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Regulation of MSK-1-mediated NF- κ B Activation Upon UVB Irradiation

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Abstract

Nuclear Factor Kappa-B (NF- κ B) is a transcription factor that controls expression of genes involved in the immune and inflammatory responses as well as being a key component in the onset of cancers. In this study we provided evidence that Mitogen- and stress activated protein kinase (MSK1) is responsible for a non-canonical late-phase activation of NF- κ B upon UVB irradiation. Our data demonstrated that following UVB irradiation, MSK1 is activated via phosphorylation at the 24 h time point coinciding with translocation of NF- κ B into the nucleus. Investigations into the signaling pathways upstream of MSK1 through the use of specific inhibitors for mitogen-activated protein kinase and p38 revealed that both kinases are required for full phosphorylation during the late-phase (24 h), while p38 is paramount for phosphorylation during the early-phase (6 h). Electromobility shift assays (EMSA) showed that inhibition of MSK1 resulted in a marked reduction in NF- κ B binding affinity without altering the nuclear translocation of NF- κ B. Supershift EMSA implicate that the p65, but not p50, isoform of NF- κ B is involved in late-phase activation in response to UVB irradiation. Together, the results of these studies shed light onto a novel pathway of MSK1 mediated late-phase activation of NF- κ B in response to UVB irradiation.

INTRODUCTION

Nuclear factor kappa-B (NF- κ B) is a transcription factor that plays a crucial role in controlling expression of genes involved in the immune and inflammatory responses, proliferation regulation, as well as being a key component in the onset of a larger number of cancers (1–5). Inactive NF- κ B resides in the cytoplasm as a dimer bound to the inhibitor of κ B (I κ B) (6, 7). In the canonical pathway of activation I κ B must first be dissociated/ degraded from the NF- κ B complex to allow the NF- κ B dimer to translocate to the nucleus via the exposure of a nuclear localization signal, thus allowing NF- κ B to bind to DNA and mediate transcription (8–11). However, a previous investigation by our lab revealed a noncanonical pathway that operates independently of I κ B degradation. It was found that in response to UV irradiation, NF- κ B was able to successfully translocate into the nucleus despite high expression levels of I κ B in a cell line incapable of inhibiting global protein synthesis (thus allowing continued synthesis of I κ B post UV irradiation). Furthermore, it was discovered that UV induced late-phase (24 h) NF- κ B activation was dependent on phosphorylation of Ser-276 upon entering the nucleus (12). This transactivation of NF- κ B via phosphorylation was hypothesized to be mediated by the activity of mitogen- and stress-

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activated protein kinase-1 (MSK1) based on a report that linked MSK1 to NF- κ B activation in response to stimulus with tumor necrosis factor alpha (TNF α) (13).

MSK1 is a nuclear kinase that resides downstream of the mitogen-activated protein kinases (MAPK) signaling pathway and is directly activated via phosphorylation by p38 and extracellular signal-regulated kinases (ERK1/2). ERKs operate downstream of tyrosine kinase receptors and G protein-couple receptors while p38 is activated in response to cellular stresses such as osmotic fluctuations, UV irradiation and cytokine signaling (14–16). Upon their activation, both ERK and p38 translocate to the nucleus where they target MSK1 (17–19). MSK1 contains two individual kinase domains that reside independently at the N- and C-terminals of the protein (20). Once p38/ERK have entered the nucleus they dock with MSK1 and phosphorylate the C-terminus. In turn, the C-terminus of MSK1 then autophosphorylates several sites that led to the activation of the N-terminus (21). The N-terminal kinase domain can then target several factors involved in transcription including activating transcription factor 1 (ATF1), cAMP response element-binding protein (CREB) and Histone H3 (22–24). In this study we sought to elucidate the time dependent dynamics of the upstream signaling pathway that is involved in MSK1-mediated activation of NF- κ B in response to UVB irradiation. Based on our results, we proposed a novel and dynamic signaling circuit for regulation of UVB induced MSK1-mediated NF- κ B activation.

MATERIALS AND METHODS

Cell Cultures

JB6 Cl41 mouse epidermal skin cells were grown in MEM medium containing 100 units/ml penicillin/streptomycin (Corning Life Sciences) and 5% FBS (Atlanta Biologicals) at 37°C and 5% CO₂ in a humidified incubator. Stably transfected JB6 Cl41 cells containing a pCMV5-Flag-MSK1-A195/NH₂ terminal kinase dead vector (a generous gift from Dr. Yong Yeon Cho, The Hormel Institute, University of Minnesota) were grown under similar conditions.

