Published in final edited form as:

Cancer Prev Res (Phila). 2010 September ; 3(9): 1104–1111. doi:10.1158/1940-6207.CAPR-10-0089.

Ultraviolet radiation inhibits 15-hydroxyprostaglandin dehydrogenase levels in human skin: evidence of transcriptional suppression

Benjamin L. Judson¹, Akira Miyaki², Vikram D. Kekatpure², Baoheng Du², Patricia Gilleaudeau³, Mary Sullivan-Whalen³, Arash Mohebbati¹, Sudhir Nair¹, Jay O. Boyle¹, Richard D. Granstein⁴, Kotha Subbaramaiah², James G. Krueger³, and Andrew J. Dannenberg²

¹Department of Surgery (Head and Neck Service), Memorial Sloan-Kettering Cancer Center

²Department of Medicine, Weill Medical College of Cornell University

³Laboratory for Investigative Dermatology, The Rockefeller University, New York, New York

⁴Department of Dermatology, Weill Medical College of Cornell University

Abstract

Elevated levels of prostaglandins (PGs) have been detected in skin following ultraviolet radiation (UVR). Prostaglandins play an important role in mediating both the acute and chronic consequences of UVR exposure. UVR-mediated induction of cyclooxygenase-2 (COX-2) contributes to increased PG synthesis. In theory, reduced catabolism might also contribute to increased PG levels. 15-hydroxyprostaglandin dehydrogenase (15-PGDH), a tumor suppressor gene, plays a major role in PG catabolism. In this study, we investigated whether UVR exposure suppressed 15-PGDH while inducing COX-2 in keratinocytes and in human skin. UVR exposure caused dose-dependent induction of COX-2, suppression of 15-PGDH and increased PGE₂ production in HaCaT cells. Exposure to UVR suppressed the transcription of *15-PGDH* resulting in reduced amounts of 15-PGDH mRNA, protein and enzyme activity. UVR exposure induced Slug, a repressive transcription factor that bound to the *15-PGDH* promoter. Silencing Slug blocked UVR-mediated down-regulation of 15-PGDH. The effects of UVR were also evaluated in the EpiDerm™ skin model, a 3-dimensional model of human epidermis. Here too, COX-2 levels were induced and 15-PGDH levels suppressed following UVR exposure. Next the effects of UVR were evaluated in human subjects. UVR treatment induced COX-2 while suppressing 15-PGDH mRNA in the skin of 9 of 10 subjects. Collectively, these data suggest that reduced expression of 15-PGDH contributes to the elevated levels of PGs found in skin following UVR exposure. Possibly, agents that prevent UVR-mediated down regulation of 15-PGDH will affect the acute or long-term consequences of UVR exposure including nonmelanoma skin cancer.

Introduction

The synthesis of prostaglandins (PGs) from arachidonic acid requires two sequential enzymatic steps. Cyclooxygenase (COX) catalyzes the synthesis of PGH₂ from arachidonic acid. There are two isoforms of COX. *COX-1* is a housekeeping gene that is expressed constitutively in

Requests for reprints: Andrew J. Dannenberg, Department of Medicine and Weill Cornell Cancer Center, 525 East 68th Street, Room F-206, New York, NY 10065. Phone: 212-746-4403; FAX: 212-746-4885; ajdannenberg@med.cornell.edu.

Disclosure of Potential Conflicts of Interest

Andrew J. Dannenberg is a member of the Scientific Advisory Board of Tragara Pharmaceuticals Inc., a company that is developing a selective COX-2 inhibitor.

most tissues (1). *COX-2* is an immediate-early response gene that is undetectable in most normal tissues including the skin but is rapidly induced by oncogenes, growth factors, cytokines, ultraviolet radiation (UVR) and tumor promoters (2-4). Specific synthases then convert PGH₂ to a variety of PGs including PGE₂ and PGF₂α (3,5).

Multiple lines of evidence suggest an important role for the COX-PG axis in the development of nonmelanoma skin cancers (5-8). Exposure to UVR induces COX-2 and PG levels in skin (4,9,10). PGE₂ stimulates cell proliferation, angiogenesis and vascular permeability while inhibiting apoptosis and immune function (3,7,11,12). Both genetic and pharmacological studies indicate a role for the COX-PG pathway in skin carcinogenesis. In UV studies, skin tumor latency was decreased and multiplicity increased in COX-2 transgenic mice compared to wild-type mice (13). Knocking out COX-2 or treatment with celecoxib, a selective COX-2 inhibitor, protected against skin carcinogenesis (14-16). In a clinical trial, celecoxib was suggested to have protective effects against basal cell carcinoma (17). Recent studies have attempted to elucidate the downstream effectors of PGE₂. PGE₂ exerts its effects by binding to and activating four G protein coupled receptors known as EP₁-EP₄. EP₂ knockout mice developed fewer skin tumors (18-20). Others have suggested that EP₁ may be important in skin carcinogenesis (21). Collectively, these EP receptor studies provide additional evidence of the importance of PGE₂ in skin carcinogenesis.

