive UVB exposure or treatment with ionizing radiation, i.e. stress-induced senescence. In vivo, SIRT4 mRNA levels were significantly upregulated in chronically sun exposed skin from neck vs intrinsically aged skin from buttock of volunteers with older age (n=16). In enzymatically separated skin samples, increased SIRT4 expression was mainly confined to the dermis. In corresponding skin samples from younger adults (n=15) no such differences were observed. As microRNAs (miRNAs) have been implicated in senescence and aging, we performed a global microarray based comparison of corresponding miRNA- and mRNA expression profiles in various in vitro senescence models. We consistently observed that a significant upregulation of SIRT4, a bona fide target of miR-15b, was associated with significantly decreased levels of miR-15b. In human skin, highest miR-15b copy numbers were detected in the epidermis, and epidermal expression was significantly reduced in photoaged skin vs intrinsically aged skin. Reduced miR-15b expression is most likely causally linked to increased SIRT4 expression because we showed that (i) miR-15b targets a conserved binding site within the 3'-untranslated region of the SIRT4 gene using luciferase reporter assays and (ii) transfection of oligonucleotides mimicking miR-15b function prevented SIRT4 upregulation in senescent cells. Thus, miR-15b may negatively regulate SIRT4 expression to antagonize mitochondrial dysfunction and photoaging of the skin.

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Evidence that CS proteins epigenetically regulate tubulin acetylation and thereby autophagy

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Cockayne syndrome (CS) is a rare progeroid disease caused by mutations in the CSB or CSA gene and partially characterized by tissue degeneration including loss of subcutaneous fat (SF). The pathogenetic mechanisms responsible for tissue degeneration in CS are not known. Using CSB-/- mice we have recently shown that spontaneous loss of SF can be aggravated by exposure to UVA radiation. Importantly, loss of SF could be prevented by the histone deacetylase (HDAC) inhibitor SAHA, indicating that CSB has epigenetic functions. We now provide evidence that CS proteins epigenetically regulate autophagy. Accordingly, loss of SF was associated with an accumulation of ubiquitinated proteins as well as the autophagic marker LC3II in the skin of CSB-/- mice. Also, in fibroblasts from CS patients UVA induced an enhanced and prolonged accumulation of ubiquitinated proteins, LC3II, autophagosomes, and of the autophagy substrate p62 when compared with normal human fibroblasts (NHF) indicating that autophagy is impaired in human and murine CS cells. In general, the clearance of autophagosomes requires the presence of acetylated microtubules. In line with this we observed that in NHF UVA irradiation time-dependently induced acetylation of α-tubulin, which was, however, markedly impaired in human CS fibroblasts. Interestingly, SAHA treatment prevented accumulation of ubiquitinated proteins and LC3II in UVA-irradiated CSB-/- mouse skin and restored α-tubulin acetylation and significantly reduced LC3II, autophagosomes, ubiquitinated proteins and p62 in human CS fibroblasts. Additional treatment of human CS fibroblasts with the microtubule inhibitor vinblastine abrogated the protective effect of SAHA, indicating that a functional tubulin network is required for the beneficial effects of SAHA. Based on these results we propose that CS proteins prevent tissue degeneration by triggering autophagy via regulation of α-tubulin acetylation and that this previously unknown function of CS proteins involves epigenetic mechanisms which might be therapeutically targeted by HDAC inhibitors.

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Ultraviolet B (UVB) stimulates central hypothalamic-pituitary-adrenal (HPA) axis

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Ultraviolet B (UVB) is the skin-specific stressor, which we believe is able to induce genomic and metabolomic changes not only in the skin, but also in distant organs. Hypothalamic-pituitary-adrenal (HPA) axis is the main regulatory algorithm maintaining body homeostasis after stress challenge. The aim of this project was to evaluate a role of UVB radiation in regulation of the HPA axis activity with the use of hypophysectomized (Hypox) mice. Normal and Hypox females of C57 BL/6 in telogen were shaved and irradiated with 400 mJ/cm2 of UVB waveband. Following 12 and 24 h time of exposure, plasma, skin, brain and adrenals were collected and extracted for RNA, peptides/proteins, and steroids or fixed for immunohistochemistry (IHC). Expression of the crucial components for HPA axis activation [corticotropin-releasing hormone (CRH), urocortin (URO), β-endorphin (β-END), adrenocorticotropin (ACTH) and corticosterone (CORT)] has been measured. Cutaneous UVB radiation led to increased CRH, URO, β-END, ACTH and CORT plasma levels in intact animals. Hypox mice did not follow this pattern, except of increased CRH in paraventricular nucleus of hypothalamus and of CRH with URO in plasma. There was no increase in ACTH and CORT plasma levels in Hypox mice after UVB treatment. Only intact animals showed increased expression of mRNAs coding melanocortin receptor 2 (Mc2R) as well as of Star and Cyp11b1 in adrenal glands. However, the "cutaneous HPA axis" was maintained and stimulated in both animal groups after UVB radiation. UVB irradiated skin expressed higher levels of CRH (by IHC), and of URO, β-END and CORT both at gene and peptide levels. In conclusion, UVB stimulates both local and central HPA axis activity in mice leading to increased concentrations of immunosuppressive CORT in plasma. The UVB-activation of central HPA axis is dependent on pituitary, since Hypox animals showed lack of stimulation of plasma levels of ACTH and CORT.

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Timecourse of recovery from UV-induced damage using a full-thickness human skin equivalent

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Solar ultraviolet (UV) radiation is known to have deleterious effects on human skin. The UVB (280-320 nm) and UVA (320-400 nm) portions of solar spectrum have been shown to affect keratinocytes (KCs), the major cellular constituent of the epidermis, by causing direct DNA damage and/or indirect DNA damage and cytotoxicity through the formation of reactive oxygen species. In the present study, a commercially available human skin equivalent (HSE) (EpiDerm-FT™) and excised human skin were exposed to solar-simulated light to gain insight into the temporal UV-induced response of human epidermal tissue. In vitro HSEs were irradiated with a single UVR dose approximately equivalent to either 6 or 9.5 minimal erythemal doses (MEDs) for an individual of skin phototype 2. Cutaneous damage and recovery were then monitored for a period of seven days. Histological analysis showed a dose dependent formation of apoptotic sunburn cells in epidermal KCs at 24 hrs post-irradiation. By day 3 post-irradiation, a thinning of the viable epidermal cell layers was evident with maximum epidermal degeneration observed at day 4. Resumption of epidermal proliferation and differentiation was evident in both 6 and 9.5 MED tissues by days 5-7, leading to regeneration of viable epidermal layers. Excised human skin tissues irradiated with the same UV doses displayed responses very similar to those observed in the in vitro HSEs. In the HSEs, DNA damage indicated by cyclopyrimidine dimer (CPD) formation was assessed using immunohistochemistry. CPD positive basal KCs decreased steadily in number each day, and were almost completely undetectable seven days post-irradiation. CPD formation could be completely blocked through topical application of OTC sunscreens. Also, elevated levels of IL-8 and MMP-1 were induced following UV-irradiation demonstrating an early inflammatory response followed by an extended period of matrix remodeling activity. These results demonstrate that HSEs are useful for UV-induced photocarcinogenesis studies and evaluation of sunscreens.

