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**Inhibition of NADPH oxidase-1 as a new approach for the prevention and treatment of cutaneous squamous cell carcinoma**

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The NADPH oxidase (NOX) family includes seven members that are involved in a multitude of physiological functions. We and others have already shown that NOX could be the source of UVB-induced ROS generation. However, it has not yet been fully defined which members of the NOX family are responsible for UVB-induced ROS generation and what precise role NOX might play in UVB-induced carcinogenesis. We found that among NOX family members, NOX1 is over-activated after acute UVB-irradiation in a biphasic manner. Indeed, UVB irradiation induced an immediate strong activation of NOX1, which followed by a second more moderate increase in NOX1 activation some hours later. Specific inhibition of both early and late NOX1 activation using a new NOX1 peptide inhibitor (InhNOX1) led to increased NER efficiency and subsequently decreased apoptosis. In contrast, when only the second peak was blocked, the NER efficiency and apoptosis were both decreased. Tumor formation following chronic UVB irradiation was markedly decreased in SKH-1 hairless mice treated topically with InhNOX1 before each irradiation. To determine whether NOX1 inhibition had a more pronounced photo-protective effect in DNA repair deficient context, XPC-deficient mice treated with InhNOX1 were exposed to chronic UVB irradiation. Treatment with InhNOX1 resulted in a significant decrease in the number and size of tumors in these mice, too. Finally, to examine the anti-cancer progression effects of InhNOX1, $XPC^{+/+}$ and $XPC^{-/-}$ mice were irradiated 22 and 13 weeks respectively and then treated topically with either scramble or InhNOX1. Results showed that InhNOX1 slowed down tumor growth significantly. Altogether, these results suggest that: NOX1 plays a key role in the determination of the ultimate fate of UVB-irradiated cells through an intrinsic ROS priming function for quenching DNA damage and promoting survival, InhNOX1 may be a new approach to the prevention and treatment of cutaneous SCC in NER-proficient and -deficient patients.

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**Postzygotic mutations of RHOA cause a mosaic neuroectodermal syndrome**

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Skin hypopigmentation along Blaschko's lines (hypomelanosis of Ito, HI) is considered a non-specific manifestation of mosaicism. Besides various mosaic chromosomal anomalies, only MTOR mutations in HI with hemimegalencephaly have been reported. Here we report on a novel mosaic neuroectodermal syndrome associated with postzygotic *RHOA* mutations. We studied 6 unrelated patients ascertained through our Mosaic Undiagnosed Skin Traits And Related Disorders (M.U.S.T.A.R.D.) project. All had a hitherto undescribed mosaic neuroectodermal syndrome, with a strikingly similar combination of linear hypopigmentation and hypotrichosis along Blaschko's lines, facial dysmorphism, ocular/dental anomalies, short fingers/toes, and asymptomatic leukoencephalopathy on MRI. We performed whole-exome sequencing (WES) in affected skin from two patients, and identified the same postzygotic variant of *RHOA*, absent in their parents' blood. This variant was also found in two unrelated individuals by targeted ultra-deep sequencing of *RHOA*. The fifth patient carried another postzygotic RHOA variant, detected by WES. In the sixth patient, no changes were detected. Transfection of NIH3T3 cells with mutant RhoA constructs resulted in reduced cell spreading, decreased stress fiber formation, and decreased phosphorylation of downstream effectors MYPT2 and MLC2, suggesting a dominant-negative effect. *RHOA* encodes a RAS-related Rho GTPase, which controls morphogenesis, chemotaxis, axonal guidance, and cell cycle progression. RhoA is a highly conserved protein particularly intolerant to amino acid substitutions. Hence, RHOA mutations are likely to be embryonic lethal and may survive only by mosaicism. Our findings highlight the role of *RHOA* in human development and disease, and expand the clinical spectrum of mosaic neuroectodermal syndromes.

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**Cole disease: Role of ENPP1 in regulation of pigmentation and epidermal differentiation**

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Cole disease is a rare autosomal dominant disorder characterized by hypopigmented macules and hyperkeratosis. However, patient may also have hyperpigmented macules. In hypopigmented macules, a normal number of pigmented melanocytes but decreased melanin content in keratinocytes, suggesting an impairment of melanosome transfer, have been reported. Five mutations in somatomedin-B-like domains of EctoNucleotide Pyrophospahatase /Phosphodiesterase 1 (ENPP1) has been identified in five families with Cole disease. To study the role of ENPP1 in pigmentation and skin differentiation, we, first, reconstructed skin with cells from one patient with Cole disease and surprisingly Cole melanocytes were able to induced thickening of epidermal reconstructs. Since this disease is rare, we transduced melanocytes and keratinocytes with lentivectors coding wild-type (WT) ENPP1 or coding the first three mutations (M) identified in ENPP1. In melanocytes, at the protein level, expression of TRP-1 and tyrosinase but not of MITF seemed inversely correlated to the level of expression of mutated ENPP1. Reconstructions with melanocytes transduced with mutated ENPP1 were not thicker than those reconstructed with melanocytes transduced with WT melanocytes. But reconstructions with cells co-transduced with WT and mutated ENPP1 forms seemed thicker than those with WT alone. We also used pharmacological inhibitor of ENPP1 on monolayer cell culture. Secretome from melanocytes treated with ENPP1 inhibitors modulated expression of keratin 5 in keratinocytes whereas direct inhibition of ENPP1 in keratinocytes seemed less effective. Thus mutations of ENPP1 seemed directly implicated in establishing and sustaining hypo or hyperpigmentation in Cole Disease. Furthermore melanocytes seemed implicated in establishment of hyperkeratosis in Cole Disease.