Although there is excellent evidence that UVR-mediated induction of COX-2 leads to increased PG synthesis, other mechanisms may also contribute to increased PG levels in skin. Reduced catabolism of PGs may lead to elevated PG levels (22). The key enzyme responsible for the degradation of PGs is NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH) (23). 15-PGDH, a 29-kDa enzyme, catalyzes the formation of 15-keto-PGs, which possess greatly reduced biological activities compared with PGs (23,24). Mice engineered to be 15-PGDH deficient have increased PG levels in tissues (22,25). Skin constitutively expresses 15-PGDH and is capable of the enzymatic degradation of PGE₂ into 15-keto metabolites (26). Therefore, it's possible that UVR mediated increases in PG levels in skin reflect down regulation of 15-PGDH in addition to up regulation of COX-2.

In the present study, we first determined that UVR exposure down regulated *15-PGDH* while inducing COX-2 and PGE₂ levels in HaCaT cells. After demonstrating that UVR had similar effects in a 3-dimensional skin model, we carried out a clinical trial. Consistent with the preclinical findings, exposure to UVR led to up regulation of COX-2 and down regulation of 15-PGDH in skin. These results provide new insights into the mechanism by which UVR alters PG levels, which is likely to be important for understanding both the acute and chronic effects of UVR.

Materials and Methods

Materials

Dulbecco's Modified Eagle Medium (DMEM) was obtained from Invitrogen. Antibodies to β-actin, L-glutamic dehydrogenase, α-ketoglutaric acid, nicotinamide adenine dinucleotide (NAD⁺) and Lowry protein assay kits were obtained from Sigma-Aldrich Corp. Antibodies to COX-2 and Slug were obtained from Santa Cruz Biotechnology. Anti-human polyclonal antiserum to 15-PGDH was from Novus Biologicals Inc. Western blot analysis detection reagents (enhanced chemiluminescence) were from PerkinElmer Life and Analytical Sciences, Inc. Nitrocellulose membranes were from Schleicher and Schuell. Enzyme immunoassay kits for PGE₂ assays were from Cayman Chemical Co. Charcoal-activated powder was from EM Science. The RNeasy Mini kit was from QIAGEN Inc. MuLV reverse transcriptase, RNase inhibitor, Oligo d(T)₁₆ and SYBR Green PCR Master mix were from Applied Biosystems.

Real-time PCR primers were synthesized by Sigma-Genosys. DharmaFECT 4 was obtained from Thermo Fisher Scientific Co. ChIP assay kits were purchased from SA Bioscience Corp.

Tissue culture

HaCaT cells were a generous gift of Dr. Sam W. Lee (Harvard University). These cells are spontaneously immortalized human epithelial keratinocytes (27). Cells were routinely maintained in DMEM supplemented with 10% FBS, 100 IU/mL penicillin, and 100 µg/mL streptomycin, grown to 70% confluence, and trypsinized with 0.125% trypsin–2 mmol/L EDTA solution. Cells were grown in basal medium (DMEM without serum) for 24 h before treatment.

UVR

UV irradiation was done in 10-cm cell culture dishes containing growth factor-free phenol red-free DMEM. For UV irradiation, a bank of six FS-40 sunlamps (Philips) in parallel connection was used. These lamps emit a continuous spectrum from 270 to 390 nm with a peak emission at 313 nm; approximately 65% of the radiation emitted by these lamps is within the UVB range (280–320 nm) (28). These bulbs also emit approximately 0.5% of their energy in the UVC region (200–280 nm) (29). Measured by an IL-1700 UVR meter (International Light), this bank of bulbs deliver an average flux of 0.9 mW per cm² at the level of exposure of the cells. Control cells were treated identically except that the UVR lamps were not turned on.

Western blot analysis

Cell lysates were prepared by treatment with lysis buffer as described previously (30). Lysates were sonicated for 8 min on ice and centrifuged at 14,000 x g for 10 min at 4°C to sediment the particulate material. The protein concentration of the supernatant was measured by the method of Lowry *et al.* (31). SDS-PAGE was performed under reducing conditions on 10% polyacrylamide gels. The resolved proteins were transferred onto nitrocellulose sheets and then incubated with antisera to COX-2, 15-PGDH, Slug and β-actin. Secondary antibody to IgG conjugated to horseradish peroxidase was used. The blots were probed with the ECL Western blot detection system. All experiments were repeated and representative results are shown.

15-PGDH activity assay

15-PGDH enzyme activity in cellular lysates was assayed by measuring the transfer of tritium from 15(S)-[15-³H] PGE₂ to glutamate by coupling 15-PGDH with glutamate dehydrogenase as described previously (32).