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Melatonin and its metabolites protect human keratinocytes against UVB-induced oxidative stress and induce DNA repair

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UV irradiation induces stress and damage to the skin. This oxidative damage is mediated by the UV-induced production of reactive oxygen species (ROS), such as hydrogen peroxide (H2O2), and nitric oxide (NO) and reduction of an important antioxidant gluthathione (GSH). Melatonin protects cells form UVR by scavenging free radicals. In this study we investigated the protective effects of melatonin and its metabolites: 6-hydroxymelatonin (6-OHM), N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), N-acetylserotonin (NAS), and 5-methoxytryptamine (5-MT) in human keratinocytes. Our data show significant reduction in ROS (50-60%) levels produced by UVB irradiated cells pretreated for 24 h and treated after exposure to different doses of UVB (25, 50, and 75 mJ/cm2) with melatonin or its derivatives. The reduction in the NO and H2O2 levels produced by UV irradiated cells was visible after 30 min of exposure. We have also seen stimulation of reduced GSH in cells by melatonin and its derivatives within 1 h after UVB exposure. Using MTS test, we have tested cell viability in UVB irradiated cells treated with melatonin or its derivatives for 48 h after UVB exposure. Data shows a dose-dependent increase in cell viability. Using dot-blot technique and immunofluorescent staining we have shown that melatonin and its metabolites enhance DNA repair capacity of UVB-induced 6-4PP or CPD generation in keratinocytes. DNA repair was also shown using Comet assay. We have tested the expression of p53 and its phosphorylated forms (Ser-15 and Ser-46) in cells irradiated with UVB and treated with melatonin and its metabolites and found an increase in p53 when compared to control. Thus, our data demonstrate that melatonin and its metabolites have strong protective effects against UVB-induced oxidative stress and DNA damage.

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Assessment of photoaging protection of the association of retinaldehyde, pretocopheryl glucopyranosyl and glycylglycine oleamide in both *in vitro* and ex *vivo* skin models

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The aim of this study was to evaluate the anti-aging properties of a combination of 3 active ingredients: Retinaldehyde (RAL, a retinoid), Pre-tocopheryl glucopyranosyl (Pretoco, an anti-oxidant) and Glycylglycine oleamide (OGG, an inhibitor of glycation). Their efficacy was evaluated on both UVA-irradiated human dermal fibroblasts and skin explants. Our results clearly showed that 48hr after UVA irradiation, the expression of mRNA coding for elastic fibers (Elastin, Fibrillin 1 and 2, Fibulin 1 and 2, and Lysyl oxidase-like 2), ECM proteins (type I collagen, nidogen 1, syndecan 4, versican) and proteins involved in cellular-MEC interactions (talin 1, tensin 1, paxillin) were strongly reduced. Furthermore, we have also showed that addition of RAL (1μM) + Pretoco (10μM) + OGG (1 μM) reduced totally or partially effects of UVA (ex: elastin: +485%, fibulin 1: +458%, versican: +94%, talin 1: +111%). An ex vivo photo-damaged human skin model confirmed that UVA strongly desorganizes the elastic network, especially the elastin and fibrillin-rich fibers in the papillary dermis. In contrast to a placebo, a RAL+Pretoco+OGG containing formulation applied topically, efficiently protected skin and well preserved the elastic system from UVA damage. Thus, the combination of RAL+Pretoco+OGG presented anti-aging properties on both in vitro and ex vivo skin models and was able to prevent UVA-induced photo-aging, suggesting that it may be useful for the development of new anti-aging dermo-cosmetic products.

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Epigenetically enhanced photodynamic therapy (ePDT) is superior to conventional PDT for inducing apoptosis in cutaneous T-cell lymphoma (CTCL)

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Conventional photodynamic therapy with aminolevulinic acid (ALA-PDT) selectively destroys diseased cells primarily by inducing apoptosis via the intrinsic (mitochondrial) pathway. It also upregulates FAS-ligand (FASL) via JNK/c-Jun but blocks FAS upregulation simultaneously, thereby limiting extrinsic pathway apoptosis. While ALA-PDT produces excellent outcomes in patients with actinic keratoses, similar results are achieved only in a subset of CTCL patients. Our previous work showed that the resistance of CTCL to FASL-mediated apoptosis correlates inversely with low expression of FAS, a protein critical to activation of the extrinsic apoptotic pathway. We also showed that methotrexate (MTX) demethylates the FAS promoter and upregulates FAS, thus acting as an epigenetic derepressor that restores the susceptibility of FAS-low CTCL to FASL-mediated cell death. These findings led us to hypothesize that the response of CTCL to ALA-PDT might be increased by MTX-mediated epigenetic manipulation, a concept we refer to as epigenetically enhanced PDT (ePDT). To test our hypothesis in vitro, we subjected CTCL cell lines with a wide range of FAS expression to conventional ALA-PDT as well as to ePDT. Protein expression was visualized and quantified in situ using multispectral image analysis of immunostained cells, a quantitative method that is 5x more sensitive than standard immunohistology for antigen detection. Compared to either conventional PDT or MTX alone, ePDT induced greater apoptosis as evidenced by increased cleaved caspase-3 and PARP cleavage products. Unlike conventional PDT, ePDT upregulated FAS and/or FASL in all cell lines resulting in significantly higher cleaved caspase-8, an indicator of enhanced extrinsic pathway apoptosis. We conclude that ePDT is superior to conventional PDT by allowing greater activation of extrinsic apoptosis, which results in enhanced tumor cell death. These data provide a rationale for clinical trials of ePDT for CTCL.

