

## PERSPECTIVES

# Immune checkpoint molecules: “new” kids on the block of skin photoimmunology



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**Abstract** Ultraviolet radiation (UVR) is a prominent etiological factor of the pathogenesis of skin diseases such as squamous cell carcinoma and melanoma. Excessive exposure to the natural sources of UVR such as sunlight or artificially from tanning lamps has been linked to the increasing incidence of skin cancers in the United States. Besides the skin inflammation, DNA damage and oncogenic mutation caused by UVR, UV exposure also plays a critical role in suppressing local and systemic immune responses which enable premalignant and cancer cells to escape immune surveillance. A variety of mechanisms have been reported to regulate the immune-suppressive effects of UVR. Here we discuss the current understanding of how UV modulates the local and systemic immunity, the recent progress in roles of immune checkpoint molecules in UVR-induced immune suppression, and how the crosstalk between the immune cells may shape the immune landscape of the skin upon UVR.

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Melanoma is the leading cause of skin cancer-associated deaths, and its incidence has been rising steadily over the last several decades. The extensive epidemiological data demonstrated that exposure to ultraviolet radiation (UVR), especially UVB, from sources like the sunlight or tanning lamps, is the most important environmental risk factor for melanoma pathogenesis.<sup>1</sup> Increasing UV exposure raises the probability of DNA damage and gene mutations which has oncogenic effect on melanoma initiation.<sup>2</sup> Transformed skin cells are considered to be excellent targets of tumor immune surveillance, due to the high mutation accumulation and rich neoantigen burden. However, UVR has been shown to suppress local immune response through damaging epidermal dendritic (Langerhans) cells, and attenuate systemic immunity by inhibiting effector and memory T cells

while activating regulatory T and B cells.<sup>3,4</sup> UVR-induced immunosuppression and immune tolerance may protect the precancerous cells and tumors from elimination by the host adaptive immune cells, which is viewed as a critical pathogenetic factor in the development of skin cancers.<sup>5</sup>

The mechanisms underlying UVR-induced immunosuppression in established tumors are complex. The immunosuppressive effect appeared to depend on the wavelength of the UVR. The UVB (290–310 nm) induces dose-dependent local immunosuppression in humans.<sup>6</sup> While the longwave UVA from 364 to 385 nm is potently immunosuppressive, short-wavelength UVA between 320 and 350 nm is ineffective to suppress immunity.<sup>7</sup> A variety of biological changes induced by UVR contribute to its immunosuppressive effect. UVR is shown to inhibit glycolysis and reduce ATP production in the epidermis, which is required for immune cells to function and immunomodulatory factors to be produced.<sup>4</sup> UVR induces cellular damage in Langerhans cells, which are epidermis-residing dendritic cells functioning as antigen-presenting

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cells. The Langerhans cells modulate T cell activation by taking up antigens within the skin microenvironment.<sup>8</sup> Primed Langerhans cells could migrate to draining lymph nodes and activate adaptive immune responses. However, UVR-induced Langerhans cell death and/or cellular damage could lead to abnormal antigen-presenting and production of immunosuppressive cytokines, such as IL-4 and IL-10, in the draining lymph nodes.<sup>9,10</sup> UVR stimulates a rapid dermal accumulation of mast cells, which are important for systemic immunosuppression by UVR.<sup>11</sup> The migration of mast cells to the B cell follicles within draining lymph nodes and consequent induction of CXCL12 in B cells have a critical role for UVR-induced immunosuppression, which can be blocked by antagonizing CXCR4.<sup>12</sup> In addition, UVR leads to reduced activation of effector and memory T cells as well as increased activation of regulatory T and B cells, which all contribute to the final outcome of UV-induced suppression to skin immunity.<sup>13</sup>