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**Asymmetric melanin distribution during the mitosis of human skin progenitor keratinocytes**

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Melanin is the protective biopolymer responsible for human skin pigmentation. It is synthesized in the epidermis basal layer by melanocytes before transfer within specific lysosome-related organelles (melanosomes) to surrounding keratinocytes. There, melanin forms protective supranuclear caps in UVR-vulnerable keratinocytes. For over a century the prevailing dogma is that melanin is degraded in the differentiating keratinocytes of the stratifying epidermis. Despite the absence of formal proof, this was inferred from the 'depletion' of melanin from the supra-basal epidermis, despite that this compartment is thought to be formed via the continuous upward movement of melanin-containing keratinocytes from the Malpighian layer. Using *in vitro / ex vivo* model systems consisting of human skin cells and tissues, together with *in vivo* assessments, we propose a wholly different fate for melanin in human skin. Melanin is asymmetrically distributed during the mitosis of keratinocyte progenitors during normal epidermis homeodynamics. Melanin granules distribute at the keratinocyte's basal pole during prophase, and thereafter preferentially segregate to the daughter cell that remains anchored to the basal epidermis, rather than to the daughter keratinocyte that differentiates and ultimately desquamates. Also, mild-moderate trauma (tape-stripping) triggered regenerative conditions that elicited an altered melanin distribution during mitosis whereby melanin becomes symmetrically distributed between daughter cells. This unexpected finding finally explains the predominant 'disappearance' of melanin in the supra-basal epidermis, and suggests that once made, melanin may be recycled for re-use in human epidermis. To our knowledge this is the first example in human biology of an organelle that undergoes a switchable context-dependant asymmetric or symmetric distribution during the mitosis of the stem/progenitor cell.

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**Prostaglandins contribute to specific ultraviolet irradiation-induced molecular alterations that are critically involved in photoaging process**

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Deterioration of type I collagen fibrils (COL1), which confer skin strength and resiliency, is a key feature of photoaging. UV irradiation (UVR) suppresses COL1 expression and enhances COL1 fragmentation. Mechanisms underlying COL1 decline and damage by UVR are not fully understood. UVR rapidly induces production of prostaglandins (PGs), which are metabolites of long-chain unsaturated fatty acids. The roles of PGs in UVR-induced COL1 deterioration are not known. To address this topic, we applied diclofenac (DCF, 0.2%), which inhibits PG synthesis, on buttock skin 24 hours before exposure to solar-simulated UVR (twice the minimal erythema dose). Skin specimens were obtained at 6 and 24 hours after UVR, and leukocyte influx (measured by levels of leukocyte-associated enzymes), PG levels (measured by enzyme immunoassay) and gene expression (measured by real-time PCR) were determined. Under the specified conditions, DCF did not reduce leukocyte influx at any time point examined. In contrast, DCF significantly attenuated induction of all detectable PGs ($PGE_2$, $PGD_2$, $PGF_{2a}$) (all data described were $p<0.05$, N=6-10). $PGE_2$, the most abundant PG, was reduced 71%. Interestingly, DCF partially protected against COL1 mRNA and protein reduction by 25% and 33%, respectively. Topical DCF also reduced induction of the matricellular protein CCN1 by 45%, which suppresses COL1 expression. In addition, DCF attenuated UVR induction of COL1-degrading matrix metalloproteinase-1 by 57%. DCF also reduced UVR-induced interleukine-6 (IL-6) mRNA and protein by 56% and 66%, respectively, but had no effects on induction of other cytokines, such as IL-1β or TNF-α. In primary cultured dermal fibroblasts, exogenous $PGE_2$, but not $PGD_2$ nor $PGF_{2a}$, suppressed transforming growth factor-β (TGF-β) dependent COL1 expression by 56%. Taken together, the above data identify specific molecular pathways by which PGs contribute to COL1 deterioration in response to UVR in human skin.

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**Poly(ADP-ribose) polymerase-1 activity modulates mitochondrial function following UVB irradiation**

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Ultraviolet B radiation (UVB) induces diverse cellular events that involve the formation of photolesions and the activation of the Poly (ADP-ribose) polymerase-1 (PARP-1). PARP-1 is a zinc finger protein with well-documented role in regulating several cellular processes. In this study, we investigated how PARP-1 inhibition by ABT-888 (Veliparib) treatment modulates the mitochondrial response of HaCaT keratinocytes following a single dose of 20 $mJ/cm^2$ and 40 $mJ/cm^2$ of UVB. Chemical inhibition of PARP-1 induced cell cycle arrest, resulted in the retention of cyclobutane pyrimidine dimers and DNA strand breaks with higher level of cell death following UVB exposure. UVB triggered mitochondrial fragmentation, disintegration of the internal membrane structure and caused a decrease in total mitochondrial area, mass and copy number, whereas PARP-1 inhibition provided partial protection against the UVB-mediated mitochondrial changes. Regarding the functionality of mitochondria, ABT-888 treatment increased total cellular ATP content which was proved to be dependent on oxidative phosphorylation. PARP inhibition also induced mitochondrial membrane depolarization, late increase in ROS production and mRNA changes in the antioxidant response genes. Finally, PARP inhibition preserved the availability of $NAD^+$, and induced mRNA upregulation of mitochondrial regulator proteins. Here we demonstrated that the elevated $NAD^+$ level and the upregulation of key mitochondrial regulators by PARP inhibition can be the underlying mechanism in the modulation of mitochondrial functionality. Our results suggest that PARP-1 is an essential player not only in UVB induced cell death and DNA repair but also in mitochondrial activity of HaCaT keratinocytes.