Real-Time PCR

Total RNA was isolated using RNeasy Mini Kit. One µg RNA was reverse transcribed using murine leukemia virus reverse transcriptase (Roche Applied Science) and oligo d(T)₁₆ primer. The resulting cDNA was then used for amplification. Each PCR was 20 µL and contained 5 µL cDNA, 2x SYBR Green PCR master mix and primers. Primer pairs were as follows: COX-2 (forward, 5'-CCCTTGGGTGTCAAAGGTAA-3' and reverse, 5'-GCCCTCGCTTATGATCTGTC-3'), 15-PGDH (forward, 5'-TCTGTTTCATCCAGTGCATGT-3' and reverse, 5'-ATAATGATGCCGCCTTCACCT-3'), β-actin (forward, 5'-AGAAAATCTGGCACCACACC-3' and reverse, 5'-AGAGGCGTACAGGGATAGCA-3') served as an endogenous normalization control. Experiments were done using a 7500 real-time PCR system (Applied Biosystems). Relative fold-induction was determined by ΔΔC_T (relative quantification) analysis.

Transient transfections

Slug siRNA was obtained from Dharmacon, Inc. HaCaT cells were transfected with 100 pmol of Slug siRNA or NS siRNA using DharmaFECT 4 transfection reagent according to the manufacturer's instructions. The medium was changed after 24 h and cells were allowed to grow in basal media for another 12 h before UVR or sham treatment.

Chromatin immunoprecipitation assay

ChIP assay was performed with a kit according to the manufacturer's instructions. Briefly, 4×10^6 cells were cross-linked in a 1% formaldehyde solution at 37°C for 10 min. Cells were then lysed and sonicated to generate 200- to 1000-bp DNA fragments. After centrifugation, the cleared supernatant was incubated with 4 µg of the indicated antibody at 4°C over night. Immune complexes were precipitated, washed, and eluted as recommended. DNA-protein cross-links were reversed by heating, and the DNA fragments were purified and used as a template for PCR amplification. Quantitative real-time PCR was carried out. *15-PGDH* promoter oligonucleotide sequences for PCR primers were forward, 5'-CTCCGCTCTCCTTCTATCCA-3' and reverse, 5'-AACCCACGACTGTGTACCT-3'. This primer set encompasses the *15-PGDH* promoter sequence from nucleotide -366 to -155. PCR was performed at 95°C for 30 s, 62°C for 30 s, and 72°C for 45 s for 40 cycles and real-time PCR was performed at 95°C for 15 s and 60°C for 60 s for 40 cycles. The PCR product generated from the ChIP template was sequenced, and the identity of the *15-PGDH* promoter was confirmed.

PGE₂ production

The amount of PGE₂ released by cells was measured by enzyme immunoassay according to the manufacturer's instructions. Production of PGE₂ was normalized to protein concentration.

Organotypic skin constructs

EpiDerm™, an established normal human epithelial keratinocyte model that exhibits *in vivo*-like morphological features and growth characteristics, was obtained from MatTek Corp. (33). These skin-like tissue structures are highly differentiated and consist of basal, spinous, granular, and cornified layers and exhibit properties similar to those of human epidermis. Cultures were grown in hydrocortisone free media. UVR exposure was performed after removing the cultures from agarose-containing 24 well plates and allowing them to equilibrate to 5% CO₂ and 37°C for 1 h in the medium supplied by MatTek. Cell lysates were prepared by snap freezing, grinding with a mortar and pestle, and then subjected to western blot analysis as described above.

Human tissue

Following approval by the Rockefeller University and Weill Cornell Medical College Institutional Review Boards, a human study was performed. The Declaration of Helsinki protocols were followed. Written informed consent was obtained from 10 healthy subjects with light skin (Fitzpatrick types I-IV). Phototesting using narrow band UVB (Philips TL-01 lamps) was performed to determine the minimal erythema dose (MED) for each subject (34). The test was read 24 h after exposure of sun-protected skin to graded doses of narrow band UVB radiation. The area exposed to the lowest dose of UV that yielded perceptible erythema was determined to be the MED. Two 6 mm punch skin biopsies were then performed. An area that received 2 MED of UVB radiation was biopsied and an adjacent, non-irradiated skin site was also biopsied. Tissue was snap frozen and stored at -80°C until analysis.

Statistical analysis

Comparisons between groups were made by a two-tailed Student's *t* test. A difference between groups of $P < 0.05$ was considered significant.

Results

UVR treatment induces COX-2 and suppresses levels of 15-PGDH in keratinocytes

Initially, we determined the effects of UVR on levels of COX-2 and 15-PGDH in HaCaT cells. As shown in Fig. 1A, UVR treatment caused dose-dependent induction of COX-2. Levels of COX-2 were markedly increased 6 h after UVR exposure and then rapidly declined. Because of its importance in PG catabolism, we also evaluated the effects of UVR on amounts of 15-PGDH. A marked dose-dependent decrease in amounts of 15-PGDH protein was found both 6 and 12 h after UVR treatment. Consistent with the western blot findings, exposure to UVR led to a dose-dependent decrease in 15-PGDH activity in cell lysates (Fig. 1B). To determine whether UVR modulated the expression of COX-2 and 15-PGDH by a pretranslational mechanism, quantitative real-time PCR was carried out. Exposure to UVR led to dose-dependent increases in levels of COX-2 mRNA with a corresponding reduction in amounts of 15-PGDH mRNA (Fig. 1C). To determine whether UVR induced changes in the expression of COX-2 and 15-PGDH had functional consequences, we next determined PGE₂ production. As shown in Fig. 2, UVR treatment led to a dose-dependent increase in PGE₂ concentration in the medium.