Treatments

Cells were pretreated with either 50 μ M PD 98059, 10 μ M SB 202190, 10 μ M H89 or a combination of the aforementioned inhibitors (Sigma-Aldrich) 1 h prior to UVB irradiation. UVB light was generated by two 15 W UVB lamps (UVP Inc.) and measured by a UVB meter (UVP Inc.) to determine intensity. Media from the cells to be treated was removed and the cells exposed to the UVB light to obtain a dose of 50 mJ/cm². After treatment the media was returned and the cells incubated until sample collection.

Western Blot Analysis

Following treatment, cell lysates were collected at 6 and 24 h time points post UVB irradiation. Whole cell lysates were collected using PhosphoSafe lysis buffer (Sigma-Aldrich) according to the manufacturer's recommended protocol. Upon collection, samples were prepped and 20–30 μ g of protein ran on 10% SDS-polyacrylamide gel. The samples were then transferred to a nitrocellulose membrane and blocked with 5% milk in TBS-T for 1–3 hrs (depending on protein of interest) before being incubated overnight (~18 hrs) with

antibodies targeting MSK1, Phospho-T581 MSK1 or β -actin (Santa Cruz Biotechnology). Bands were visualized using the appropriate HRP-conjugated secondary antibodies (Santa Cruz Biotechnology) and exposed to radiosensitive film (HyBlot CL, Denville Scientific).

Electromobility Shift Assay

Cytoplasmic and nuclear lysates were prepared in a step wise fashion using Sucrose Buffer (0.32 M sucrose, 10 mM Tris HCl, 3 mM CaCl_2 , 2 mM MgOAc, 0.1 mM EDTA, 0.5% NP-40, 1 mM DTT) to collect cytoplasmic proteins and a Low Salt Buffer (20 mM HEPES, 1.5 mM MgCl_2 , 20 mM KCl, 0.2 mM EDTA, 25% glycerol, 0.5 mM DTT) and High Salt Buffer (Low Salt Buffer with 800 mM KCl and 1% NP-40) to collect nuclear proteins. NF- κ B consensus sequence (Santa Cruz Biotechnology) was radiolabeled with ^{32}P according to the following procedure: 200 ng oligo was incubated with 150 μCi (50 μmol) [γ - ^{32}P]-ATP (3000 Ci/mmol 10 mCi/ml, Perkin Elmer), 20 units T4 Kinase, 10X Labeling Buffer (New England Biolabs) and dH_2O (to give a total of 50 μL) for 1 h at 37° C. The probe was then purified with a QIAquick Nucleotide Removal Kit (Qiagen) according to the manufacturers protocol. The resultant solution was then diluted 15 fold (200 μL to 3000 μL) in TE buffer. For the binding reaction, 30 μg of nuclear lysates were incubated with 1 μL labeled probe, 2 μg poly-dIdC, 5X Binding Buffer (50 mM Tris HCl pH 8.0, 750 mM KCl, 2.5 mM EDTA, 0.5% Triton-X 100, 62.5% glycerol, 1 mM DTT) and dH_2O (to give 40 μL) for 30 min at room temperature. Samples were then loaded onto a 5% non-denaturing polyacrylamide gel and ran in 0.5X TBE Buffer. Gels were dried and exposed to radiosensitive film at -80° C for 12–48 h to visualize bands. For competition tests and supershift assays, 20 ng unlabeled probe or 1 μg antibodies specific for p50/p65 NF- κ B (respectively) were added to the binding reaction prior to the addition of the labeled probe.

Immunohistochemical staining

Cells were plated into a 12-well plate containing glass slide cover slips to allow for mounting then treated with UVB irradiation and specific inhibitors as previously mentioned. Cells were then fixed with a 3.7% formaldehyde/PBS solution for 15 min, washed in PBS and permeabilized in a solution of PBS containing 0.1% TritonX-100 (Sigma-Aldrich). Following an additional PBS rinse, cells were blocked for 1 h (2 mg/mL BSA in PBS) and incubated overnight (~18 h) at 4° C with primary p65 NF- κ B antibody binding buffer (200 fold antibody [Santa Cruz Biotechnology] dilution in PBS containing 10 mg/ml BSA and 0.3% TritonX-100). Cells were then rinsed with PBS and incubated with FITC-conjugated secondary antibody buffer (400 fold antibody [Santa Cruz Biotechnology] dilution in PBS containing 10 mg/ml BSA and 0.3% TritonX-100) for 1 h at room temperature, rinsed with PBS and mounted on slides using VectaShield mounting medium (Vector Laboratories, Inc.) and visualized on a Nikon Eclipse E600 microscope.

