Upregulation of PD-L1 via HMGB1-Activated IRF3 and NF-κB Contributes to UV Radiation-Induced Immune Suppression

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Abstract

Solar ultraviolet radiation (UVR) suppresses skin immunity, which facilitates initiation of skin lesions and establishment of tumors by promoting immune evasion. It is unclear whether immune checkpoints are involved in the modulation of skin immunity by UVR. Here, we report that UVR exposure significantly increased expression of immune checkpoint molecule PD-L1 in melanoma cells. The damage-associated molecular patterns molecule HMGB1 was secreted by melanocytes and keratinocytes upon UVR, which subsequently activated the receptor for advanced glycation endproducts (RAGE) receptor to promote NF-κB– and IRF3-dependent transcription of PD-L1 in melanocytes. UVR exposure significantly reduced the susceptibility of melanoma cells to CD8+ T-cell–dependent cytotoxicity, which was mitigated by inhibiting the HMGB1/TBK1/IRF3/NF-κB cascade or by blocking the PD-1/PD-L1 checkpoint. Taken together, our findings demonstrate that UVR-induced upregulation of PD-L1 contributes to immune suppression in the skin microenvironment, which may promote immune evasion of oncogenic cells and drive melanoma initiation and progression.

Significance: These findings identify PD-L1 as a critical component of UV-induced immune suppression in the skin, which facilitates immune evasion of oncogenic melanocytes and development of melanoma.

See related commentary by Sahu, p. 2805

Introduction

Solar ultraviolet radiation (UVR) is a key epidemiologic factor causing skin cancers, such as cutaneous melanoma (1). As an environmental genotoxic stressor, UVR induces DNA damage, elicits inflammation, as well as alters genome structure and function in skin cells, which all contribute to the development of skin cancers and aging. Within the solar UV spectrum, UVB and UVA are of major environmental significance to skin carcinogenesis, because UVC is mostly absorbed by ozone in the earth’s atmosphere. UBV can penetrate into the dermis papillary area and induce DNA damage in skin-residing keratinocytes, melanocytes, and dendritic cells, resulting in its much higher carcinogenicity than UVA (2). The influence of UVR in oncogenic mutation of melanoma was further supported by the TCGA melanoma study, which identified the UVR-associated mutation signature in 76% of primary tumors and 84% of metastatic samples in patients with melanoma (3). Besides leading to genomic mutation, UVR could suppress the local immune response through damaging and expelling skin Langerin+ antigen-presenting dendritic (Langerhans) cells. In addition, UVR attenuates systemic immunity by inhibiting effector and memory T cells while activating regulatory T and B cells (4). The resulting immunosuppressive microenvironment of UVR-exposed skin enables premalignant skin cells and tumor cells to escape immune surveillance and facilitates cutaneous melanoma initiation and progression. Consistently, increased risk of invasive melanoma was observed in organ transplant patients who normally underwent medical immunosuppression to prevent graft rejection (5). Therefore, reinvigorating the immunosuppressive microenvironment of the skin after UVR could play a pivotal role in reducing incidence and progression of invasive melanoma.

Recent advances in understanding the critical role of immune checkpoints in regulating tumor-infiltrating T-cell activity has led to a radical shift in cancer immunotherapy and remarkable success in treating patients with invasive melanoma with immune checkpoint blockers, such as humanized antibodies antagonizing cytotoxic T lymphocyte antigen 4 (CTLA4, CD152), programed death-1 (PD-1, CD279), or its ligand (PD-L1, CD274; ref. 6). Naïve T-cell activation requires T-cell receptor (TCR) activation by recognition of specific antigen presented by antigen-presenting cells (APC), and costimulatory or coinhibitory signals to further modulate T-cell activation (7). Costimulatory signals, such as CD28 ligation with B7-1/CD80 or B7-2/CD86, are required for
effective activation of T-cell immunity. On the contrary, coinhibitory signals, such as CTLA4 binding with B7-1/B7-2 and PD-1/PD-L1 ligation, function as immune checkpoints to prevent tissue damage from overactivated T-cell immunity and maintain peripheral immune tolerance. Tumor cells can exploit the immune checkpoints by expressing increased ligands for coinhibitory receptors, such as PD-L1 and PD-L2, and induce an immunosuppressive tumor microenvironment, thereby escaping from antitumor immunity (8). Thus, blocking immune checkpoint signals mediated by CTLA4 and PD-1/PD-L1 significantly enhances antitumor immunity and has shown durable efficacy in treating various types of cancer, including invasive melanoma.

Although the immune-suppressive effect of UVR has been well established, whether immune checkpoint activation is involved in the UVR-dependent immune suppression is not completely understood. Gene expression profiling using neonatal melanocytes from mouse skin exposed to UVR revealed an IFN response signature that includes CTLA4 induction (9). This increased CTLA4 transcription is likely dependent on macrophage-produced IFNγ within the skin microenvironment (9, 10). Here, we show that UVR also induces PD-L1 upregulation in melanocytes and melanoma cells, which is independent of IFN signaling. Instead, UVR induces HMGB1 release from skin cells, which engages the receptor for advanced glycation endproducts (RAGE) receptor and activates the NF-κB/IRF3 transcriptional complex in melanocytes. The NF-κB/IRF3 complex was enriched on the PD-L1 promoter upon UVR and was responsible for transcriptional upregulation of PD-L1. Consistently, PD-L1 levels were significantly correlated with activation of NF-κB and IRF3 gene signature in melanoma patient samples. Moreover, blocking the HMGB1/RAGE/NF-κB/IRF3 signaling cascade or using PD-1/PD-L1 checkpoint blocker dramatically enhanced the susceptibility of melanoma cells to CD8+ T-cell-mediated cytoxicity after UVR exposure. Overall, our findings support a critical role of UVR-induced PD-L1 upregulation in promoting an immunosuppressive microenvironment in the skin after UVR, which facilitates the evasion of premalignant melanocytes and melanoma cells from tumor immune surveillance, leading to melanoma initiation and progression.