UVR suppresses 15-PGDH transcription

Next we attempted to elucidate the mechanism by which UVR exposure led to reduced expression of 15-PGDH. Slug, a member of the Snail family of transcription factors, has been reported to be induced by UVR (35) and to suppress *15-PGDH* transcription in lung cancer (36). Hence, we investigated whether Slug was responsible for UVR-mediated suppression of 15-PGDH in HaCaT cells. UVR led to rapid induction of Slug protein (Fig. 3A). To further evaluate the potential role of Slug in regulating 15-PGDH expression, we determined whether UVR stimulated the binding of Slug to the *15-PGDH* promoter. Repressive transcription factors, e.g., Snail family members, bind to a region of the *15-PGDH* promoter that contains E-boxes (36,37). ChIP assays were carried out using a primer set that included the *15-PGDH* promoter segment containing E-boxes. As shown in Fig. 3B, treatment with UVR caused approximately a 1-fold increase in the binding of Slug to the *15-PGDH* promoter. Next it was important to evaluate whether Slug played a role in UVR-mediated down-regulation of 15-PGDH expression. Silencing of Slug blocked UVR-mediated down-regulation of 15-PGDH (Fig. 3C). Together, these results suggest that UVR-mediated induction of Slug is responsible for the down-regulation of *15-PGDH* transcription. Given these findings in HaCaT cells, we also evaluated whether UVR would induce similar effects in a model that more closely mimics human skin. Hence, the EpiDerm™ skin model, a 3-dimensional highly differentiated model of the human epidermis, was employed. These organotypic cultures were exposed to 0, 100 or 150 mJ/cm² UV and then harvested 6, 12 or 24 h later. Western blot analysis was performed for COX-2 and 15-PGDH (Fig. 4). Consistent with the findings in HaCaT cells, UVR treatment induced COX-2 while suppressing 15-PGDH protein levels. These effects were observed for up to 24 h following UVR exposure.

UVR induces COX-2 and suppresses levels of 15-PGDH in human skin

Based on the consistency of the UVR effects in two well-established preclinical models, we next conducted a clinical study to determine if these findings extended to human skin. As detailed in the Materials and Methods section, 10 subjects with light skin (Fitzpatrick types I-IV) were recruited. The skin was UV irradiated to establish the minimal erythema dose (MED).

Twenty-four hours later, each subject underwent biopsies of both unexposed skin (non-irradiated) and skin that most closely corresponded to 2 MED. Quantitative PCR was used to determine amounts of COX-2 and 15-PGDH mRNAs in the biopsies. As shown in Fig. 5, UVR treatment led to marked increases in COX-2 mRNA with a corresponding reduction in amounts of 15-PGDH mRNA in 9 of 10 subjects. UVR failed to either induce COX-2 or suppress levels of 15-PGDH in the skin of subject 6. There was nothing in the way of skin type or medications used that provided a potential explanation for the difference in response observed in this subject.

Discussion

In this study, we found that UVR exposure suppressed the expression of 15-PGDH in human skin. This is important because 15-PGDH is the key enzyme for inactivating PGs including PGE₂ (23,38). UVR is known to cause increased amounts of both COX-2 and PGs in the skin (4,9,10). Based on our results, it seems likely that the increased levels of PGs found in skin following UVR exposure reflect reduced catabolism in addition to increased synthesis. In support of this possibility, a recent human study reported increased levels of PGE₂ in skin 24 h after UVB exposure in the absence of a similar increase in 13,14-dihydro-15-keto PGE₂, a catabolic product (10). Future studies will be needed to determine whether the UVR-mediated changes in COX-2 and 15-PGDH expression occur in the same cells or not. Previously, we showed that levels of 15-PGDH were reduced in inflammatory bowel disease (39). Given that levels of 15-PGDH are down regulated in both inflammatory bowel disease and UVR exposed skin, it is highly likely that reduced PG catabolism will be found in a range of inflammatory conditions. Considering the importance of PGs in inflammation and carcinogenesis (3,10,40, 41), future studies should be done to evaluate the expression of 15-PGDH in other inflammatory conditions.