RESULTS

UVB-induces MSK1 phosphorylation via p38 and ERK in a time dependent manner

Activation of MSK1 requires phosphorylation from upstream kinases p38 and/or ERK. These kinases target Thr-581 and Ser-360, which then allow MSK1 to autophosphorylate into its active form (21). In this study, western blot analysis was performed to determine

expression levels of active (Thr-581 phosphorylated) as well as total MSK1. MSK1 was found to be phosphorylated at Thr-581 well above basal levels at both 6 and 24 h following UVB irradiation (Fig. 1A lane 2 vs. 1 and 1B lane 2 vs. 1, respectively). This phosphorylation was present at 2 fold higher levels at 6 h post UVB than at 24 h (Fig. 1C lane 2 vs. 5).

In order to determine the contribution of ERK and p38 upstream pathways in MSK1 phosphorylation, cells were treated with specific inhibitors 1 h prior to irradiation. Treatment with inhibitor PD 98059 for MEK (upstream of ERK) showed a slight reduction in phosphorylation at 6 h post UVB (Fig. 1A lane 4 vs. 2) while treatment with SB 203580, a p38 inhibitor, was able to effectively block phosphorylation at 6 h (Fig. 1A lane 6 vs. 2). These results suggest that p38 is the predominant kinase responsible for MSK1 phosphorylation at the 6 h time point. However, at 24 h post UVB irradiation, treatment with either inhibitor resulted in a return to basal phosphorylation levels (Fig. 1B lanes 4 and 6 vs. 1) demonstrating a requirement for both kinases during the late-phase response. Combined treatment with the inhibitors was also able to prevent phosphorylation of MSK1 at 24 h (Fig. 1C lane 7 vs. 1) but only resulted in a slight reduction at 6 h (Fig. 1C lane 4 vs. 2). Control treatments with either inhibitor, and without UVB, resulted in a small decrease in basal phosphorylation levels at both time points (Fig. 1A/B lanes 3 and 5 vs. 1), whereas combined inhibitor treatment showed a slight increase (Fig. 1C lanes 3 and 6 vs. 1).

Exposure to UVB also resulted in a decrease of total MSK1 expression levels at 6 h with levels remaining comparably reduced at 24 h (Fig. 1A/B lane 2 vs. 1 and Fig. 1C lanes 2 and 5 vs. 1). Interestingly, the reduction of MSK1 phosphorylation via treatment with specific inhibitors resulted in rescued levels of total MSK1 expression (Fig. 1A/B lanes 4 and 6 vs. 2 and Fig. 1C lanes 4 vs. 2 and 7 vs. 5). These findings suggest reduced levels of MSK1 expression following irradiation may rely on a phosphorylation dependent pathway of degradation.

Inhibition of MSK1 decreases late-phase NF- κ B Binding Affinity

In order to assess NF- κ B activation during the early- and late-phases following UVB irradiation, an electromobility shift assay was performed to determine the relative binding affinity of NF- κ B to its consensus sequence. The EMSA revealed a strong DNA binding capacity of NF- κ B at 24 h post UVB irradiation while binding at 6 h increased approximately 23% (not statistically significant) (Fig. 2A/B lanes 2 and 5 vs. 1). In addition to stimulation with UVB, cells were treated with specific inhibitors for MSK (H89), as well as its upstream activators, p38 (SB 203580) and MEK (PD 98059) in order to assess their role in activation of NF- κ B. Inhibition of MSK1 resulted in a significant reduction in NF- κ B binding affinity at 24 h (Fig. 2B lane 7 vs. 5), signifying its importance in transactivation of NF- κ B in response to UVB irradiation. Inhibition of MEK also resulted in a marked reduction in binding affinity at 24 h (Fig. 2A lane 6 vs. 5). However, inhibition of p38 alone or combined with inhibition of MEK resulted in a marked increase in NF- κ B binding affinity at 24 h (Fig. 2A lane 7 vs. 5 and 2B lane 6 vs. 5). Similar results were observed at 6 h where inhibition of p38 alone or combined with inhibition of MEK resulted in an increase in binding affinity (Fig. 2A lane 4 vs. 2 and Fig. 2B lane 3 vs. 2). Inhibition of MSK at 6 h

also showed a slight increase in NF- κ B binding affinity (Fig. 2B lane 4 vs. 2). Control treatments with the inhibitors alone showed no increase in DNA binding capacity (Data not shown).