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microRNA-21 and microRNA- 29 act in concert with the TGF-β pathway to down-regulate synthesis of type I collagen in response to UV irradiation in human skin fibroblasts

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TGF-β pathway is a major regulator of dermal extracellular matrix (ECM) production by human skin fibroblasts. Ultraviolet (UV) irradiation down-regulates TGF-β pathway, thereby impairing dermal ECM structure and function. UV impairment of TGF-β pathway is mediated by induction of microRNA-21 (miR-21), which directly binds to sequences in the 3′ untranslated region (UTR) of the type II TGF-β receptor (TβRII) transcript, thereby reducing its translation. We report here that miR-21-mediated down regulation of TβRII induces miR-29, which acts to reduce type I procollagen alpha1 (COL1α1), the most abundant protein in the dermal ECM. Transfection of miR-21 mimic into skin fibroblasts significantly increased miR-29 (5.4-fold, n=6, p<0.01). This induction was abrogated by co-transfection of miR-21 mimic with TβRII expression vector. Interestingly, TβRII expression not only prevented miR-21 induction of miR-29, but also reduced miR-29 below basal levels, indicating that TβRII exerts tonic control on basal miR-29 expression. Conversely, transfection of miR-21 antagonist significantly induced TβRII protein (2.4-fold, n=3, p<0.05), and reduced miR-29 (50%, n=3, p<0.05). In UV exposed fibroblasts, transfection of miR-21 antagonist completely prevented reduction of TβRII (n=3, p<0.01), and induction of miR-29 (n=3, p<0.05). miR-21 antagonist also significantly prevented UV irradiation reduction of COL1α1 (70% reduction with control scrambled antagonist vs. 25% reduction with miR-21 antagonist, n=3, p<0.05). miR-29 mimic reduced COL1α1 protein level 49.2% (n=6, p<0.05), and this reduction was mediated by direct targeting of the 3′ UTR of COL1α1 transcript, determined by pMIR-REPORT luciferase reporter assays. These data reveal a novel pathway in which UV induction of miR-21 down-regulates TβRII, leading to upregulation of miR-29, which in turn suppresses COL1α1 production. Inhibiting miR-21/29 may protect skin from UV-induced dermal ECM damage and its sequela.

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Platelet-activating factor mediates ultraviolet B radiation systemic immunosuppression via mast cell products and IL-33

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Platelet-activating Factor (PAF) is a glycerophosphocholine-derived lipid mediator that activates a variety of cell types via the PAF-receptor (PAF-R). Systemic PAF-R activation exerts pro-inflammatory, as well as delayed systemic immune suppression effects in vivo. Many pro-oxidative stressors including UVB lead to systemic immunosuppression as measured by an inhibition of contact hypersensitivity (CHS) reactions via the production of PAF agonists produced by free radical oxidation of phosphocholines. Of importance, this same process can result in the augmentation of experimental tumor growth. Yet, the mechanism by which the PAF-R mediates UVB systemic immunosuppression remains largely uncharacterized. The work herein seeks to establish the importance of the PAF-R on mast cells (MC) and to identify the mediators that MCs release which lead to the systemic immune suppression. Studies, including our own, demonstrate that mast cells (MC) and T regulatory cells partake in PAF-R mediated immune suppression. In fact, UVB-mediated immune suppression is lost in MC deficient mice. The present studies demonstrate that dermal reconstitution of MC-deficient mice with WT but not PAF-R-/- bone-marrow derived MC (BMDMC) rescues PAF-R-mediated inhibition of CHS reactions. Additionally, the use of cyclooxygenase-2 (COX-2) inhibitors and IL-10 neutralizing antibodies has identified the importance of COX-2 and IL-10 in PAF-R mediated immune suppression in that they attenuate CHS responses. We also demonstrate that PAF-R activation up-regulates the expression of MC derived mediators, TGF-beta, COX-2 and TNF-alpha, as measured by qRT-PCR. Activation of the MC PAF-R also releases histamine, another mediator which induces systemic immunosuppression. Finally, we report that PAF-R mediated immunosuppression is attenuated in the absence of IL-33, indicating that IL-33 is necessary for UVB/PAF-R-mediated immunosuppression. In summary, these studies provide a mechanism involving MC and MC-derived mediators in the systemic immunosuppressive effects of UVB induced by PAF-R activation.

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Anacardic acid increases type I procollagen expression following ultraviolet irradiation via inhibition of DNA methylation in the COL1A2 promoter region

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The photoaged skin following repeated exposure to solar ultraviolet irradiation shows largely accumulated damage in cutaneous connective tissue, which is composed mainly of type I procollagen. In the present study, we showed the roles of DNA methylation and histone acetylation in UV-induced regulation of COL1A2 transcription in human dermal fibroblasts (HDFs) by using anacardic acid (AA). UV irradiation decreased type I procollagen expression and decrease phosphorylation of Smad3. Using chromatin immunoprecipitation assays, we observed that the recruitment of acetyl-H3, p300, and Smad3 were consistently decreased by UV to a distinct region (-1446/-1320) adjacent to the p300 binding site (-1406/-1393) in the COL1A2 promoter. However, the recruitment of acetyl-H3, p300, and Smad3 was increased by AA treatment to this region of COL1A2 promoter. Using bisulfite modification and methylation specific PCR (MSP), we observed that DNA methylation was increased by UV at a distinct region (-1450/-1349) adjacent to the putative p300 binding site (-1406/-1393) in COL1A2 promoter, while the UV-induced DNA methylation was inhibited by AA, indicating that AA may be inhibitor of DNA methyltransferase. Inhibition of UV-induced DNA methylation by treatment with a DNMT inhibitor (5-AZA-2′-deoxycytidine; 5-AZA-dC) resulted in increased recruitment of acetyl-H3 and p300 to this region of COL1A2 promoter. Also, at this region, a decreased DNA methylation was observed by 5-AZA-dC. These results indicate that DNA methylation of the putative p300 binding site (-1406/-1393) in COL1A2 promoter inhibits recruitment of p300 and acetyl-H3 to this site, leading to less binding of Smad3 to its response elements and decreased synthesis of procollagen. Our results suggest that DNA methylation plays a critical role in the COL1A2 transcription by UV-irradiation.

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Atomic hydrogen surrounded by water molecules, H(H2O)m, modulates basal and UV-induced gene expressions in human skin *in vivo*

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Recently, there has been much effort to find effective ingredients which can prevent or retard cutaneous skin aging after topical or systemic use. Here, we investigated the effects of the atomic hydrogen surrounded by water molecules, H(H2O)m, on acute UV-induced responses and as well as skin aging. Interestingly, we observed that H(H2O)m application to human skin prevented UV-induced erythema and DNA damage. And H(H2O)m significantly prevented UV-induced MMP-1, COX-2, IL-6 and IL-1b mRNA expressions in human skin in vivo. We found that H(H2O)m prevented UV-induced ROS generation and inhibited UV-induced MMP-1, COX-2 and IL-6 expressions, and UV-induced JNK and c-Jun phosphorylation in HaCaT cells. Next, we investigated the effects of H(H2O)m on intrinsically aged or photoaged skin of elderly subjects. In intrinsically aged skin, H(H2O)m application significantly reduced constitutive expressions of MMP-1, IL-6, and IL-1b mRNA. Additionally, H(H2O)m significantly increased procollagen mRNA and also decreased MMP-1 and IL-6 mRNA expressions in photoaged facial skin. These results demonstrated that local application of H(H2O)m may prevent UV-induced skin inflammation and can modulate intrinsic skin aging and photoaging processes. Therefore, we suggest that modifying the atmospheric gas environment within a room may be a new way to regulate skin functions or skin aging.