Additional studies were carried out to define the mechanism by which UVR suppressed levels of 15-PGDH. Our results suggest that UVR suppressed the transcription of *15-PGDH* leading to reduced levels of 15-PGDH mRNA and protein. We also report that suppression of 15-PGDH reflects UVR-mediated induction of Slug, a Snail family zinc-finger transcription factor. These findings are a logical extension of previous work. UVR is known to induce Slug in keratinocytes (35). Moreover, Slug-mediated suppression of *15-PGDH* transcription is believed to explain the reduced levels of 15-PGDH in non-small cell lung cancer (36). Slug and Snail bind to E-boxes in the *15-PGDH* promoter and thereby inhibit transcription (36, 37). In the current study, ChIP analysis indicated that UVR exposure stimulated the binding of Slug to a region of the *15-PGDH* promoter containing three E-boxes. To evaluate the functional significance of increased Slug binding, siRNA was used. Silencing of Slug blocked UVR-mediated down regulation of 15-PGDH. It is intriguing to note that Slug-knockout mice exhibit an attenuated cutaneous inflammatory response to UVR (42). The current findings suggest the possibility that UVR will cause less suppression of 15-PGDH in Slug-knockout mice resulting in less PG-related skin inflammation.

The fact that coordinated changes in both PG synthetic and catabolic pathways occur in UVR exposed skin suggests that elevated levels of eicosanoids are likely to play a significant role in the UV response. In fact, there is extensive evidence that UVR-mediated induction of PGE₂ is important in stimulating vasodilatation, vascular permeability and cell proliferation while inhibiting apoptosis and immune function (5,10,12,43-45). Consistent with these effects, increased levels of PGE₂ appear to be important for wound healing (46). Inhibitors of COX-derived PGE₂ production including selective COX-2 inhibitors suppress several components of the acute inflammatory response to UVR exposure (44-47). As mentioned above, there is extensive evidence that PGs also play a significant role in the development of nonmelanoma skin cancer (5-7,14-18). A number of different mechanisms may contribute to carcinogenesis.

For example, it is hypothesized that UVR-mediated induction of PGE₂ levels protect against apoptosis increasing the likelihood of DNA photoproducts becoming mutations (7). Recent studies have suggested that 15-PGDH behaves as a tumor suppressor for several tumor types (48-50). It's possible, therefore, that UVR-mediated down regulation of 15-PGDH will contribute to increased levels of PGs in skin and thereby increase the risk of carcinogenesis. Based on this constellation of findings, it will be worthwhile to determine whether knocking out or over expressing 15-PGDH in the skin impacts on either the acute or chronic consequences of UVR exposure. Inducers of 15-PGDH have been identified (51). Whether inducers of 15-PGDH will alter the acute or chronic effects of UV exposure should be evaluated. In addition to PGs, some other eicosanoids including lipoxin A₄ are excellent substrates for 15-PGDH. It is possible, therefore, that down regulation of 15-PGDH will have significant effects independent of changes in PG levels. Taken together, it is likely that short-term elevation of PGE₂ in skin following UVR exposure is an adaptive mechanism that enhances wound healing, whereas chronic elevation of PGE₂ promotes the development of nonmelanoma skin cancer. The results of the current study highlight the potential significance of UVR-mediated down regulation of PG catabolism in mediating these effects.

Acknowledgments

Grant Support

NIH Grants UL1RR024143 and T32 CA09685

References

1. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. *Annu Rev Biochem* 2000;69:145–82. [PubMed: 10966456]
2. Subbaramaiah K, Telang N, Ramonetti JT, et al. Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells. *Cancer Res* 1996;56:4424–9. [PubMed: 8813136]
3. Dannenberg AJ, Subbaramaiah K. Targeting cyclooxygenase-2 in human neoplasia: rationale and promise. *Cancer Cell* 2003;4:431–6. [PubMed: 14706335]
4. Buckman SY, Gresham A, Hale P, et al. COX-2 expression is induced by UVB exposure in human skin: Implications for the development of skin cancer. *Carcinogenesis* 1998;19:723–729. [PubMed: 9635856]
5. Muller-Decker K, Furstenberger G. The cyclooxygenase-2-mediated prostaglandin signaling is causally related to epithelial carcinogenesis. *Mol Carcinog* 2007;46:705–710. [PubMed: 17546626]
6. Butler GJ, Neale R, Green AC, Pandeya N, Whiteman DC. Nonsteroidal anti-inflammatory drugs and the risk of actinic keratoses and squamous cell cancers of the skin. *J Am Acad Dermatol* 2005;53:966–72. [PubMed: 16310056]
7. Rundhaug JE, Fischer SM. Cyclo-oxygenase-2 plays a critical role in UV-induced skin carcinogenesis. *Photochem Photobiol* 2008;84:322–329. [PubMed: 18194346]
8. Chen W, Tang Q, Gonzales MS, Bowden GT. Role of p38 MAP kinases and ERK in mediating ultraviolet-B induced cyclooxygenase-2 gene expression in human keratinocytes. *Oncogene* 2001;20:3921–3926. [PubMed: 11439356]
9. Einspahr JG, Bowden GT, Alberts DS, et al. Cross-validation of murine UV signal transduction pathways in human skin. *Photochem Photobiol* 2008;84:463–476. [PubMed: 18248498]
10. Rhodes LE, Gledhill K, Masoodi M, et al. The sunburn response in human skin is characterized by sequential eicosanoid profiles that may mediate its early and late phases. *FASEB J* 2009;23:3947–3956. [PubMed: 19584301]
11. Nishimura K, Setoyama T, Tsumagari H, et al. Endogenous prostaglandins E₂ and F_{2α} serve as an anti-apoptotic factor against apoptosis induced by tumor necrosis factor-α in mouse 3T3-L1 preadipocytes. *Biosci Biotechnol Biochem* 2006;70:2145–53. [PubMed: 16960384]