Materials and Methods

Cells and reagents

The SK-Mel-28, SK-Mel-2, MeWo, A375, HaCat, and B16 cells were obtained from ATCC, and maintained in DMEM or RPMI1640 media supplemented with 10% FBS following standard culture conditions. Human primary epidermal melanocytes (HEM) were purchased from Lifeline and cultured with DermaLife Melanocyte Complete Medium following the manufacturer’s protocol. The WM35 and WM1341 cell lines were generous gifts from Dr. Meenhard Herlyn (Wistar Institute, Philadelphia, PA), and were cultured in DMEM supplemented with 10% FBS. ATCC cell lines were characterized by short tandem repeat profiling, and cells resuscitated from original passage were used up to 20 passages within 6 months in all experiments. Mycoplasma contamination was routinely tested by DAPI staining. SK-Mel-28 KO lines (TBK1 KO, HMGB1 KO, RAGE KO, and IRF3 KO) were generated with CRISPR/Cas9. lentCRISPR v2 was a gift from Feng Zhang (MIT McGovern Institute, Cambridge, MA; Addgene plasmid # 52961). The following sgRNA sequences were used: sg1TBK1 CAT AAG CCT TCT TCG TCC AG; sg2TBK1 GAA GAA CCT TCT AAT GCC TTA; sg1HMGB1 GGA GAT CCT AAG AAG; sg2HMGB1 GAG AAG TTG ACT GAA GCA TC; sg1RAGE TAT TTC CCA GGG GCA GTA GT; and sg2RAGE GTG GCT CAC CCC ACA GAC TC. sg1IRF3 TTG AGC AGA GGA GGA CCG GAG and sg2IRF3 ATC TAC TAC TTT GTG AAC TC.

Electrophoretic mobility supershift assay

Briefly, 10 μg of total cellular extract was incubated with 32P-labeled double-stranded oligonucleotides (NF-κB: 5’-TCA ACA GAG GGG ACT TTC GAG phosphate, 1 mmol/L sodium orthovanadate, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 10 μmol/L p-nitrophenyl phosphate, and 10 μmol/L sodium fluoride). Five percent of total lysates were used as input for each sample. The remaining lysate was incubated with 1 μg of primary antibody (or control IgG) on the rotator at 4°C overnight. Protein G sepharose was then added and incubated for another 4 hours at 4°C. Protein G sepharose–enriched complexes were resolved on SDS-PAGE gels and transferred onto polyvinylidene difluoride membranes. Protein signals were detected by specific antibodies and visualized by chemiluminescence.

Chromatin immunoprecipitation

In brief, cells were cross-linked with 1% formaldehyde, sonicated, and then immunoprecipitated with antibodies against p65 or IRF3 in dilution buffer (1% Triton X-100, 2 mmol/L EDTA, 50 mmol/L NaCl, 20 mmol/L Tris-HCl, pH 8.1). The chromatin immunoprecipitation (ChIP)-qPCR primers were designed to amplify the promoter regions containing respective binding sites in PD-L1 promoter. For ChIP-reChIP experiments, the p65 immunoprecipitate-containing beads were washed and resuspended in reChIP buffer (dilution buffer containing 10 mmol/L DTT) and incubated at 37°C for 50 minutes. The sample was then diluted 40 times in dilution buffer, and subjected to secondary immunoprecipitation with anti-IRF3 antibody. Enriched chromatin was analyzed by qPCR with respective primer sets.

Conditioned media preparation and extracellular HMGB1 enrichment

Centricon-10 (Amicon) was used to concentrate the extracellular HMGB1 protein. Cell culture media (10 mL) were collected after respective treatments and applied to the concentrators followed with centrifugation at 5,000 × g for 2 hours. The media fraction containing molecules larger than 10 kDa (HMGB1; 25 kDa) were enriched in a volume around 50 μL. The extracellular HMGB1 level was examined by Western blot analysis.

Immunofluorescence and flow cytometry

To measure cell surface PD-L1 protein level, cultured cells were washed with PBS and incubated with recombinant human PD-1 Fc protein (R&D Systems) at room temperature for 1 hour. The Alexa Fluor 488-conjugated anti-human (Life Technologies) was
used as a secondary antibody. After mounting, the cells were visualized using an EVOS fluorescence microscope (Thermo Fisher Scientific). To determine the PD-L1 level in cells by flow cytometry, unfixed cells were incubated with Alexa Fluor 488-conjugated PD-1 Fc protein on ice for 30 minutes and analyzed with Accuri 6 flow cytometer (BD Biosciences).

To prepare tumor cell suspension for flow cytometry analysis, xenograft tumors were harvested, washed extensively in PBS, and minced before digestion with type IV collagenase (2 mg/mL), hyaluronidase (0.01%), and DNase I (20 µg/mL; Sigma-Aldrich) in Hank’s balanced salt solution at 37°C for 2 hours. The cell suspensions were filtered through a cell strainer (70 µm) and washed in PBS containing 2 mmol/L EDTA and 2% FBS. Cell suspensions were then incubated with anti-CD8 (53-6.7)-FITC and anti-granzyme B [anti-GZMB; (GB11)-PE (all from Invitrogen)] for 1 hour at 4°C and analyzed with Accuri 6 flow cytometer.

CD8⁺ T-cell isolation and activation

Human peripheral blood mononuclear cells (PBMC) were isolated from apheresis rings obtained from the Blood Donor Center at St. Jude Children’s Hospital (Memphis, Tennessee). Written informed consent was obtained from blood donors for their discarded blood products to be used for research purposes. This consent form has been approved by the Institutional Review Board at St. Jude Children’s Research Hospital (Memphis, Tennessee) and is in accordance with the Declaration of Helsinki. The PBMCs were isolated using lymphocyte separation medium and density centrifugation, followed by expansion medium. Naïve CD8⁺ T cells were positively selected using CD8 microbeads (Ly-2; Miltenyi Biotec) as per the manufacturer’s instructions. Naïve CD8⁺ CD25⁻ CD44⁺ CD62L⁺ OT-I cells were then sorted using a Sony Synergy. Naïve OT-I cells were suspended in complete Click’s medium and activated with anti-CD3 (clone 2C11, 5 µg/mL) and anti-CD28 (5 µg/mL) antibodies in the presence of IL2 (100 U/mL) for two days, removed from the antibodies, and expanded in IL2-containing medium for three additional days. The cells were maintained in IL2 and IL15-containing medium prior to use in cytotoxicity assays or tumor experiments.