Lately, immune checkpoint blockade has captured spotlights in immune-based therapies for the treatment of human malignancies including melanoma. Recent data on the efficacy of immune checkpoint inhibition in melanoma indicates that melanoma requires immunosuppression and local mitigation of immune surveillance to evade host immune response.<sup>14,15</sup> To date, little is known about whether immune checkpoint blockade molecules contribute to the UVR-suppressed immune surveillance during early melanomagenesis and melanoma progression. Interestingly, cytotoxic T lymphocyte antigen 4 (CTLA4) was found to be significantly upregulated in epidermal melanocytes in response to UVB exposure.<sup>16</sup> CTLA4 is a potent coinhibitory receptor that functions as an immune checkpoint and suppresses the cytotoxic T lymphocyte (CTL) activation by competing with T cell costimulatory receptor CD28 for binding of their shared ligands CD80 and CD86 (also known as B7-1 and B7-2 respectively).<sup>17</sup> Upregulation of CTLA4 in activated T cells negatively regulates antitumor activity by promoting T cell tolerance and anergy, which lead to immunoevasion of tumors.<sup>18</sup> An interferon response signature, including immunoevasion-associated genes, was identified in a study on the genomic response in mouse epidermal melanocytes to UVR, which is associated with aberrant growth and migration of melanocytes.<sup>16</sup> Interferon-gamma (IFN- $\gamma$ ) released from immigrated skin macrophages is essential for the melanocytic cell survival/immunoevasion promoted by UVR. Further investigation revealed that IFN- $\gamma$  could induce the upregulation of CTLA4 transcription in melanocytes by activating the JAK-STAT1 pathway.<sup>19</sup> This IFN- $\gamma$ -dependent CTLA4 induction may also contribute to the upregulation of CTLA4 in tumor cells in response to activated effector T cells which secrete IFN- $\gamma$  to modulate anti-tumor immunity. Therefore, the induction of immune checkpoint molecule CTLA4 likely also contributes to the immune suppression by UVR, which facilitates premalignant cells and tumor cells to evade immune surveillance during skin cancer initiation.

We recently reported that another immune checkpoint molecule PD-1 ligand (PD-L1/CD274) was significantly upregulated following UVB exposure in melanocytes and a variety of melanoma cells.<sup>20</sup> Inhibitory signals from PD-1/

PD-L1 keep T cells' activity in check and attenuate cytotoxic CD8<sup>+</sup> T-cell (CTL)-mediated tumoricidal effects.<sup>15</sup> We observed that UVB treatment induced a robust activation of transcription factor NF- $\kappa$ B in melanocytes, keratinocytes, and melanoma cells in a dose-dependent manner. UVB-induced PD-L1 upregulation was substantially attenuated in RelA/p65-depleted cells or by inhibiting NF- $\kappa$ B signaling kinase IKK. Interestingly, we found conditioned media (CM) from UVB-treated cells could activate NF- $\kappa$ B, suggesting that secreted molecule(s) in CM from UVB-treated cells is sufficient for activating NF- $\kappa$ B. Further analyses revealed that HMGB1 (High Mobility Group Box 1), as an alarmin,<sup>21</sup> was released from keratinocytes, melanocytes, and melanoma cells after UVB exposure. HMGB1 secretion upon UVB exposure has a critical function in mediating NF- $\kappa$ B activation in an autocrine and/or paracrine fashion. Through screening a panel of inhibitors, we found RAGE (receptor for advanced glycation endproducts) is an essential receptor to mediate HMGB1-induced NF- $\kappa$ B activation in skin cells. In addition to NF- $\kappa$ B, transcription factor IRF3 and its upstream kinase TBK1 (TANK-binding kinase 1) were also activated in melanocytes exposed to UVR. TBK1 was originally identified as a TRAF2/TANK-associated kinase activating NF- $\kappa$ B through directly phosphorylating IKK $\beta$ .<sup>22,23</sup> In accordance, we found that UVB-activated TBK1 is required for IKK/NF- $\kappa$ B activation and the phosphorylation of IRF-3 in skin cells.