12. Chung HT, Burnham DK, Robertson B, Roberts LK, Daynes RA. Involvement of prostaglandins in the immune alterations caused by the exposure of mice to ultraviolet radiation. *J. Immunol* 1986;137:2478–2484. [PubMed: 3463622]
13. Fischer SM, Pavone A, Mikulec C, Langenbach R, Rundhaug JE. Cyclooxygenase-2 expression is critical for chronic UV-induced murine skin carcinogenesis. *Mol Carcinog* 2007;46:363–371. [PubMed: 17219415]
14. Tiano HF, Loftin CD, Akunda J, et al. Deficiency of either cyclooxygenase (COX)-1 or COX-2 alters epidermal differentiation and reduces mouse skin tumorigenesis. *Cancer Res* 2002;62:3395–3401. [PubMed: 12067981]
15. Fischer SM, Lo HH, Gordon GB, et al. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, and indomethacin against ultraviolet light-induced skin carcinogenesis. *Mol Carcinog* 1999;25:231–240. [PubMed: 10449029]
16. Pentland AP, Schoggins JW, Scott GAG, Khan KNM, Han R. Reduction of UV-induced skin tumors in hairless mice by selective COX-2 inhibition. *Carcinogenesis* 1999;20:1939–1944. [PubMed: 10506108]
17. Tang JY, Aszterbaum M, Athar M, et al. Basal cell carcinoma chemoprevention with nonsteroidal anti-inflammatory drugs in genetically predisposed PTCH^{+/−} humans and mice. *Cancer Prev Res* 2010;3:25–34.
18. Sung YM, He G, Fischer SM. Lack of expression of the EP₂ but not EP₃ receptor for prostaglandin E₂ results in suppression of skin tumor development. *Cancer Res* 2005;65:9304–11. [PubMed: 16230392]
19. Chun KS, Langenbach R. A proposed COX-2 and PGE₂ receptor interaction in UV-exposed mouse skin. *Molec Carcinog* 2007;46:699–704. [PubMed: 17570497]
20. Brouxhon S, Konger RL, VanBuskirk J, et al. Deletion of prostaglandin E₂ EP₂ receptor protects against ultraviolet-induced carcinogenesis, but increases tumor aggressiveness. *J Invest Dermatol* 2007;127:439–46. [PubMed: 16977324]
21. Tober KL, Wilgus TA, Kusewitt DF, Thomas-Ahner JM, Maruyama T, Oberyszyn TM. Importance of the EP₁ receptor in cutaneous UVB-induced inflammation and tumor development. *J Invest Dermatol* 2006;126:205–211. [PubMed: 16417238]
22. Coggins KG, Latour A, Nguyen MS, Audoly L, Coffman TM, Koller BH. Metabolism of PGE₂ by prostaglandin dehydrogenase is essential for remodeling the ductus arteriosus. *Nat Med* 2002;8:91–2. [PubMed: 11821873]
23. Tai HH, Ensor CM, Tong M, Zhou H, Yan F. Prostaglandin catabolizing enzymes. Prostaglandins Other Lipid Mediat 2002;69:483–493. [PubMed: 12432938]
24. Pichaud F, Delage-Mourroux R, Pidoux E, Jullienne A, Rousseau-Merck MF. Chromosomal localization of the type-I 15-PGDH gene to 4q34–q35. *Hum Genet* 1997;99:279–81. [PubMed: 9048936]
25. Hughes D, Otani T, Yang P, Newman RA, Yantiss RK, Altorki NK, et al. NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase regulates levels of bioactive lipids in non-small cell lung cancer. *Cancer Prev Res* 2008;1:241–249.
26. Fincham N, Camp R. Novel prostaglandin dehydrogenase in rat skin. *Biochem J* 1983;212:129–134. [PubMed: 6575778]
27. Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J Cell Biol* 1988;106:761–771. [PubMed: 2450098]
28. Rivas JM, Ullrich SE. Systemic suppression of delayed-type hypersensitivity by supernatants from UV-irradiated keratinocytes: an essential role for keratinocyte-derived IL-10. *J Immunol* 1992;149:3865–3871. [PubMed: 1460278]
29. Nghiem DX, Walterscheid JP, Kazimi N, Ullrich SE. Ultraviolet radiation-induced immunosuppression of delayed-type hypersensitivity in mice. *Methods* 2002;28:25–33. [PubMed: 12231185]
30. Subbaramaiah K, Yoshimatsu K, Scherl E, et al. Microsomal prostaglandin E synthase-1 is overexpressed in inflammatory bowel disease. Evidence for involvement of the transcription factor Egr-1. *J Biol Chem* 2004;279:12647–58. [PubMed: 14722058]

31. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265–75. [PubMed: 14907713]
32. Tong M, Tai HH. Induction of NAD⁺-linked 15-hydroxyprostaglandin dehydrogenase expression by androgens in human prostate cancer cells. *Biochem Biophys Res Commun* 2000;276:77–81. [PubMed: 11006085]
33. Zhao JF, Zhang YJ, Kubilus J, et al. Reconstituted 3-dimensional human skin as a novel *in vitro* model for studies of carcinogenesis. *Biochem Biophys Res Commun* 1999;254:49–53. [PubMed: 9920731]
34. Coven TR, Burack LH, Gilleaudeau P, Keogh M, Ozawa M, Krueger JG. Narrowband UVB produces superior clinical and histopathological resolution of moderate-to-severe psoriasis in patients compared with broadband UV-B. *Arch Dermatol* 1997;133:1514–1522. [PubMed: 9420535]
35. Hudson LG, Choi C, Newkirk KM, et al. Ultraviolet radiation stimulates expression of snail family transcription factors in keratinocytes. *Molec Carcinog* 2007;46:257–268. [PubMed: 17295233]
36. Yang L, Amann JM, Kikuchi T, et al. Inhibition of epidermal growth factor receptor signaling elevates 15-hydroxyprostaglandin dehydrogenase in non-small cell lung cancer. *Cancer Res* 2007;67:5587–93. [PubMed: 17575121]
37. Miyaki A, Yang P, Tai HH, Subbaramaiah K, Dannenberg AJ. Bile acids inhibit NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase transcription in colonocytes. *Am J Physiol Gastrointest Liver Physiol* 2009;297:G559–G566. [PubMed: 19608733]
38. Ensor CM, Tai HH. 15-Hydroxyprostaglandin dehydrogenase. *J Lipid Mediat Cell Signal* 1995;12:313–9. [PubMed: 8777575]
39. Otani T, Yamaguchi K, Scherl E, et al. Levels of NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase are reduced in inflammatory bowel disease: evidence for involvement of TNF- α . *Am J Physiol Gastrointest Liver Physiol* 2006;290:G361–G368. [PubMed: 16195422]
40. Wang D, DuBois RN. Eicosanoids and cancer. *Nature Rev Cancer* 2010;10:181–193. [PubMed: 20168319]
41. Narasimha AJ, Watanabe J, Ishikawa T, et al. Absence of myeloid COX-2 attenuates acute inflammation but does not influence development of atherosclerosis in apolipoprotein E null mice. *Arterioscler Thromb Vasc Biol* 2010;30:260–268. [PubMed: 19926832]
42. Newkirk KM, Duncan FJ, Brannick EM, Chandler HL, Parent AE, Kusewitt DF. The acute cutaneous inflammatory response is attenuated in Slug-knockout mice. *Lab Invest* 2008;88:831–841. [PubMed: 18458671]
43. Kabashima K, Nagamachi M, Honda T, et al. Prostaglandin E₂ is required for ultraviolet B-induced skin inflammation via EP₂ and EP₄ receptors. *Lab Invest* 2007;87:49–55. [PubMed: 17075575]
44. Wilgus TA, Koki AT, Zweifel BS, Kusewitt DF, Rubal PA, Oberyzyzn TM. Inhibition of cutaneous ultraviolet light B-mediated inflammation and tumor formation with topical celecoxib treatment. *Molec Carcinog* 2003;38:49–58. [PubMed: 14502644]
45. Chun KS, Akunda JK, Langenbach R. Cyclooxygenase-2 inhibits UVB-induced apoptosis in mouse skin by activating the prostaglandin E₂ receptors, EP₂ and EP₄. *Cancer Res* 2007;67:2015–21. [PubMed: 17332329]
46. Lee JL, Mukhtar H, Bickers DR, Kopelovich L, Athar M. Cyclooxygenases in the skin: pharmacological and toxicological implications. *Toxicol Appl Pharmacol* 2003;192:294–306. [PubMed: 14575647]
47. Wilgus T, Ross M, Parrett M, Oberyzyzn T. Topical application of a selective cyclooxygenase-2 inhibitor suppresses UVB mediated cutaneous inflammation. *Prostaglandins Other Mediat* 2000;62:367–384.
48. Ding Y, Tong M, Liu S, Moscow JA, Tai HH. NAD⁺-linked 15-hydroxyprostaglandin dehydrogenase (15-PGDH) behaves as a tumor suppressor in lung cancer. *Carcinogenesis* 2005;26:65–72. [PubMed: 15358636]
49. Wolf I, O'Kelly J, Rubinek T, et al. 15-Hydroxyprostaglandin dehydrogenase is a tumor suppressor of human breast cancer. *Cancer Res* 2006;66:7818–23. [PubMed: 16885386]
50. Myung SJ, Rerko RM, Yan M, et al. 15-Hydroxyprostaglandin dehydrogenase is an *in vivo* suppressor of colon tumorigenesis. *Proc Natl Acad Sci U S A* 2006;103:12098–102. [PubMed: 16880406]