Xenograft studies

All animal studies were conducted in accordance with NIH animal use guidelines and following protocols approved by Institutional Animal Care and Use Committee at University of Tennessee Health Science Center (UTHSC; Memphis, TN). NOD. Cg Prkarski ic Il2rgtm1Wij/SzJ (NSG) mice (age 6 weeks) were maintained in the UTHSC animal facility. SK-Mel-28 cells (Luciferase-labeled) were treated with UVB (50 mJ/cm²) or mock treated. After 24 hours, 1 × 10⁶ SK-Mel-28 cells and 2.5 × 10⁶ TA-CTLs were subcutaneously coinjected into the flanks of these mice (2 per mouse, n = 5 per group) following a cotransplantation model reported previously (13). Tumor volume was assessed by caliper measurement using the formula (width² × length/2). Mice were also imaged by Xenogen IVIS system (Perkin Elmer). The mice were treated with anti-PD-1 mAb (BioXCell, clone J116, 100 µg/mouse) or isotype on day 4 after transplantation then every other day for two weeks as indicated. At the endpoint, the mice were sacrificed and primary tumors were isolated for further analyses.

For syngeneic mouse melanoma xenograft model, C57/BL6J mice (age 7 weeks) were maintained in the UTHSC animal facility. B16-OVA cells were treated with UVB (50 mJ/cm²) or mock treated. After 24 hours, 5 × 10⁶ B16-OVA cells and 1 × 10⁶ OTI-CD8⁺ T cells were subcutaneously coinjected into the flanks of C57/BL6 mice (2 per mouse, 4 male + 3 female mice per group). The mice were treated with anti-PD-1 mAb (BioXCell, clone 10F.9G2, 100 µg/mouse) or isotype on day 1 after transplantation then every other day for two weeks as indicated. Tumor growth was monitored by caliper measurement and harvested tumors were analyzed as above.

Results

UV radiation induces PD-L1 upregulation in melanoma cells in an NF-κB–dependent manner

UVB radiation enhances the expression of the immune checkpoint molecule CTLA4 in melanocytes in neonatal mouse skin, which is dependent on macrophage-secreted IFNγ within the skin microenvironment upon UVR (9, 10). We further analyzed the melanocytic gene expression profile and revealed that another immune checkpoint molecule PD-1 ligand (PD-L1/CD274) was also significantly upregulated following UVB exposure (Supplementary Fig. S1A). To determine whether UVR influenced the expression of PD-L1 in melanoma cells, we treated SK-Mel-28 cells with UVB. Expression of PD-L1 was significantly increased at both the mRNA and protein levels in response to UVB treatment (Fig. 1A). UVB exposure increased PD-L1 levels in SK-Mel-28 cells in a time-dependent manner (Supplementary Fig. S1B).
Consistently, PD-L1 level on cell surface also was substantially increased in SK-Mel-28 cells upon UVB exposure (Fig. 1B and C). UVB exposure also increased the expression of PD-L1 in a panel of human melanoma cell lines and mouse B16 melanoma cells (Fig. 1D), suggesting that PD-L1 induction is a conserved stress response to UV exposure in melanocytes and melanoma cells.

In the tumor microenvironment, PD-L1 expression can be induced by inflammatory cytokines such as IFNγ (6), which activates STAT1 and increases PD-L1 transcription (14). We found PD-L1 induction by UVB was not affected by momelotinib (CYT387, JAK1/2 inhibitor) or WP1066 (JAK2 inhibitor), although UVB-induced activation of STAT1 and STAT3 was blocked (Supplementary Fig. S1C), suggesting IFNγ likely is dispensable for UVB-induced PD-L1 in melanoma cells. UVR also activates the transcription regulator NF-κB, which was suggested to be a downstream mediator of IFNγ-induced PD-L1 induction (15). We observed that UVB treatment induced NF-κB activation in melanoma cells (SK-Mel-28, WM35, and WM1341), melanocytes (HEM), and keratinocytes (HaCaT) in a dose-dependent manner (Fig. 1E; Supplementary Fig. S1D and S1E). NF-κB has been reported to upregulate PD-L1 transcription in ovarian, lung, and breast cancer cells (16–18). Indeed, we found that UVB-induced PD-L1 upregulation in melanoma cells was blocked by an IKK2 inhibitor TPCA-1 (Supplementary Fig. S1F). Moreover, depletion of RelA/p65 of NF-κB family remarkably attenuated PD-L1 induction in SK-Mel-28 cells exposed to UVB (Fig. 1F and G, compare blue and green lines). All these data suggested that UVB-induced NF-κB activation plays a critical role in mediating PD-L1 induction in melanoma cells.

UVB-induced NF-κB activation depends on TBK1 and IKK

NF-κB activation by UVR, especially by UVC, depends on casein kinase 2 (CK2)-mediated p38α activation, which, in turn, phosphorylates IκBα and promotes its degradation (19). However, inhibiting either p38 or CK2 minimally affected NF-κB activation in UVB-treated melanoma cells (Supplementary Fig. S2A). We previously reported that UVC-induced NF-κB activation was regulated by DNA damage response kinases ATM.
and ATR (20, 21). Nevertheless, inhibiting ATM, ATR, or DNA-PK with specific inhibitors or by genetic deletion did not substantially affect UVB-induced NF-κB activation (Supplementary Fig. S2B-S2D). IκB kinases (IκK) were shown to be required for UV-induced NF-κB activation (20, 22, 23), and we found IκK inhibition, by either a pharmacologic inhibitor or genetic deletion, did not substantially affect UVB-induced NF-κB activation (Supplementary Fig. S2B–S2D). IκBα kinases (IKK) were shown to be required for UV-induced NF-κB activation (20, 22, 23), and we found IKK inhibition, by either a pharmacologic inhibitor or genetic deletion, abrogated NF-κB activation by UVB (Fig. 2A and B), supporting an essential role of IKKs in mediating UVB-induced NF-κB signaling. Supershift analysis further revealed that the p65/p50 heterodimer was the major NF-κB complex activated by UVB in SK-Mel-28 melanoma cells (Supplementary Fig. S2E). Because IKK activity is required for UVB-induced NF-κB activation, we examined whether the IKK upstream kinase TAK1 is required for this signaling cascade. Surprisingly, depletion of TAK1 barely affected UVB-induced phosphorylation of IκBα and p65, suggesting that TAK1 is not essential for IKK activation in response to UVB (Supplementary Fig. S2F).

TBK1 was originally identified as a TRAF2/TANK–associated kinase activating NF-κB (24). We found inhibiting TBK1 abolished UVB-induced NF-κB activation, which was comparable to that by inhibiting IKK (Fig. 2A). TBK1-dependent phosphorylation of IKKβ was reported to activate canonical IKK activity by directly phosphorylating IKKβ (25). In accordance, we found that UVB-activated TBK1 phosphorylates IKKβ in vitro, which depends on TBK1 kinase activity (Fig. 2C). UVB treatment induced a dose-dependent activation of TBK1 and phosphorylation of its canonical downstream substrate IRF-3 (Supplementary Fig. S2G). Moreover, UVB-induced p65 phosphorylation was abolished in TBK1-knockout (KO) SK-Mel-28 cells (Fig. 2D). PD-L1 induction by UVB was also inhibited in IKKαβ−/− MEFs, TBK1-KO cells, and cells cotreated with inhibitors of IKK or TBK1 (Fig. 2A–D), supporting that IKK and TBK1 are essential for UVB-induced NF-κB activation and subsequent PD-L1 induction.

