Role of p38 MAPK in UVB-Induced Inflammatory Responses in the Skin of SKH-1 Hairless Mice

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The p38 mitogen-activated protein kinase (MAPK) signaling pathway is activated by numerous inflammatory mediators and environmental stresses. We assessed the effects of ultraviolet B (UVB) on the p38 MAPK pathway and determined whether cyclooxygenase (COX)-2 expression is downstream of this kinase in the skin of UVB-irradiated SKH-1 mice. SKH-1 mice were irradiated with a single dose of UVB (360 mJ per cm^2), and activation of the epidermal p38 MAPK pathway was assessed. UVB-induced phosphorylation of p38 MAPK occurred in a time-dependent manner. Phosphorylation of MAPK-activated protein kinase-2 (MAPKAPK-2) also was detected and correlated with an increase in its kinase activity. Phosphorylation of heat shock protein 27 (HSP27), a substrate for MAPKAPK-2, also was detected post-irradiation. Oral administration of the p38 inhibitor, SB242235, prior to UVB irradiation, blocked activation of the p38 MAPK cascade, and abolished MAPKAPK-2 kinase activity and phosphorylation of HSP27. Moreover, SB242235 inhibited expression of the pro-inflammatory cytokines interleukin (IL)-6 and KC (murine IL-8) and COX-2. Our data demonstrate that UVB irradiation of murine skin activates epidermal p38 MAPK signaling and induces a local pro-inflammatory response. Blockade of the p38 MAPK pathway may offer an effective approach to reducing or preventing skin damage resulting from acute solar radiation.

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p38 mitogen-activated protein kinase (MAPK) belong to a highly conserved family of serine/threonine protein kinases that include extracellular signal-regulated protein kinases (ERK) and c-Jun N-terminal kinases (JNK). p38 MAPK is involved in numerous cellular processes, such as cell growth and survival, differentiation, development, cell cycle regulation, and cell death, depending on the cell type and stimulus (Juretic *et al*, 2001; Yosimichi *et al*, 2001). The ERK pathway is generally activated by mitogenic stimuli, whereas the JNK and p38 pathways are activated by pro-inflammatory or stressful stimuli. Various stressors, such as ultraviolet (UV) radiation, oxidative injury, heat shock, cytokines, and other pro-inflammatory stimuli, are known to trigger induction of the p38 MAPK-dependent signaling cascade. p38 MAPK exists as four isoforms (α, β, γ, and δ) and is activated by several upstream kinases *in vitro*, including MAP kinase kinases (MKK) 3, 4, and 6 (Kyriakis and Avruch, 2001). Phosphorylation of p38 MAPK in turn activates numerous downstream substrates, including p90 MAPK-activated protein kinase-2 (MAPKAPK-2) (Maizels *et al*, 2001) and mitogen and stress-activated kinase-1/2 (MSK1/2) (Deak *et al*, 1998). p90 MAPKAPK-2 and MSK1/2 function to phosphorylate heat shock protein 27 (HSP27) and cAMP-response element binding protein transcriptional factor, respectively (Kato *et al*, 2001; Wiggin *et al*, 2002). Other transcription factors, including activating transcription factor 2, Elk, CHOP/GADD153, and myocyte enhancer factor 2, are known to be regulated by these kinases (Shi and Gaestel, 2002).

Exposure of skin to both acute and chronic solar UVB radiation is the major known environmental agent driving the development of basal cell carcinomas and squamous cell carcinomas (SCC), also known as non-melanoma skin cancers. These are the most common types of human malignancy affecting more than one million Americans each year. In addition to its direct mutagenic effects, UVB induces inflammatory responses that are critical for tumor induction (Wilgus *et al*, 2002). The UVB-induced inflammatory response is characterized by the acute development of edema and erythema, and increases in dermal inflammatory cell infiltrates and augmented prostaglandin synthesis (Hruza and Pentland, 1993; Terui and Tagami, 2000).

Cyclooxygenases (COX) convert free arachidonic acid into a series of pro-inflammatory eicosanoids including prostaglandins. The COX-1 isoform serves as a housekeeping enzyme and is expressed in most tissues, whereas

Abbreviations: ATF2, activating transcription factor 2; COX-2, cyclooxygenase 2; ERK, extracellular signal-regulated kinase; HSP27, heat shock protein 27; IL, interleukin; JNK, c-Jun N-terminal kinase; KC, mouse KC/N51 originally identified as a PDGF (platelet-derived growth factor)-induced immediate early gene, known as the functional homolog of IL (interleukin)-8; MAPK, mitogen-activated protein kinase; MAPKAPK-2, MAPK-activated protein kinase-2; MKK, MAP kinase kinase; MSK1/2, mitogen and stress-activated kinase-1/2; NFκB, nuclear factor-κB; TNF-α, tumor necrosis factor-α; UVB, ultraviolet B

COX-2 is an inducible isoform generated in response to pro-inflammatory stimuli, including cytokines. The role of p38 MAPK in UVB-induced COX-2 expression has been documented by several laboratories using a human keratinocyte cell line, HaCaT. In this cell system, p38 MAPK, but not ERK, was required for UVB-induced COX-2 expression (Chen *et al*, 2001). In contrast, Ashida *et al* (2003) recently reported that UVB induction of COX-2 is associated with the activation of EGFR, ERK, p38 MAPK, and PI (phosphatidylinositol) 3-kinase. p38 MAPK transcriptionally regulates COX-2 in HaCat cells following UVB irradiation (Chen *et al*, 2001; Tang *et al*, 2001). The involvement of p38 MAPK in regulation of the stability of the COX-2 message was also demonstrated in interleukin (IL)-1-treated HeLa cells and lipopolysaccharide-treated primary human monocytes (Ridley *et al*, 1998; Dean *et al*, 1999; Lasa *et al*, 2000). These data suggest that UVB-induced p38 MAPK activity and its attendant pro-inflammatory signaling are involved in the pathogenesis of skin disorders including SCC.