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Incidence of lung cancer in patients with systemic sclerosis treated with extracorporeal photopheresis

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Introduction: Systemic sclerosis (SSc) is a chronic disease of the connective tissue, which is associated with fibrosis and thickening of the skin and internal organs. There is evidence that SSc is associated with an increased risk of lung cancer. The treatment of systemic sclerosis is still unsatisfying and depends on the severity of the disease and the affected organ systems. Extracorporeal photopheresis (ECP), a treatment almost free of side-effects, in many cases improves skin sclerosis substantially. The aim of the present study was to determine the risk of lung carcinoma in ECP-treated SSc-patients. Methods: We performed a cohort study with an anonymous retrospective analysis of 71 patients with SSc who were treated with ECP between 1991 and 2013 at the Photopheresis Unit of the Department of Dermatology at the Medical University of Vienna, Austria. Results: Lung cancer was detected in 7 (= 10%) of 71 patients with SSc. In the Austrian general population the cumulative risk to develop lung cancer before the age of 75 is 2.1% for women and 4.5% for men. SSc patients of our cohort had a risk 10% and thus an enhanced risk to develop lung cancer. A total of 54 % of our collective had developed a non-specific interstitial pneumonia (NSIP). Remarkably, all of the lung carcinoma patients had been diagnosed with a NSIP before the development of lung carcinomas. In our study, patients with systemic sclerosis have a standardized incidence rate (SIR) of 2.34 (95% CI 0.84 to 4.58) for developing lung cancer. Conclusion: In accordance with previously published studies, patients with systemic sclerosis have an increased risk of developing a lung cancer. NSIP in systemic sclerosis may be a risk factor for the development of lung cancer, since lung cancers appeared only in SSc patients previously diagnosed with an NSIP.

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Evaluation of the large spectral range photoprotective efficacy of a new sunscreen formulation against solar radiation-induced oxidative stress

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Sunscreens are becoming widely used to prevent sunburn around the world. Recently, we developed a new broad-spectrum UVB+A photoprotective system, a patented association of 4 filters that has been shown to prevent solar radiation-induced DNA damage and apoptosis (poster 1242, IID 2013). Since this SPF50+ formulation also contains tocopheryl glucoside (TG), a vitamin E precursor, the aim of this study was to evaluate its efficacy to protect skin from oxidative stress in both the ultraviolet (UV) and visible/infrared (VIS/IR) spectral ranges of the solar light. The UV irradiation of a reconstructed human epidermis with a dose of 16.5 J/cm2 revealed a strong decrease of the antioxidant peptide glutathione (GSH). GSH depletion was detected by a luminescent assay 4h after UV exposure, persisted 24h, and correlated to the induction of the caspase 3/7 activity. Topical sunscreen application at 2 mg/cm2 allowed to maintain GSH pool and tissue integrity in irradiated skin affording an almost complete UV photoprotection. The VIS/IR irradiation of ex vivo porcine ear skin showed a significant radical formation in this solar wavelength range as assessed by electron paramagnetic resonance spectroscopy using a test radical (EPR technique). Sunscreen revealed a medium radical protection factor (RPF) value of 45x1014 radicals/mg and had the highest antioxidant properties as compared to placebo without sunfilters and TG or formula with TG only (RPF 10 and 15, respectively). It also presented high scattering properties and decreased the radical production in the skin after VIS/IR irradiation (41% versus 23% for placebo). Thus, the new sunscreen formulation provides skin protection against oxidative stress covering the entire solar spectrum from UV to IR, and may be useful for the development of new suncare products.

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Central role of MC1R in the photobiological response of human melanocytes

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Exposure to UV up regulates the synthesis of α-Melanocortin (α-MSH), endothelin-1 (ET-1), and 1, 25(OH)2 vitamin D3 (vit D) by keratinocytes. α-MSH binds the melanocortin 1 receptor (MC1R), a Gs protein-coupled receptor expressed on melanocytes (MC), the product of a gene that is important for determining human pigmentation and melanoma susceptibility. We have reported that activation of the MC1R, which stimulates melanogenesis, reduces the generation of reactive oxygen species (ROS), and enhances repair of DNA photoproducts and oxidative DNA damage in UV-irradiated human MC. We found that expression of loss-of- function MC1R alleles that are associated with increased melanoma risk results in compromised DNA repair capacity. ET-1 and vit D also reduce the extent of UV-induced DNA photoproducts and apoptosis. We have reported that α-MSH and ET-1 synergistically stimulate human MC proliferation and melanogenesis and reduce UV-induced ROS generation and apoptosis. Here we show that α-MSH and ET-1 augment the phosphorylation of the stress MAP kinases p38 and JNK and their downstream target ATF-2, a transcription factor that regulates expression of cell cycle, DNA repair, and apoptosis genes. These effects of α-MSH are absent in MC expressing loss of function MC1R. α-MSH, ET-1, and vitamin D up regulate MC1R gene expression, which is expected to enhance the response of MC to α-MSH. Given the significance MC1R/α-MSH axis in modulating the UV response of MC, we have developed tri- and tetrapeptide analogs of α-MSH that are selective for MC1R, and mimic α-MSH in its effects on human MC. Using human skin explants or skin equivalents, the tetrapeptide analogs were found to enhance repair of UV-induced DNA photoproducts in melanocytes, and to stimulate melanogenesis after prolonged treatment. Our goal is to use these analogs in a chemoprevention strategy to inhibit photocarcinogenesis, including melanoma.