HMGB1 secretion upon UVB is required for NF-κB activation

We next asked whether TBK1/NF-κB activation by UVB depends on a signal from the extracellular milieu after UVB treatment. We found conditioned media (CM) from UVB-treated SK-Mel-28 cells could activate NF-κB, which was mediated by TBK1 and IKK, but not CK2 (Fig. 3A). CM from UVB-treated cells induced IRF-3 activation and increased TBK1 activity, suggesting an autocrine mechanism for TBK1 activation by UVB (Supplementary Fig. S3A–S3C). We also found that UVB-induced IRF3 activation and TBK1 activity were abrogated in TBK1-KO SK-Mel-28 cells (Supplementary Fig. S3D). In accordance, UVB-induced phosphorylation of p65 was abolished in TBK1-KO cells (Fig. 3B). PD-L1 induction by CM from UVB-treated SK-Mel-28 cells was also inhibited in TBK1-KO cells (Fig. 3C). These results support the essential role of TBK1 and IKK in activating NF-κB signaling by UVB.

Figure 2. UVB-activated NF-κB depends on IKK and TBK1. A, SK-Mel-28 cells were pretreated with the IKK inhibitor TPCA-1 (1 μmol/L, IKKi) and TBK1 inhibitor amlexanox (20 μmol/L, TBK1i), and then were exposed to UVB (50 mJ/cm²). After 4 hours, the cell lysates were examined by EMSA (NF-κB) and Western blot as indicated. B, Wild-type (IKKαβ+/−) or IKKαβ−/− MEFs were treated with UVB (50 mJ/cm²), and the activation of NF-κB, TBK1, and IRF3 was examined by Western blots. C, SK-Mel-28 cells were exposed to UVB (50 mJ/cm²) with or without TBK1i. Cells were harvested at 4 hours after UVR, and TBK1 was immunoprecipitated (IP) and used for TBK1 kinase assay with GST-IKKβ as substrate. D, WT and TBK1-knockout (KO) SK-Mel-28 cells were treated and analyzed as in B.
Figure 3.
UVR-induced HMGB1 secretion is required for NF-κB activation by UVB. A, CM from SK-Mel-28 cells treated with UVB (50 mJ/cm²) or mock treated were collected at 4 hours after exposure. Fresh SK-Mel-28 cells were incubated with CM with or without inhibitors of IKK (TPCA-1, IKKi), TBK1 (amlexanox, TBKi), or CK2 (TBB, CK2i) as shown for 4 hours. Then, the cells were harvested and analyzed with EMSA (NF-κB) or Western blot analysis. B, CM from HEM were harvested at 4 hours after UBV (50 mJ/cm²) exposure and treated as indicated before adding to fresh HEM culture. HEM cells were harvested at 4 hours after CM incubation and whole-cell extracts were analyzed by Western blotting with the indicated antibodies. C, SK-Mel-28 cells were treated by UVB (50 mJ/cm²) and whole-cell extracts (WCE) of treated cells were harvested at the times as shown, and Western blotting with the indicated antibodies was performed. D, HaCat keratinocytes were treated with UVB (50 mJ/cm²) and fixed at 2 hours after treatment. The intracellular localization of HMGB1 was visualized by anti-HMGB1 antibody with immunofluorescence. Nuclei were stained with DAPI. E, Parental (WT) and HMGB1-knockout (KO) SK-Mel-28 cells generated with two different sgRNAs for CRISPR were treated with UVB (50 mJ/cm²). Whole-cell lysates were analyzed by Western blot with indicated antibodies. F, CM from parental (WT) or HMGB1-KO SK-Mel-28 cells with or without UVB treatment were collected. Fresh SK-Mel-28 cells were incubated with indicated CM for 4 hours, and whole-cell lysates were analyzed by Western blot analysis. G, SK-Mel-28 cells were treated with recombinant HMGB1 (1 μg/mL) as shown. Cells were harvested at 4 hours after treatment and analyzed with EMSA (NF-κB) or Western blot analysis. H, SK-Mel-28 cells were treated by UVB (50 mJ/cm²) with or without inactivating HMGB1-BoxA fragment at indicated doses. Cells were harvested at 4 hours after treatment, and whole-cell lysates were analyzed with EMSA (NF-κB) or Western blot with indicated antibodies. I, SK-Mel-28 cells were incubated with CM from WT or HMGB1-KO cells treated by UVB or mock treated as shown. After 24 hours, PD-L1 expression was examined by flow cytometry.
primary melanocytes also effectively induced NF-κB activation in cells that were not exposed to UVB (Fig. 3B), suggesting that secreted molecule[s] in CM from UVB-treated cells is sufficient for inducing NF-κB activation. Moreover, this activity was sensitive to heat inactivation, but not nuclelease treatment, suggesting that secreted proteins are essential for NF-κB activation upon UVB (Fig. 3B).

Secretion of the nuclear protein HMGB1, as a DAMP (danger-associated molecular patterns) molecule, from keratinocytes has been linked with skin inflammation upon UVR (26,27). We found HMGB1 was released from melanocytes and melanoma cells as early as 0.5 hours after UVB exposure (Fig. 3C, Supplementary Fig. S2H). The increased extracellular HMGB1 was likely due to active secretion instead of passive release from necrotic cells, because little cell necrosis was observed until 2 hours after UVB radiation. The subcellular translocation and release of HMGB1 was further confirmed by immunofluorescence showing a substantial decrease of nuclear HMGB1 in response to UVB (Fig. 3D). To determine whether HMGB1 is essential for UVB-induced N-kB activation in melanoma cells, we generated HMGB1-KO SK-Mel-28 cells. Deletion of HMGB1 abolished the activation of TBK1 and phosphorylation of p65 and IRF-3 in response to UVB (Fig. 3E). Moreover, CM from UVB-treated HMGB1-KO SK-Mel-28 cells failed to activate NF-κB in parental SK-Mel-28 cells (Fig. 3F). These results strongly support that HMGB1 secretion upon UVB exposure plays a critical role in mediating NF-κB activation in an autocrine and/or paracrine fashion.