NF- $\kappa$ B has been reported to upregulate PD-L1 transcription in ovarian, lung and breast cancer cells.<sup>24–26</sup> We detected a significant increase of p65 enrichment at the promoter of PD-L1 in melanocytes and melanoma cells in response to UVR. Surprisingly, UVB-induced IRF-3 enrichment was also detected at NF- $\kappa$ B-binding site in the promoter of PD-L1, while genetic deletion of IRF-3 abrogated PD-L1 induction by UVB in melanoma cells. IRF-3 was reported to interact with p65, and the nuclear IRF3-p65 complex was required for transactivation of IRF3-target genes such as interferon induction by LPS.<sup>27</sup> Chromatin IP analysis validated that IRF-3/p65 complexes are enriched on the PD-L1 gene promoter in response to UVR, which collaboratively upregulated the PD-L1 transcription. These findings suggest that UVB-induced activation of TBK1/IRF-3/NF- $\kappa$ B axis upregulates PD-L1 in melanocytes and melanoma cells, which may promote their escape from T cell-mediated anti-melanoma immunity. Consistent with this notion, UVB exposure significantly reduced the susceptibility of human and mouse melanoma cells to CTL-dependent cytotoxicity, and this inhibition can be attenuated by pharmacological inhibition and genetic deletion of HMGB1/RAGE/IRF-3/NF- $\kappa$ B signaling. Notably, a recent study showed that UVB upregulated-PD-L1 expression could be mediated by NRF2 activation in human primary keratinocytes and human primary melanocytes, which suggested a potential cell-type-specific mechanism by which UVB regulates PD-L1 transcription.<sup>28</sup> NRF2 may function alternatively and/or collaboratively with NF- $\kappa$ B/IRF3 to regulate PD-L1 expression by UVR in primary melanocytes.

Using a co-transplantation animal model by injecting melanoma cells, exposed to UVB or mock-treated, with or without CTLs into the flanks of immunodeficient NOD scid gamma mice, we showed that activated CTLs dramatically