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New evidence implicates communication between epidermal keratinocytes and dermal mast cells in UVB-induced inflammation of the skin

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Ultraviolet-B radiations (UVB) cause sunburn characterized by an acute inflammatory response and immuno-suppression. Mast cells (MCs) are resident cells of the skin present in the dermis. Following UVB irradiation, the number of MCs in the dermis increase. Many hypothesis have been proposed for MC involvement in Skin irradiation, including immuno-suppression, the signals that call mast cells to the surface is still unknown. Knowing that UVB affects the basal layer of the epidermis, while MCs are deeper in the dermis, we investigated a possible cross talk between MCs and NHK after UVB irradiation. We tested different potential mediators susceptible to activate MCs, including TLR3 ligands, double-stranded RNA and interleukins and obtained negative results. However, UVB-irradiated keratinocytes are known to secrete lipids that could possibly activate MCs. We then hypothesized that UVB-irradiated epidermal keratinocytes could activate dermal MCs through the secretion of a lipid messenger. First, in vivo experiments showed that UVB-irradiation of the back skin of C57BL6 wild type mice triggers recruitment of MCs toward the outer section of the dermal layer. Then, with a co-culture of BMMCs, with Normal human epidermal keratinocytes (NHEKs), we demonstrated that UVB-irradiated NHEKs could induce degranulation, the release of histamine of BMMCs, as well as the secretion of cytokines such as interleukin-6 (IL-6) and the tumor necrosis factor alpha (TNFα). Finally, we showed that UVB-irradiated NHEKs enhanced the migration of BMMCs, which can be reversed by the inhibition of the Sphingosine-1-phosphate (S1P) receptors on BMMCs. S1P is released by apoptotic NHEKs after UVB-irradiation and can consequently activate the BMMCs. Taken together, these results demonstrate an active dialogue between MCs and NHEKs after UVB irradiation, and a new role of lipid S1P as a key messenger during UVB irradiation of the skin.

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A novel mediator of UVA-induced cutaneous photooxidative stress: The endogenous arylhydrocarbon receptor ligand 6-formylindolo[3,2-b]carbazole (FICZ) is a nanomolar photosensitizer in epidermal keratinocytes and reconstructed human skin

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Endogenous UVA-chromophores may act as sensitizers of photooxidative stress underlying cutaneous photoaging and carcinogenesis, but the molecular identity of non-DNA key chromophores displaying UVA-driven photodyamic activity in human skin remains largely undefined. Here we demonstrate for the first time that 6-formylindolo[3,2-b]carbazole (FICZ), a tryptophan photoproduct and endogenous high affinity ligand of the mammalian aryl hydrocarbon receptor (AhR), displays nanomolar activity as a photosensitizer potentiating UVA-induced oxidative, proteotoxic, and genotoxic stress. In human HaCaT and primary epidermal keratinocytes, induction of apoptosis occurred in response to the combination of solar simulated UVA (3.3 J/cm^2) and FICZ (10 nM), an effect not observed upon exposure to the isolated action of UVA or FICZ. Similarly, in a human epidermal tissue reconstruct sunburn cell formation and caspase 3 activation were detectable only upon combined exposure to FICZ and UVA. Array analysis revealed FICZ-potentiation of UVA-induced cellular heat shock (*HSPA6*, *HSPA1A*), ER stress (*DDIT3*), and oxidative stress (*TXNRD1*, *HMOX1*, *AKR1C2*, *SPINK1*) response gene expression. In contrast, FICZ upregulation of the AhR-target gene *CYP1A1* occurred independently of UVA exposure. FICZ photosensitization was associated with intracellular photooxidative stress counteracted by inclusion of singlet oxygen quenchers (NaN_3, DABCO). ΦX174-plasmid cleavage and cellular comet assays revealed introduction of FPG-sensitive DNA lesions consistent with genotoxic effects downstream of FICZ/UVA-induced photooxidative stress. Taken together, our data demonstrate that the endogenous AhR ligand FICZ displays nanomolar photodynamic activity potentially representing a novel mediator of UVA-induced photooxidative stress and photodamage in human skin.

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Voriconazole promotes UV-DNA damage by increasing oxidative stress

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Immunosuppression in organ transplant recipients necessitates long-term use of antimicrobial agents to prevent potentially fatal infections like aspergillosis. Voriconazole (Vcz), a second generation triazole antifungal agent, is the first-line prophylactic therapy for aspergillosis and is used widely in immunosuppressed patients. Clinical use of Vcz is associated with an increased incidence of cutaneous squamous cell carcinoma (cSCC) leading to significant morbidity. To better understand the pro-carcinogenic effect of Vcz, we hypothesized that Vcz may induce UV-induced cSCCs by promoting DNA damage. Vcz-treated primary human keratinocytes (PHKs) (25uM) exhibited significantly increased DNA damage ($P<0.001$) by TUNEL assay compared to Fluconazole (Fcz)-treated controls. Vcz-treated PHKs also demonstrated sustained elevation of DNA damage markers including ph-p53, ph-H2AX and ph-ATM leading to elevated p21 levels post-UVB (25mJ/cm2). PHKs treated with Vcz demonstrated greater UVB-induced growth inhibition compared to mock and Fcz-treated cells. Interestingly, Vcz had no effect on the formation or the repair of cyclobutane pyrimidine dimers indicating that the Vcz-associated DNA damage likely involves other mechanisms. Since ATM phosphorylation is associated with reactive oxygen species, the effect of Vcz on oxidative stress, a cause of DNA damage, was assessed. Vcz-treated PHKs exhibited decreased levels of NADPH (2.1nM) as compared to mock or Fcz-treated cells (3.3 and 3.4nM, respectively) indicating Vcz exposure is associated with increased oxidative stress. Nrf2, a transcriptional regulator of genes that counter oxidative stress, Aryl hydrocarbon receptor and antioxidant genes such as glutathione synthetase and CYP1B1 were decreased by Vcz treatment. These data support the hypothesis that Vcz treatment increases oxidative stress and UVB-induced DNA damage in PHKs. The Vcz-dependent increase in DNA damage secondary to UVB likely increases the risk of cSCC development.

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Toll-like receptor-4 cooperates with inflammasome to mediate ultraviolet radiation induced cutaneous inflammation and tumor development

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Ultraviolet (UV) irradiation of the skin induces inflammation, and is linked to the progression of skin cancer. Our previous studies indicate that Toll-like receptor 4 (TLR4), a component of innate immunity, plays an important role in regulation of UVB induced DNA damage. When mice were exposed to multiple doses of UVB radiation (200 mJ/cm2) for 40 weeks, cutaneous carcinogenesis was retarded in terms of tumor incidence, and tumor latency, in TLR4 deficient mice compared to TLR4 proficient mice, whereas significantly greater ($p<0.05$) numbers of tumors occurred in TLR4 proficient mice. There was significant ($p<0.05$) up-regulation of inflammatory markers like cyclo-oxygenase (COX)-2, prostaglandin (PG)E2, inducible nitric oxide synthase (iNOS), and S100A8/9 in the skin of TLR4 proficient mice compared to skin of TLR4 deficient mice. Furthermore, we found that TLR4 proficient mice had significantly ($p<0.05$) higher number of Gr1+CD11b+ myeloid cells than TLR4 deficient mice (18.8±1.64% versus 6.24±0.87%). Nod-like receptor family, pyrin domain-containing 3 (NLRP3) known as the NLRP3 inflammasome is known to assemble with active caspase-1 to cleave pro IL-1β to active IL-1β. IL-1β was found to be significantly up-regulated ($p<0.05$) in TLR4 proficient mice compared to TLR4 deficient mice. Since, there is a cross-talk between TLR and NLR signaling to trigger inflammatory responses, including production of IL-1β, we assessed the expression level of NLRP3 and caspase-1 in the skin of UVB exposed TLR4 proficient and TLR4 deficient mice. Our results clearly show that the level of NLRP3 and caspase-1 was significantly ($p<0.05$) higher in UVB exposed TLR4 proficient mice compared to UVB exposed TLR4 deficient mice. Together, our data indicate that crosstalk between TLR4 and NLRP3 enhances the development of UVB induced skin tumors. These findings may allow us to develop preventive and therapeutic approaches for management of UVB induced skin cancer.