51. Hazra S, Batra RK, Tai HH, Sharma S, Cui X, Dubinett SM. Pioglitazone and rosiglitazone decrease prostaglandin E₂ in non-small-cell lung cancer cells by up-regulating 15-hydroxyprostaglandin dehydrogenase. *Mol Pharmacol* 2007;71:1715–20. [PubMed: 17412838]

Fig. 1.

UVR treatment induces COX-2 and suppresses levels of 15-PGDH in HaCaT cells. A, cells were sham treated or exposed to 3 or 6 mJ/cm² of UV and harvested 6 h or 12 h later. Cellular lysate protein (50 µg/lane) was loaded onto a 10% SDS–polyacrylamide gel, electrophoresed and subsequently transferred onto nitrocellulose. Immunoblots were probed for COX-2, 15-PGDH and β-actin. B, cells were sham treated or exposed to 3 or 6 mJ/cm² of UV and harvested 12 h later. Cellular levels of 15-PGDH enzyme activity were determined as described in Materials and Methods. Enzyme activity is expressed as pmol/min per mg protein. Columns, means (n=5); bars, SD. ***, P < 0.001 compared to sham treated keratinocytes. C, cells were sham treated or exposed to 3 or 6 mJ/cm² of UV and harvested 6 h later. Total RNA was isolated, and quantitative real-time PCR was performed. Values for COX-2 and 15-PGDH were normalized to values obtained for β-actin. Columns, means (n=4); bars, SD. **, P < 0.01; ***, P < 0.001 compared to sham treated keratinocytes.

Fig. 2.

UVR treatment stimulates PGE₂ production by keratinocytes. HaCaT cells were either sham treated or exposed to 3 or 6 mJ/cm² of UV. Levels of PGE₂ were measured in the media 12 h after UV exposure. Columns, means (n = 6); bars, SD. ***, P < 0.001.

Fig. 3.

Slug plays a critical role in UVR-mediated down regulation of *15-PGDH* transcription. A, HaCaT cells were sham treated or exposed to 6 mJ/cm² of UV. Cells were harvested 1, 2 or 3 h following UV exposure. Cellular lysate protein (100 µg/lane) was loaded onto a 10% SDS-polyacrylamide gel, electrophoresed and subsequently transferred onto nitrocellulose. Immunoblots were probed for Slug and β-actin. B, Chromatin immunoprecipitation (ChIP) assays were performed. HaCaT cells were sham treated or exposed to 6 mJ/cm² of UV. Three h later, cells were harvested. Chromatin fragments were immunoprecipitated with antibodies against Slug, and the *15-PGDH* promoter was amplified by PCR (top) or real time PCR (bottom). DNA sequencing was carried out and the PCR product was confirmed to be the *15-*

PGDH promoter. The *15-PGDH* promoter was not detected when normal IgG was used or antibody was omitted from the immunoprecipitation step (data not shown). Columns, means (n=3); bars, SD, *P<0.05. C, HaCaT cells were transfected with 100 pmol of Slug siRNA or nonspecific (NS) control siRNA. Following transfection, cells were sham treated or exposed to 6 mJ/cm² of UV. Six h after irradiation, cells were harvested for western blot analysis. Cellular lysate protein (100 µg/lane) was loaded onto a 10% SDS–polyacrylamide gel, electrophoresed and subsequently transferred onto nitrocellulose. Immunoblots were probed for Slug, 15-PGDH and β-actin.

Fig. 4.

Treatment with UVR induces COX-2 and suppresses levels of 15-PGDH in the EpiDermTM skin model. Organotypic cultures were exposed to 0, 100 or 150 mJ/cm² UV and then harvested 6, 12 or 24 h later. Cellular lysate protein (50 µg/lane) was loaded onto a 10% SDS-polyacrylamide gel, electrophoresed and subsequently transferred onto nitrocellulose. Immunoblots were probed for COX-2, 15-PGDH and β-actin as indicated.

Fig. 5.

UVR induces COX-2 and suppresses 15-PGDH mRNA levels in human skin. Ten subjects with light skin (Fitzpatrick types I-IV) were recruited. The skin was UV irradiated to establish the minimal erythematous dose (MED). Twenty-four h later, each subject underwent biopsies of both unexposed skin (sham-treated) and skin that corresponded to 2 MED (UV treated). Total RNA was isolated, and quantitative real-time PCR was performed. Values for COX-2 and 15-PGDH were normalized to values obtained for β -actin.