To determine whether HMGB1 is sufficient for activating NF-κB in melanoma cells, we treated SK-Mel-28 cells with recombinant human HMGB1 (rHMGB1). NF-κB activation was substantially increased in SK-Mel-28 cells exposed to rHMGB1 (Fig. 3G). Moreover, treatment with recombinant HMGB1-BoxA fragment, which serves as an inactivating competitor for the HMGB1 receptor (28), decreased UVB-induced NF-κB activation in melanoma cells in a dose-dependent manner (Fig. 3H). Importantly, PD-L1 induction was detected in melanoma cells exposed to UVB CM from wild-type (WT) SK-Mel-28 cells, but not from HMGB1-KO SK-Mel-28 cells, likely due to the inability to induce NF-κB activation (Fig. 3I).

SIRT1 inactivation by UVB promotes HMGB1 secretion

Active secretion of HMGB1 has been associated with increased HMGB1 acetylation at its nuclear localization signals, which blocks the re-entry of shuttling HMGB1 into the nucleus (29). We found UVB treatment significantly increased HMGB1 acetylation (Supplementary Fig. S3A). Moreover, depletion of the deacetylase SIRT1 further enhanced UVB-induced HMGB1 acetylation (Supplementary Fig. S3B), which is consistent with previous reports showing that SIRT1-dependent deacetylation inhibits HMGB1 secretion (30, 31). Accordingly, deletion of SIRT1 further enhanced UVB-induced TBK1 activation and subsequent phosphorylation of IRF3 and p65 (Supplementary Fig. S3C). We found the interaction between HMGB1 and SIRT1 was decreased in response to UVB treatment (Supplementary Fig. S3D). Inhibiting SIRT1 activity by nicotinamide (NAM) promoted HMGB1 secretion from SK-Mel-28 cells, comparable with that by UVB treatment (Supplementary Fig. S3E). Moreover, WT SIRT1, but not the catalytically inactive H355A mutant, suppressed UVB-induced TBK1/NF-κB activation in melanoma cells (Supplementary Fig. S3F). We also found treatment with the SIRT1-activating agent, resveratrol, inhibited HMGB1 secretion by UVB (Supplementary Fig. S3G), which was correlated with decreased TBK1 activation and reduced phosphorylation of IRF3 and p65 in cells exposed to UVB (Supplementary Fig. S3H). SIRT1 is a NAD+-dependent deacetylase and decreased NAD+/NADH ratio suppresses SIRT1 function (32). We found that the intracellular NAD+/NADH ratio was significantly decreased in SK-Mel-28 cells in response to UVB (Supplementary Fig. S3I), which may be responsible for decreased SIRT1 activity upon UVB exposure. All these results indicate that UVB may promote HMGB1 acetylation and secretion by suppressing SIRT1 in melanoma cells, which results in increased activation of NF-κB/IRF3.

RAGE mediates HMGB1-induced TBK1/NF-κB activation upon UVB

To identify the receptor responsible for HMGB1-induced NF-κB activation upon UVB exposure, we treated SK-Mel-28 cells with UVB in the presence of inhibitors of Toll-like receptor 4 (TLR4) or RAGE (receptor for advanced glycation endproducts). Inhibiting RAGE with FPS-ZM1 abolished UVB-induced NF-κB activation to a similar extent as inhibition of either IKKβ or TBK1, while treatment with TLR4 inhibitor had little effect (Fig. 4A). Inhibiting RAGE also abrogated UVB-induced TBK1/NF-κB activation and IRF3 phosphorylation in another melanoma cell line MeWo (Fig. 4B). UVB-induced TBK1/NF-κB activation was absent in RAGE-KO SK-Mel-28 cells (Fig. 4C). Inhibiting RAGE also blocked NF-κB activation by conditioned medium (CM) from UVB-treated melanoma cells (Fig. 4D). Moreover, the CM from UVB-treated melanoma cells failed to induce TBK1/NF-κB activation in RAGE-KO cells, whereas the same CM was able to induce TBK1/NF-κB activation in HMGB1-KO cells (Fig. 4E). In accordance, UVB-induced PD-L1 upregulation was also diminished by RAGE inhibition (Fig. 4B and F) or in RAGE-KO SK-Mel-28 cells (Supplementary Fig. S4A). These data support an essential role of RAGE in mediating the HMGB1-induced activation of NF-κB and IRF3.

IRF3 and NF-κB form a complex on the PD-L1 promoter and enhance its transcription

It was recently reported that NF-κB activation by TNFα could increase PD-L1 levels by stabilizing PD-L1 protein, which depends on upregulation of the deubiquitinate CSN5 and subsequent deubiquitination of PD-L1 (33). PD-L1 mRNA levels were not significantly changed in breast cancer cells treated with TNFα. In contrast, we found UVB exposure significantly increased PD-L1 mRNA expression (Fig. 1A), which was attenuated by inhibiting RAGE, IKK, or TBK1 (Fig. 5A). The increased PD-L1 transcription by UVB was also diminished in HMGB1-KO cells (Fig. 5B), suggesting that UVB/HMGB1-activated NF-κB directly enhanced PD-L1 transcription. Accordingly, we detected a substantial enrichment of RelA/p65 on the PD-L1 promoter region upon UVB exposure (Fig. 5C), which supports a role of NF-κB in upregulating PD-L1 transcription in melanoma cells after UVR. Interestingly, we found that UVB-induced PD-L1 upregulation was also decreased in IRF3-KO melanoma cells (Fig. 5D) or cells transfected with IRF3-targeting siRNAs, in which UVB-induced TBK1/NF-κB activation was not affected (Supplementary Fig. S4B). This observation indicates that UVB-activated NF-κB may not be sufficient for upregulating PD-L1 in melanoma cells, and IRF3 also plays an important role in mediating PD-L1 induction by UVB. Scanning the promoter sequence of PD-L1 revealed two putative IRF-binding sites (ISRE) along with the NF-κB-binding.
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Figure 4.
RAGE is required for HMGB1-promoted NF-κB/IRF3 activation in response to UVB. A, SK-Mel-28 cells were treated by UVB (50 mJ/cm²) with or without inhibitors of IKK (TPCA-1, IKKi), TBK1 (amlexanox, TBK1i), TLR4 (CLI-095, 1 μg/mL, TLR4i), and RAGE (FP5-2M, 10 μM/L, RAGEi). Cells were harvested at 4 hours after treatment and analyzed with Western blot analysis. B, MeWo cells were treated by UVB (50 mJ/cm²) with or without RAGEi. Cells were harvested at 4 hours after treatment and analyzed with Western blot analysis as shown. C, RAGE-knockout (KO) SK-Mel-28 cells generated by CRISPR/Cas9 using two different sgRNAs and parental cells were treated by UVB (50 mJ/cm²). Whole-cell lysates were analyzed by Western blot analysis with indicated antibodies. D, SK-Mel-28 cells were incubated with UVB CM along with indicated inhibitors for 4 hours. Whole-cell lysates were analyzed with EMSA. E, CM were harvested from SK-Mel-28 cells treated with or without UVB. Parental (WT), HMGB1-KO, and RAGE-KO SK-Mel-28 cells were incubated with indicated CM for 4 hours, and whole-cell lysates were analyzed by Western blot analysis with indicated antibodies. F, SK-Mel-28 cells treated with UVB (50 mJ/cm²) along with inhibitors as indicated. Cells were analyzed by flow cytometry for PD-L1 expression at 24 hours after the treatment.