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Baicalin protects keratinocytes from Toll like receptor-4 mediated DNA damage and inflammation following ultraviolet irradiation

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UVB radiation causes both direct and indirect damage to the skin including the generation of free radicals and reactive oxygen species (ROS), inflammatory responses, immunosuppression, and gene mutations, which can ultimately contribute to photocarcinogenesis. A plant-derived flavonoid, baicalin, has been shown to have a wide range of pharmacological properties, such as antioxidant, anti-inflammatory, and free radical scavenging activities. Previous studies from our laboratory have shown that Toll like receptor-4 (TLR4) enhanced both UVB-induced DNA damage and inflammation in murine skin. The aim of the present study is to investigate the efficacy of baicalin against TLR4 mediated processes in the murine keratinocyte PAM 212 cell line. Our results demonstrate that treating keratinocytes with baicalin before and after UV radiation (100 mJ/cm2) significantly inhibited the level of intracellular ROS and reduced markers of DNA damage, including cyclobutane pyrimidine dimers (CPDs) and 8-Oxo-2′-deoxyguanosine (8-oxo-dG). Furthermore, baicalin treatment inhibited TLR4 and its downstream signaling molecules, MyD88, TRIF, TRAF6, and IRAK4. TLR4 pathway inhibition resulted in NF-κB inactivation and down-regulation of iNOS and COX-2 protein expression. Based on these results, we conclude that baicalin treatment effectively protected keratinocytes from UVB-induced inflammatory damage by modulating the TLR pathway.

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Effects of ascorbic acid on simulated solar radiation-induced melanogenesis in human keratinocytes

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The melanin pigment produced in the skin is essential for protecting against the damaging effects of ultraviolet radiation (UVR) from sunlight. UVR stimulates the keratinocyte p53 pathway expression of proopiomelanocortin (POMC)-related protein ligands that bind to melanocytes and lead to the expression of microphthalmia-associated transcription factor (MITF). MITF is important for the expression of rate-limiting enzymes necessary for melanin production. Although MITF has been identified as an essential molecule in melanogenesis, a knowledge gap exists in the current understanding of its regulators. The aim of this study was to determine the effect of ascorbic acid (AA) on MITF, POMC, MCR1 and TYR gene expression following UVR. Primary human keratinocytes, with or without AA pre-treatment, were exposed to simulated solar radiation (SSR) instrument emitting wavelengths in the UVA and UVB spectrum (290 to 400 nm) closely resembling the ultraviolet portion of natural sunlight. AA was administered at a dose of 100 nM for 24 hours and the UVR dose delivered was equivalent to 40 or 60mJ/cm2 UVB, which approximates the sunburn threshold (1.0 and 1.5 MED, respectively) of an individual with Fitzpatrick Skin Type III. Quantitative RT-PCR performed 4 hours after UV demonstrated that AA pre-treatment suppressed expression of MITF and MCR1 at this time point. This indicates that AA may inhibit UV-induced pigmentation through targeting specific steps in the pathway.

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UVB irradiation of human keratinocytes causes translational repression via discordant eIF2 kinase stress response

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This study addressed the mechanisms by which UV irradiation regulates protein synthesis in human keratinocytes and its importance in cell survival. Phosphorylation of eukaryotic initiation factor 2 (eIF2~P) is an important process for control of global translation. Concurrent with repression of general protein synthesis, eIF2~P enhances translation of cytoprotective gene transcripts, including the transcriptional activator ATF4. To determine how UV stress affects this pathway, we irradiated human keratinocytes with either UVB or UVC. Both UV stresses induced eIF2~P in keratinocytes to levels measured with a pharmacological inducer of endoplasmic reticulum stress, a known potent trigger of eIF2~P. However, despite induction of eIF2~P neither ATF4 protein and mRNA levels were enhanced by UV irradiation. The eIF2~P is suggested to be cytoprotective in response to UV stress, and we wished to determine if forced expression of ATF4 alters these protective qualities in keratinocytes. If ATF4 protein expression was induced by salubrinal, a small molecular inhibitor of eIF2~P dephosphorylation, prior to UVB irradiation, the keratinocytes showed significantly enhanced apoptosis. These results suggest that UV irradiation elicits a discordant eIF2 kinase stress response in skin cells that is markedly different from that of the prototypical eIF2 kinase stress response. Furthermore, repression of a key downstream target of the eIF2 kinase stress response, ATF4, is suggested to provide to provide a survival advantage during UV stress. These studies suggest that precise implementation of the eIF2 kinase pathway is central for the viability of skin to the UV irradiation and could provide insight into defects that occur in related skin diseases.

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Role of UVB-induced neutrophils in collagenase induction, and collagen fragmentation

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Short-term irradiation of mice with UVB (100 mJ/cm^2/day for 5 days) causes collagen fragmentation and an increase in pro-inflammatory cells influx, especially neutrophils in the dermis. Previous studies have shown that TNFα inhibition decreases inflammatory cell infiltration, but not collagen degradation, while the source of MMPs leading to degradation could be keratinocytes, fibroblasts, neutrophils, or other inflammatory cells. We now sought to determine how specific depletion of neutrophils affects collagen fragmentation. Female C57BL/6J mice were treated with anti-neutrophil antibody (400μg/day for 7 days) with daily UVB irradiation for 5 days, followed by harvesting of skin samples 3h after the 5th exposure to UVB. We found that UVB significantly increased the density of dermal neutrophils relative to sham-irradiated controls (143.21±11.43 vs 14.0±0.5 cells per high-power field, mean±SEM, n=6, p<0.001). Pretreatment with anti-neutrophil antibody significantly inhibited UVB-induced neutrophil influx into the dermis (11.0±0.7, p<0.001). Skin samples were homogenized and collagen and other proteins extracted. Dermal collagen fragmentation was evaluated by immunoblot for Type I collagen. UVB-irradiated and anti-neutrophil antibody-treated UVB-irradiated mice had similar numbers and intensity of bands of ¾ size collagen fragments. MMP-13, a major collagenase in mouse skin, was assayed by immunoblot. UVB significantly increased MMP-13 compared to sham-irradiated and anti-neutrophil antibody-treated mice (1.02±0.21 UVB vs. 0.042±0.002 vs 0.041±0.003; p <0.001). MMP-13 levels in the skin of UVB-irradiated mice treated with anti-neutrophil antibody were statistically lower than UVB-treated mice (0.60±016; p<0.01). Overall, our results indicate that collagen fragmentation of UVB-irradiated skin occurs in neutrophil-depleted mice, suggesting a prominent contribution of enzymes derived from non-neutrophil cell types.