In this study, we have assessed the *in vivo* effects of UVB irradiation on acute inflammatory responses in SKH-1 hairless mice, a well-established model for UVB-induced skin carcinogenesis. Our data indicate that acute UVB exposure induces the rapid activation of the p38 kinase signaling cascade, leading to COX-2 and pro-inflammatory cytokine production. Furthermore, oral administration of a p38 MAPK inhibitor abrogated these UVB-mediated inflammatory responses.

Results

UVB activates p38 MAP kinase signaling in murine skin Irradiation of SKH-1 mice with a single dose of 360 mJ per cm^2 of UVB-induced consistent visible erythema and edema, and this dose was used throughout this study. To demonstrate that UVB-induced activation of p38 MAPK signaling *in vivo*, mice were irradiated and sacrificed at various time points thereafter (0.5, 2, 8, and 24 h). Dorsal skin patches were excised and the dermis was removed by scraping, and extracts of the epidermal samples were prepared as described in Materials and Methods. Rapid phosphorylation of p38 MAPK was documented following analysis of these extracts by western blotting using a phospho-specific antibody (Fig 1). p38 MAPK phosphorylation was detected as early as 30 min post-irradiation, and was maximal at 24 h. No phosphorylation was detected in non-irradiated skin. Phosphorylation of MAPKAPK-2, a downstream substrate of p38 MAPK, was detected 30 min post-irradiation and gradually increased to its maximum level by 24 h. The total endogenous p38 MAPK and MAPKAPK-2 levels were not altered by UVB irradiation (Fig 1). Western blot analysis also demonstrated that kinases upstream of p38 (MKK3/6) were phosphorylated immediately following UVB irradiation (data not shown).

UVB induces phosphorylation of MAPKAPK-2 at Thr 222 and Thr 334 MAPKAPK-2 activation by p38 MAPK requires the phosphorylation of two threonine sites at positions 222 and 334. We assessed the phosphorylation pattern of these two residues after UVB irradiation. Skin sections from UVB-irradiated and non-irradiated mice were subjected to immunohistochemical staining using Thr222- and Thr334-phospho-specific MAPKAPK-2 antibodies. Phosphorylated MAPKAP-2 proteins in both residues were detectable throughout the epidermis following UVB irradiation, and 24 h later, all epidermal cells stained positively for the phospho-MAPKAPK-2 protein (Fig 2). The staining was minimal in the non-irradiated control skin. To distinguish the basal layer staining, the skin section (1 h post-UVB) was stained for proliferating cell nuclear antigen (PCNA), which is an established marker of cellular proliferation in both normal and neoplastic tissues and is required for DNA replication. PCNA expression was noted to be more intense in the basal layer (Fig 2*f*).

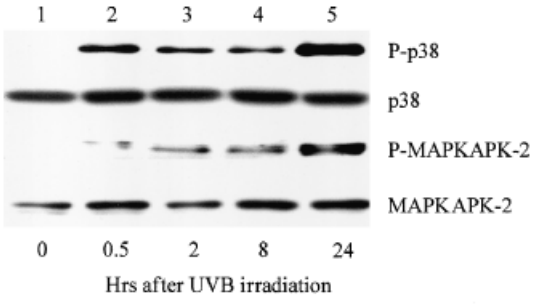

Figure 1
p38 mitogen-activated protein kinase (MAPK) pathway is activated by ultraviolet B (UVB) irradiation of murine skin *in vivo*. Total and phosphorylated levels of p38 MAPK and MAPK-activated protein kinase-2 (MAPKAPK-2) were detected by western blotting of epidermal extracts prepared from non-irradiated control (*lane 1*) and UVB-irradiated skin (*lanes 2–5*). Mice were sacrificed at 30 min (*lane 2*), 2 h (*lane 3*), 8 h (*lane 4*), and 24 h (*lane 5*) after UVB irradiation. Each lane contains 80 μg of protein lysate.

UVB-induced MAPKAP kinase-2 activity correlates with HSP27 phosphorylation MAPKAPK-2 kinase activity was assessed in UVB-irradiated skin extracts by immunoprecipitating with an anti-MAPKAPK-2 antibody and assaying these complexes for kinase activity, using an MAPKAPK-2 substrate peptide; incorporation of ^{32}P-ATP into the substrate peptide was measured by scintillation counting. Figure 3*B* compares MAPKAP kinase-2 activity in the non-irradiated control skin and the UVB-irradiated skin at 1, 3, and 24 h post-irradiation. Compared with the non-irradiated control, a 5-fold ($p = 0.018$) increase in kinase activity was observed at 3 h, and increases of 2.5-fold ($p = 0.032$) and 4.4-fold ($p = 0.003$) were observed at 1 and 24 h, respectively. Moreover, activation of MAPKAPK-2 correlated with the appearance of phospho-HSP27 substrate (Fig 3*A*). These data indicate that a single skin exposure to UVB activates the p38 MAPK signaling cascade in the skin of SKH-1 hairless mice. This finding is consistent with studies demonstrating that p38 kinase is involved in responses to UVB in murine and human cells *in vitro* (Bulavin *et al*, 2001).

The p38 MAPK inhibitor SB242235 abolishes MAPKAPK-2 activity and HSP27 phosphorylation To further explore the role of p38 in mediating UVB-induced inflammatory responses, we administered the p38 MAPK inhibitor