The p65 site within the proximal region of PD-L1 promoter. We also detected significantly increased IRF3 binding at the distal ISRE2 site within the PD-L1 promoter upon UVB (Fig. 5E). Unexpectedly, UVB-induced IRF3 enrichment was also detected at NF-κB–binding site in the promoter, while p65 recruitment was specifically at NF-κB–binding site upon UVB exposure. To determine the importance of ISRE2 and NF-κB–binding site in regulating PD-L1 transcription upon UVB, we generated a luciferase reporter whose transcription was controlled by the PD-L1 promoter. We found deletion of ISRE2 did not significantly reduce the reporter activity in response to UVB, whereas mutating NF-κB–binding site diminished UVB-induced transactivation (Fig. 5F), supporting an essential role of the NF-κB-binding site in enhancing PD-L1 transcription upon UVB. IRF3 was previously showed to interact with NF-κB/p65, and the IRF3–p65 complex was required for transactivation of IRF3-target genes such as IFN induction by LPS (34). We confirmed that IRF3 and p65 formed a complex in melanoma cells treated by UVB (Fig. 5G). Moreover, we found the p65–IRF3 complex was enriched on the NF-κB–binding site in the PD-L1 promoter in response to UVB exposure (Fig. 5H), suggesting that UVB-induced transactivation of PD-L1 was regulated by p65–IRF3 regulatory complex.

UVB-induced PD-L1 upregulation inhibited cytotoxic T-cell-mediated antimelanoma immunity
The coinhibitory signals from PD-1/PD-L1 keep T cells’ activity in check and attenuate cytotoxic CD8+ T-cell (CTL)-mediated tumoricidal effects (6). We found that UVB treatment significantly reduced the susceptibility of SK-Mel-28 (Fig. 6A) or B16 melanoma cells (Supplementary Fig. S5A) to CTL-dependent cytolysis. The decreased CTL susceptibility was observed in SK-Mel-28 cells exposed to CTL cells activated by either anti-CD3 or CD28 antibodies (Supplementary Fig. S5B, AA T cells, black lines) or tumor-specific antigens from IR-inactivated melanoma cells (Supplementary Fig. S5B, TA T cells, red lines). Inhibiting RAGE or TBK significantly increased the specific lysis of UVB-exposed melanoma cells by CTLs (Fig. 6B). Moreover, depletion of IRF3 also enhanced the susceptibility of melanoma cells to CTL-mediated cytotoxicity after UVB exposure (Fig. 6C). Blocking PD-L1/PD-1 signaling with an anti-PD-1 antibody significantly decreased the survival of UVB-treated SK-Mel-28 cells cocultured with activated CTLs (Fig. 6D), which was associated with dramatically increased CTL-dependent cytolyis in UVB-reposed melanoma cells (Fig. 6E). These findings suggest that UVB-induced activation of TBK1/IRF3/NF-κB promotes melanoma cell escape from CTL-mediated antimelanoma immunity by upregulating PD-L1.

To determine whether the level of PD-L1 correlates with NFκB activation and/or IRF3 activity in melanoma patient samples, we analyzed the genomic data from the TCGA cutaneous melanoma study (3). We found that the PD-L1/CD274 expression level is significantly correlated with NFκB-p65-p536 levels, a surrogate of NFκB activity, determined by reverse-phase protein analysis (RPPA) in patients with...
melanoma (Fig. 6F; Supplementary Fig. S5C). Because IRF3 was not included in the RPPA analyses, we used an IRF3-upregulated gene signature (35) as a readout for measuring IRF3 activity in the patient samples. High IRF3 activity in melanoma patient samples was also correlated with PD-L1/CD247 transcription level (Fig. 6F and G), supporting a critical role of IRF3 activation in promoting PD-L1 expression.