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IGF-1 receptor regulates repair of UVB-induced DNA damage in human keratinocytes

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The activation status of the insulin-like growth factor-1 receptor (IGF-1R) regulates the cellular response of keratinocytes to UVB exposure, both in vitro and in vivo. Briefly, geriatric skin is deficient in IGF-1 expression resulting in an aberrant IGF-1R-dependent UVB response which contributes to the development of aging-associated squamous cell carcinoma. Furthermore, our lab and others have reported that geriatric keratinocytes repair UVB-induced DNA damage less efficiently than young adult keratinocytes. Here, we show that IGF-1R activation influences DNA damage repair in UVB-irradiated keratinocytes. When immortalized N-TERT keratinocytes were irradiated with increasing doses of UVB in the presence or absence of IGF-1R activation, the rate of DNA repair was greater in N-TERT keratinocytes with an activated IGF-1R. However, no difference was observed in the removal of total thymine dimers (TD) at 48 hours post-irradiation. In contrast, when these studies were replicated using primary human keratinocytes, inactivation of the IGF-1R inhibited UVB-induced TD DNA repair (both the rate and total repair) following exposure to low doses of UVB. The influence of the IGF-1R on DNA repair waned with increasing doses of UVB. UVB-irradiation of human skin xenografted on immunocompromised mice yielded a 5-fold increase in TD(+) basal layer keratinocytes when the IGF-1R was inhibited using a topical IGF-1R inhibitor compared to vehicle-treated skin at 24 hours post-irradiation. Similar results were also observed in basal keratinocytes isolated using a novel isolation procedure using anti-α6 integrin antibodies from intact human skin irradiated with varying doses of UVB in the presence or absence of activated IGF-1Rs. These results suggest that deficient UVB-induced DNA repair in geriatric keratinocytes may be due in part to silenced IGF-1R activation in geriatric skin and that the IGF-1 pathway could possibly play a role in the initiation of squamous cell carcinoma in geriatric patients.

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UVB photoconverts voriconazole N-oxide to a UVA sensitizer

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Voriconazole is a phototoxic triazole antifungal agent that has also been associated with the development of aggressive cutaneous squamous cell carcinomas. The mechanisms by which voriconazole is phototoxic and might be carcinogenic are unknown. We explored the photochemistry and photobiology of voriconazole and its major hepatic metabolite, voriconazole N-oxide (VNO), using spectroscopic, cell viability, and fluorescence microscopy approaches. Voriconazole and VNO have varying degrees of ultraviolet B (UVB) absorption but, at 30 μM, do not acutely sensitize cultured human keratinocytes following exposure to UVB doses up to 700 J/m^2, as measured by viability in a thiazolyl blue assay. However, UVB exposures from 100-4000 J/m^2 produce significant dose-dependent changes in the absorption and emission spectra of both compounds, but especially of VNO which acquires an ultraviolet A (UVA) band with an isosbestic point, suggesting formation of a discrete photoproduct. In phosphate-buffered saline, voriconazole and VNO pre-irradiated with 800 J/m^2 UVB were modestly toxic to keratinocytes in the dark (20-40% reduction in viability, p<0.01), but did not further sensitize keratinocytes to subsequent UVB exposure. However, VNO's UVB photoproduct additionally sensitized cells by 3-fold (p<0.05) relative to controls following 10 J/m^2 UVA. Using a dihydrodichloromethyl-fluorescein derivative as a reporter, we observed increases in reactive oxygen species (ROS) of 45% and 58% (p<0.0001) relative to diluent-treated controls at 2.0 and 2.5 J/m^2 UVA, respectively, in cells incubated with VNO's UVB photoproduct. No significant increases in ROS occurred in cells treated with voriconazole or pre-irradiated voriconazole relative to controls. These results suggest that voriconazole induces phototoxicity and photocarcinogenesis through a multi-step mechanism: Initial hepatic conversion to VNO, followed by photoconversion by UVB to form a UVA-sensitizing agent capable of generating ROS that may damage DNA and other molecules involved in genomic stability.

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Proteomic analysis of UVB irradiation effects on human epidermal skin using label free mass spectrometry

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The deleterious effects of acute UVR exposure result in multi-faceted cell damage in the skin. Global changes in protein expression of the specific pathways that lead to these effects have not been fully characterized. We aimed to utilize a discovery proteomic-based analysis to determine changes in protein expression levels in human skin after in vivo simulated solar radiation (SSR). Two 2.5 cm2 areas of full-thickness suction blister epidermis were collected from twelve volunteers (age: 19-53; FST I-IV). Each volunteer provided non-irradiated control and SSR-irradiated (1 MED) skin. One dimensional label free GeLC-MS/MS quantitative approach, together with predictive statistical modeling of the free label free LC-MS/MS and Ingenuity pathway analysis (IPA), was used to examine proteins that are differentially expressed in control vs SSR-irradiated skin. An additional 3 subjects provided skin punch biopsies of control and SSR-irradiated skin for immunofluorescence staining. Using epidermal samples, we found 8% of the 2000 differentially expressed proteins were statistically significant and validated published UV-induced effects such as changes in keratins and heat shock proteins. IPA revealed the top canonical pathway to center on beta catenin. Validation by immunofluorescence staining of punch biopsies showed an increase in its cytoplasmic (3.12 fold, $p< 0.001$) and nuclear (1.52-fold, $p< 0.001$) expression. Detailed analysis of phosphorylation status at the serine 552 site showed UV induced alterations. We hypothesize that the constitutive phosphorylation of beta catenin that maintains its structural membrane function is disrupted by UV, releasing it to exert transcription factor effects.