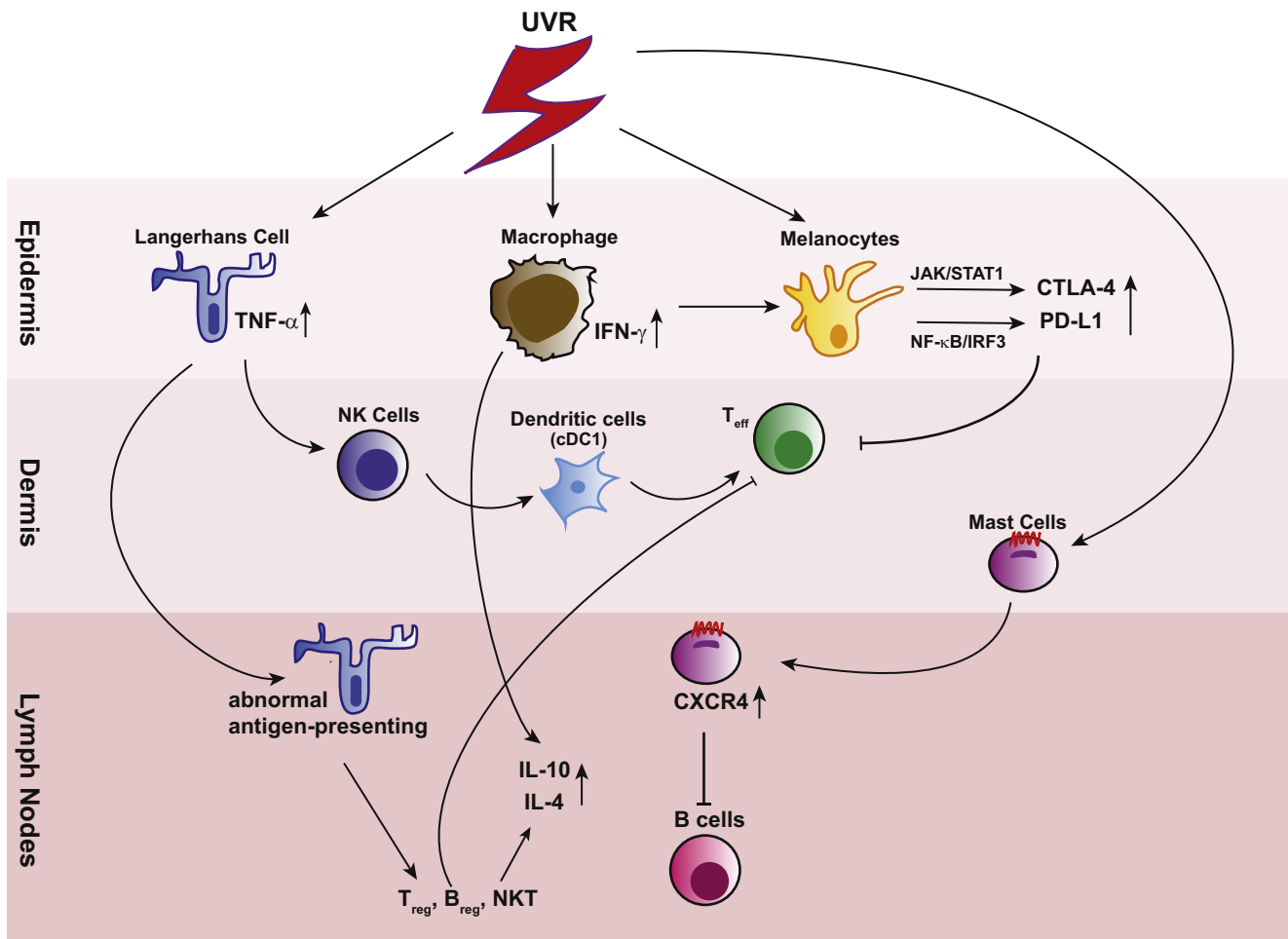


Figure 1 UVR-induced skin immune suppression.

suppressed melanoma xenograft tumor growth. Consistent with *in vitro* results, UVB exposure substantially enhanced melanoma xenograft growth even in the presence of tumor-reactive CTLs, which was attenuated by anti-PD1 treatment. These data suggested that the upregulation of PD-L1 in melanoma cells by UVB could inhibit the anti-tumor activity of CTLs and promote melanoma progression *in vivo*. Indeed, we detected substantially increased expression of PD-L1 in xenografts from UVB-treated melanoma cells, which correlated with increased TBK1/IRF-3/NF-κB activation in these tumors. Consistent results were also observed in a syngeneic B16 melanoma model, in which UVB exposure substantially decreased the susceptibility of B16-OVA melanoma to OVA-specific OTI-CTLs. Treatment with anti-PD-L1 antibody significantly enhanced CTL-dependent anti-tumor immunity while minimally affecting UVB-induced TBK1/IRF3/NF-κB signaling in B16-OVA tumors. Taken together, these results support that UVB-induced PD-L1 induction could promote immunoevasion of premalignant melanocytes and melanoma cells from CTL-mediated anti-tumor immunity, which may also serve as an integral mechanism underlying UVR-induced immune suppression in the skin.

The immune suppression of UVR is orchestrated through the crosstalk among the immune cells within the skin

microenvironment and draining lymph nodes.<sup>29</sup> (Fig. 1) Quickly increased mast cell density in the skin after UVR correlates with the increased mast cells in the skin-draining lymph nodes, suggesting that mast cells may transmit immune suppressive signals from the UV-exposed skin to the proximate lymph nodes. Migration of UV-damaged Langerhans cells from the epidermis to the draining lymph nodes activates the Treg cells, regulatory B cells, and immunosuppressive natural killer T (NKT) cells, leading to increased levels of IL-4 and IL-10, and systemic immune suppression. The skin immune response to UVR is likely also modulated by other immune cells. Natural killer (NK) cells were found to be recruited into the epidermis in a manner dependent on Langerhans cell activation, which may be regulated by Langerhans cell-secreted TNFα.<sup>30</sup> UVR leads to the activation of Langerhans cells in the skin but does not suppress basal and inducible NK cell activity.<sup>31</sup> NK cell activation may indirectly recruit and activate effector T cells by enhancing the cDC1 dendritic cell population in the melanoma microenvironment.<sup>32,33</sup> which may partially mitigate the immunosuppression of effector T cells by UVR. Taken together, modulating the crosstalk among skin immune cells by selectively activating or suppressing a specific immune cell population may alleviate the immune suppression by UVR in the skin,

leading to enhanced immune surveillance and reduced skin tumor incidence, which warrants further exploration.

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Wei Wang

Department of Pathology and Laboratory Medicine,  
University of Tennessee Health Science Center, Memphis,  
TN, USA  
Center for Cancer Research, University of Tennessee  
Health Science Center, Memphis, TN, USA

Zhao-Hui Wu\*

*Department of Pathology and Laboratory Medicine,  
University of Tennessee Health Science Center, Memphis,  
TN, USA*

*Center for Cancer Research, University of Tennessee  
Health Science Center, Memphis, TN, USA  
Department of Radiation Oncology, USA*

\*Corresponding author. University of Tennessee Health  
Science Center, 19 S. Manassas St., Memphis, TN, 38163,  
USA. Fax: +1 901 448 3910.  
*E-mail address: [zwu6@uthsc.edu](mailto:zwu6@uthsc.edu) (Z.-H. Wu)*

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