UVB promotes melanoma evasion from CTL-mediated immunity by upregulating PD-L1
To determine whether UVB exposure promotes melanoma growth by escaping antimelanoma immunity, we stimulated human CD8+ T cells with inactivated SK-Mel-28 cells. UVB treatment of tumor antigen–activated CTLs had little effect on their cytotoxicity toward SK-Mel-28 cells in vitro, while UVB
exposure of the SK-Mel-28 cells decreased their cytotoxicity (Supplementary Fig. S6A). After a cotransplantation animal model using melanoma cells and human tumor–reactive T cells (13), we coinjected SK-Mel-28 cells, exposed to UVB or mock-treated, with or without activated CTLs into the flanks of immunodeficient NSG mice. As expected, activated CTLs dramatically suppressed SK-Mel-28 xenograft tumor growth (Fig. 7A–C). Consistent with our in vitro results, UVB exposure substantially enhanced SK-Mel-28 xenograft growth in the presence of tumor-reactive CTLs, which was attenuated by anti-PD1 treatment (Fig. 7A–C). These data suggested that upregulation of PD-L1 in melanoma cells by UVB could suppress the tumoricidal activity of CTLs to promote melanoma progression. Indeed, we detected substantially increased expression of PD-L1 in xenografts from UVB-treated melanoma (Fig. 7D and E), which correlated with increased TBK1/IRF3/NF-κB activation in these tumors (Fig. 7D). CTL cytotoxicity against melanoma cells, visualized by granzyme B staining and quantified by percentage of GZMB⁺ cells in the CD8⁺ population isolated from tumors, was significantly suppressed in SK-Mel-28 tumors exposed to UVB, which was reinvigorated by anti-PD1 treatment (Supplementary Fig. S6B; Fig. 7F). Moreover, injection of PD-1 antibody did not suppress TBK1/IRF3/NF-κB activation by UVB and consequent PD-L1 induction (Fig. 7D and E), suggesting that the improved tumor regression by anti-PD1 was
Figure 7. Blocking PD-1/PD-L1 checkpoint enhances CTL-dependent antimelanoma immunity after UVR. A, SK-Mel-28-Luc cells treated with UVB or mock treated were cotransplanted with tumor antigen–specific CD8+ T cells/CTLs as indicated (n = 5 per group). Mice with xenograft tumors were treated with anti-PD1 (100 μg/mouse) or isotype control antibodies as shown. Tumor growth was measured by calipers. The blue line shows tumors resulting from SK-Mel-28-Luc cells not receiving UVB and transplanted without CTLs. B, Images of xenograft tumors (left) and tumor weights (right) harvested at endpoint. C, Tumor growth on day 18 was monitored by IVIS imaging. D, Lysates from tumor samples (three per group) were analyzed by Western blot with the indicated antibodies. E, PD-L1 expression in xenograft tumors was determined by IHC. Scale bar, 20 μm. F, Percentage of granzyme B–positive (GZMB+) cell in CD8+ cell population isolated from xenograft tumors of respective group was quantified by flow cytometry. G, A model illustrating UVR-induced PD-L1 upregulation through activating NF-kB and IRF3, which facilitates melanoma cell immune evasion. *, P < 0.05; **, P < 0.01.
mediated by improved CTL activity. Consistent results were also observed in a syngeneic B16 melanoma xenograft model, in which UVB exposure substantially decreased the susceptibility of B16-OVA melanoma to activated OVA-specific OTI-CTLs (Supplementary Fig. S6C). In accordance to SK-Mel-28 xenograft model, treatment with anti-PD-L1 antibody significantly enhanced CTL-dependent antitumor immunity while minimally altering UVB-induced TBK1/IRF3/NF-κB signaling (Supplementary Fig. S6D and S6E) in B16-OVA tumors.

We also compared the growth of xenograft tumors from UVB-treated WT or IRF3-KO SK-Mel-28 cells cotransplanted with tumor-reactive CTLs. Although both of these cells were exposed to UVB, IRF-3 deficiency significantly reduced tumor growth (Supplementary Fig. S6F), likely owing to increased melanoma sensitivity to CTL-mediated antitumor immunity. Consistent with this notion, PD-L1 levels in IRF3-KO SK-Mel-28 xenograft tumors were substantially decreased compared with that in wild-type xenografts (Supplementary Fig. S6G and S6H). Taken together, our results support that UVB-induced IRF3/NF-κB activation promotes melanoma cell’s evasion from CTL-mediated antitumor immunity, which is dependent on the upregulation of PD-L1.

Discussion

UV-induced immunosuppression plays critical roles in promoting skin cancer (36). UVB-dependent expulsion of Langerhans cells from the skin microenvironment substantially reduces the antigen-presenting capacity of the skin-resident dendritic cells, which stimulates the premalignant cells and early-stage melanoma cells to escape from tumor immune surveillance (36, 37). UVB also induces immunosuppressive cytokine production from keratinocytes, such as IL4, IL10, and prostaglandin (PGE2). Moreover, exposure of UVB enhances migration of UVB-damaged Langerhans cells into the regional lymph nodes, which induces the expansion of regulatory T cells and reduces effector and memory T cells, resulting in systemic inhibition of adaptive immunity (4, 37). Here we showed that UVB also increased expression of PD-L1 in melanocytes and melanoma cells, which could facilitate premalignant and melanoma cells to inhibit effector T-cell activity, thereby reducing antitumor immunity in the skin microenvironment. Moreover, PD-L1 has been shown to be present on the surface of the exosomes secreted from tumor cells, which are found in the circulation and are expected to suppress T-cell activity systemically (38). Therefore, PD-L1 induction in skin cells may participate in local and systemic immune suppression by UVB, and could play an important role in immune evasion of melanoma cells from tumor immune surveillance (Fig. 7G). Targeting UVB-induced HMGB1/RAGE/TBK1 signaling could abrogate PD-L1 induction in melanocytes and melanoma cells exposed to UVB, which may serve as potential pharmacologic targets for mitigating immune escape of the premalignant melanocytes from immune surveillance.

Our previous studies have shown that transcription factor NF-κB can be activated by a variety of genotoxic agents in human cancer cells (39, 40). As a DNA-damaging agent, UVB has been shown to activate NF-κB in a p38 and CK2-dependent manner (19). Moreover, we found that UVC-induced NF-κB activation was also regulated by the DNA damage response kinases ATM and ATR (20, 21). Inhibition of ATM or ATR could suppress or augment UVC-induced NF-κB activation, respectively. In contract to UVC, our data indicated that UVB-induced NF-κB activation in human keratinocytes and melanoma cells was not affected by inhibiting p38/CK2 or by ATM/ATR deletion. Moreover, another upstream kinase TAK1, which we have previously shown to be essential for DNA damage-induced NF-κB signaling (41), was dispensable for UVB-induced NF-κB activation in skin cells. All these data suggest that UVB could activate NF-κB in skin cells through a distinct signaling cascade. Indeed, we found HMGB1, secreted from skin cells as a stress response to UVB, was essential for NF-κB activation by UVB exposure. UVB-enhanced HMGB1 acetylation, which was mediated by inactivation of SIRT1, may play an essential role in promoting HMGB1 secretion from the skin cells. In the skin microenvironment, HMGB1 binds to the DAMP receptor RAGE and leads to downstream kinase TBK1 activation, which is required for UVB-induced NF-κB activation. Consistent with previous studies (22, 23, 42), IKKβ was required for UVB-induced NF-κB activation. There was strong evidence that IKKs are indispensable for UVB-induced IkBα proteolysis, as UVB-induced IkBα degradation and NF-κB activation were not detected in IKK-KO cells, particularly in IKKβ-deficient (IKKβ⁻/⁻) cells. Besides phosphorylating IkBα in cytoplasm, the nuclear IKKβ also functions as an adaptor protein for IkBβ association with β-TrCP in the nucleus, which results in IkBβ degradation and subsequent NF-κB activation in response to UVB (42). Furthermore, computational modeling also suggested that constitutive phosphorylation of IkBα by basal IKK activity, which affects the turnover rate of IkBα, and IkBα synthesis inhibition are crucial for UVB-induced NF-κB activation (23, 43). We found UVB-activated TBK1 could directly phosphorylate IKKβ, which may promote IKK activation. TBK1-dependent phosphorylation of IKKβ was reported to either activate or inhibit canonical IKK activity depending on the upstream stimuli (23, 44). In skin cells exposed to UVB, TBK1 activity is essential for IKK activation and subsequent NF-κB activation. This remains to be further delineated how RAGE promotes TBK1 activation in cells exposed to UVB. Nevertheless, our findings demonstrated that UVB activates NF-κB in skin cells through a signaling pathway initiated from the cell surface receptor RAGE and HMGB1 stimulation from the skin microenvironment, which is distinct from the atypical nucleus-to-cytoplasm NF-κB activation signaling utilized by other genotoxic agents, including UVC.