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Tumor necrosis factor alpha (TNF-α)-dependent augmentation of ultraviolet B-induced apoptosis and inflammation as well as a profound defect in contact hypersensitivity responses are seen in mice lacking epidermal peroxisome proliferator activated receptor gamma (PPARγ)

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We have previously demonstrated that UVB induces the production of PPARγ ligands through the oxidation of membrane lipids. Moreover, we demonstrated that mice lacking PPARγ (*Pparg*-/-^{epi}) exhibit an augmentation in UVB-induced apoptosis, inflammation, and photocarcinogenesis. Inasmuch as PPARγ is known to suppress TNF-α production, we examined the role of downstream TNF-α in acute UVB responses in *Pparg*-/-^{epi} mice. *Pparg*-/-^{epi} mouse skin had increased basal levels of TNF-α mRNA expression relative to WT mouse skin. At 24 hrs after irradiation with 1500 J/m^2 UVB, *Pparg*-/-^{epi} mice exhibited a significant augmentation in UVB-induced TNF-α mRNA expression. Increased UVB-induced TNF-α in the epidermis was verified by immunohistochemical staining. Pretreatment of *Pparg*-/-^{epi} mice with anti-TNF-α neutralizing antibodies, but not isotype control antibodies, completely abolished the augmented UVB-induced inflammatory response as measured by skin thickness as well as the augmented apoptotic response as measured by activated caspase 8 assay. UVB induces both local and systemic immunosuppression that can be measured by contact hypersensitivity (CHS) reactions to contact allergens such as DNFB. Local UVB-induced immunosuppression is TNF-α-dependent. We therefore examined whether *Pparg*-/-^{epi} mice exhibited a defect in CHS responses. CHS responses to topical DNFB application were profoundly suppressed in non-UVB treated *Pparg*-/-^{epi} mice and this immunosuppression was not altered by UVB irradiation. Using the DNFB CHS model, pretreatment of normal C57Bl/6 mice with oral rosiglitazone for 10 days prior to UVB-irradiation blocked both local and systemic UVB-induced immunosuppression. Studies are ongoing to determine the role of TNF-α in *Pparg*-/-^{epi} mouse resistance to CHS responses well as whether anti-tumor immune responses are also altered.

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Light emitting diode-generated red light generates reactive oxygen species and decreases fibroblast migration

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Skin fibrosis is characterized by increased fibroblast proliferation and extracellular matrix deposition. Skin fibrosis-derived fibroblasts also demonstrate increased migration speed compared to fibroblasts derived from normal human skin. We found that light emitting diode-generated red light (LED-RL) 633 nm +/- 15 nm decreases normal human skin fibroblast migration speed in a dose-dependent manner as measured by time-lapse video microscopy. To control for LED-RL induced temperature change, each condition was matched with a temperature regulated "bench control plate" (BCP). Fluences of 320, 480, 640, and 800 J/cm2 decreased fibroblast migration speed in a dose dependent manner to 86.7%, 83.0%, 74.4%, 58.6% of the BCP respectively ($p<0.05$). We previously published that resveratrol inhibits reactive oxygen species (ROS) in vitro in a dose dependent manner. We hypothesized that LED-RL inhibits fibroblast migration speed through generation of ROS and may be reversed by resveratrol. To test our hypothesis, fibroblasts were irradiated with LED-RL at 160 J/cm2 which increased cellular ROS compared to BCP (mean fluorescence intensity 5931.3 versus 5594.6, $p<0.05$) measured immediately post-irradiation. Decreases in LED-RL migration speed post-irradiation at 320, 480, 640, and 800 J/cm2 were reversed by 0.001% resveratrol (94.7%, 103.7%, 99.0%, 97.4% relative to matched control, respectively, $p<0.05$). These findings demonstrate that mechanistically, decreased migration speeds are likely due to LED-RL generated ROS and reversed by the anti-oxidant resveratrol. Our findings contribute to the mechanistic understanding of the effect of LED-RL in vitro and provide a foundation for future mechanistic and translational studies that contribute to the light-based management of skin fibrosis.

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HO-1 regulation in photodynamic therapy in mycosis fungoides

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Heme Oxygenase-1 (HO-1) plays an important role in protecting cells against oxidative injury and its expression is known to alter in response to UVR and photodynamic therapy (PDT). PDT with silicon phthalocyanine (Pc) 4 is a potential treatment for cutaneous malignancy. Data from a Phase 1 study demonstrated partial responses in the majority of mycosis fungoides (MF) patients following a single dose of Pc 4-PDT1. The aim of this study was to investigate the changes in the oxidative stress-related gene expression of HO-1 in patients with early stage MF following the topical application of 0.1 mg/mL Pc 4 plus 675 nm red light (50-150 J/cm2). HO-1 immunohistochemical staining revealed an increase of nuclear staining in the epidermis of lesional MF relative to skin of normal volunteers; HO-1 was also upregulated with 4 MEDs of UVB, consistent with activation in association with inflammation signaling. Epidermal nuclear staining in biopsies obtained 4 hours post Pc 4-PDT, there was significant down-regulation of the increased nuclear HO-1, which appeared dose related. Interestingly, quantitative RT-PCR of Pc 4-PDT treated MF skin biopsies showed minimal change, except at the highest light dose, where an increase in the transcription of HO-1 relative to the untreated lesional skin. Degradation or alteration of inflammation-associated nuclear HO-1 by Pc 4-PDT may reflect changes in oxidative defense or modification of disease process, and may be useful in optimizing treatment protocols.

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Efficacy of a dietary supplement versus placebo on skin protection after UV exposure

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The immunosuppressive effect of UV is well known and is evidenced by inhibition of contact hypersensitivity (CHS) reactions after allergen application on a skin site exposed to UV. Recent studies indicate that probiotics have many immunomodulatory functions in the gut and skin. Lactobacillus johnsonii (La1, NCC533) is a probiotic that was isolated by Nestlé Research Center and has shown potential in maintaining the skin's defense mechanisms, regulating the immune system and enhancing recovery from photo-induced damage. We aimed to evaluate the effect of dietary La1 supplementation on UV suppression of CHS response to dinitrochlorobenzene (DNCB). This was a double-blind randomized controlled trial. Caucasian males 20-40 years old, FST II-III were enrolled in the study and randomized into 1 of 2 groups. Subjects took a daily dose of either placebo (maltodextrin) or La1 formulated as a 10g powder (1x109 CFU) that was dissolved in water. After 8 weeks of intake, UV exposure via 1 kW solar simulator with WG320/1.5 and UG11/1 filters was performed on the upper buttock, followed 2-3 days later by DNCB sensitization. DNCB challenge was performed 2 weeks later on the arm. Both skin fold thickness and visual evaluation of CHS (NACDG scoring) were performed. 98 men completed the study, (48 placebo, 48 La1). The 2 groups were comparable in age, FST and baseline MED.Both placebo and La1 were well tolerated orally. There was a significant difference in the CHS response between the 2 groups in the subjects who received 2 MED, in favor of the La1 group, indicating the capacity of La1 to protect against UV suppression of skin immune responses.