A number of transcriptional regulators have been shown to modulate PD-L1 expression. In the tumor microenvironment, PD-L1 can be induced by inflammatory cytokines such as IFNγ, which induces activation of STAT1 and increased PD-L1 transcription (6, 14). Other transcriptional regulators, including HIF1α, Myc, STAT3, AP-1, and NF-κB, have also been shown to enhance PD-L1 transcription in response to a variety of upstream signals (45). In addition, NRF2 was recently reported to be required for UVB-induced PD-L1 upregulation in primary melanocytes and keratinocytes isolated from the human skin (46). Our data indicated that PD-L1 induction by UVB was minimally affected in NRF2-depleted human melanoma cells examined, suggesting a potential cell-type–specific mechanism for regulating PD-L1 transcription in primary cells. We found NF-κB, in association with IRF3, plays an essential role in enhancing PD-L1 transcription in melanoma cells upon UVB. Also, NF-κB and IRF3 were indispensable for PD-L1 upregulation by UVB in primary melanocytes, in which they may mediate PD-L1 transcription with NRF2 alternatively and/or collaboratively. Besides directly promoting PD-L1 gene transcription, NF-κB has been shown to indirectly stabilize PD-L1 by upregulating the deubiquitinase
CSN5, which inhibits the ubiquitination and proteasomal degradation of PD-L1 in breast cancer cells (33). We showed that, in melanoma cells, both the mRNA and protein levels of PD-L1 were increased after UVR. Moreover, the increased PD-L1 transcription by UVR was attenuated by inhibiting NF-κB upstream kinases IKKβ or TBK1. We further confirmed the enrichment of NF-κB on the PD-L1 promoter in melanoma cells after UVR. All these findings support that UVB-induced NF-κB activation directly increases PD-L1 transcription in melanocytes and melanoma cells.

Interestingly, we found that IRF3 also plays a critical role in promoting PD-L1 induction by UVR by forming a transcriptional complex with NF-κB/p65. To our knowledge, the transcriptional regulatory role for IRF3 in PD-L1 expression has not been reported. A recent study showed that DNA damage–induced PD-L1 upregulation in breast cancer cells was regulated in a STING-dependent manner (47). As the primary downstream transcriptional regulator of STING, IRF3 likely plays a role in regulating PD-L1 transcription in breast cancer cells treated with chemotherapeutic drugs. Furthermore, our previous studies demonstrated that NF-κB is activated upon chemotherapeutic treatment in breast cancer cells (48). It is tempting to speculate that NF-κB may also collaborate with IRF3 in promoting PD-L1 transcription in breast cancer cells treated with chemotherapeutics. IRF3 was previously shown to interact with NF-κB/p65, and the IRF3–p65 complex was required for transactivation of IRF3-target genes in response to LPS (33). In UVB-treated melanocytes, IRF3 was recruited onto the NF-κB–binding site within PD-L1 promoter through association with p65, which coordinately promoted PD-L1 transcription. The finding that the upstream kinase TBK1 is responsible for activation of both NF-κB and IRF3 upon UVR may have provided an attractive pharmacologic target for antagonizing PD-L1 induction by UVR by simultaneously blocking the activation of NF-κB and IRF3.

Cancer immune surveillance plays an essential role in preventing tumor incidence and effectively eliminates oncogenic cells at precancerous stages (8). Therefore, the occurrence of many cancers can be considered a breach of immune control, which is fueled by additional oncogenic mutations. The immune-suppressive landscape caused by UVR provides a permissive environment for premalignant cells to escape from tumor immunity and promote skin cancer initiation and progression. In addition to the well-established local and systemic immunosuppressive mechanisms by UVR (4), our studies indicated that activating PD-1/PD-L1 immune checkpoints by upregulating PD-L1 in melanocytes and melanoma cells also play an essential role in facilitating immune evasion of malignant skin cells from tumor immunity. Genome-wide examination of neonatal melanocytes after UVR identified an interferon gene signature, which promotes melanocytic cell survival and immune evasion (9). Secretion of HMGB1 from skin cells in response to UVR may promote an immunosuppressive microenvironment by activating PD-1/PD-L1 checkpoint, along with other previously reported immunosuppressive cytokines, such as IL4, IL10, PGE2, and TGFβ. Our data indicate that blocking PD-1/PD-L1 significantly enhanced CD8+ T-cell–mediated antitumor immunity against melanoma cells after UVB exposure, suggesting that immune checkpoint blockers may also mitigate UVR-promoted immune evasion during melanoma development. Treatment with anti-PD-1 blocking antibody has been shown to increase the number of CD8+ memory T cells in the tumor microenvironment (49). Using small molecules, such as BMS-202 (50), which inhibit the PD-1/PD-L1 interaction, may serve as an immune prevention approach to mitigate immune suppression by UVR, increase effector and memory T-cell skin infiltration, and antagonize oncogenic cell immune evasion, thereby preventing the initiation and progression of invasive melanoma after UVR.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References

PD-L1 Induction Promotes UVR-Induced Skin Immune Suppression
Correction: Upregulation of PD-L1 via HMGB1-Activated IRF3 and NF-κB Contributes to UV Radiation-Induced Immune Suppression

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In the original version of this article (1), the image used in Fig. 7E to represent immunostaining of PD-L1 in UVB+CTL+α-PD1 was inadvertently taken from UVB+CTL. The image has been replaced with the intended image for UVB+CTL+α-PD1. The error does not affect the conclusion and has been corrected in the latest online HTML and PDF versions of the article. The authors regret this error.

Reference

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Upregulation of PD-L1 via HMGB1-Activated IRF3 and NF-κB Contributes to UV Radiation-Induced Immune Suppression

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