In order to assess the involvement of p50, p65 or the p50/p65 heterodimer in response to NF- κ B activation following UVB irradiation, an electromobility super-shift assay was performed. A clear band shift upon sample incubation with an antibody specific for p65 was observed at 24 h (Fig. 3 lane 7 vs. 6) and 6 h (over-exposure of same figure not shown). However, incubation with an antibody specific for p50 failed to elicit a shift (Fig. 3 lane 8 vs. 6). A cold-competition test was run to ensure that the binding observed in this, and previous EMSA studies, were specific for the NF- κ B binding sequence. Results confirmed this ascertainment, as bands were lost at 24 h (Fig. 3 lane 9 vs. 6) and 6 h (over-exposure of same figure not shown).

p38 inhibition-induced NF- κ B activation and MEK inhibition-induced NF- κ B inactivation is dependent on MSK1

In order to aid in our studies, we utilized a stable JB6 Cl41 cell line expressing a pCMV5-Flag-MSK1-A195/NH₂ vector for a dominant-negative N-terminal kinase dead MSK1 mutant. This cell line allowed us to analyze the upstream signaling to NF- κ B in a system mostly devoid of functional MSK1. Results from an electromobility shift analysis revealed an induction of NF- κ B DNA binding affinity 24 h post-UVB irradiation (Fig. 4A/B lane 5 vs. 1) while binding remained near basal levels at 6 h (Fig. 4A/B lane 2 vs. 1). In contrast to the wild-type cell line, treatment with a single inhibitor for MEK or p38 did not affect NF- κ B binding affinity (Fig. 4A lanes 6 and 7 vs. 5). These results indicate that p38 inhibition-induced NF- κ B activation and MEK inhibition-induced NF- κ B inactivation (Fig. 2A lanes 7 and 6 vs. 5) observed in wild type cells post irradiation are dependent on proper MSK1 function. However, upon combined treatment an increase in binding affinity was observed at 24 h post-UVB irradiation with a slight decrease occurring at 6 h (Fig. 4B lanes 6 and 3 vs. 5 and 2). Additionally, treatment of this cell line with a MSK inhibitor resulted in a slight increase NF- κ B binding affinity at 6 h and slight reduction at 24 h post irradiation (Fig. 4B lanes 4 and 7 vs. 2 and 5).

MSK1-mediated NF- κ B activation is independent of NF- κ B nuclear translocation

In order to assess the mechanism of MSK1-mediated NF- κ B activation upon UVB irradiation, translocation of NF- κ B was visualized via immunohistochemical staining. Following staining, NF- κ B was observed to translocate to the nucleus during the late-phase in response to UVB irradiation, but no detectable change during the early-phase was observed (Fig. 5). Immunostaining of the cells following treatment with UVB and specific inhibitors did not result in significant changes in translocation in comparison to the UVB-only treated cells (Fig. 5). These findings indicate that changes in NF- κ B binding affinity following treatment with specific inhibitors are not the result modified NF- κ B translocation within the nucleus of the cell.

DISCUSSION

Misregulation of NF- κ B is a hallmark amongst various forms of skin cancer (25, 26). Suppression of UV-induced NF- κ B activation has been suggested as a potential chemopreventive method for photocarcinogenesis (27–30). However the mechanism of NF- κ B activation in response to UV irradiation is not fully understood. In this study we sought to elucidate the pathways involved in MSK1-mediated NF- κ B activation in response to UVB irradiation and to determine the time dependent dynamics through which this activation is achieved. Our data indicated that MSK1 in JB6 cells is strongly phosphorylated at Thr-581, which is a marker for activation, at 6 h post irradiation with lowered, but continued, activation occurring at the 24 h time point (Fig. 1C lanes 2 and 5 vs. 1). However, only the late-phase MSK1 activation coincides with NF- κ B nuclear translocation and activation (Figs. 1, 2 and 5). Inhibition of MSK1 with H89 results in the inhibition of UVB-induced NF- κ B activation (Fig. 2B lane 7 vs. 5) indicating that MSK1 mediates late-phase activation of NF- κ B. In addition to H89, inhibition of MEK with PD 98059 resulted in a decrease in NF- κ B binding affinity at 24 h post UVB irradiation (Fig. 2A lane 6 vs. 5), which correlates to the downstream decrease in MSK1 phosphorylation (Fig. 1B lane 4 vs. 2). Contrarily, inhibition of p38 with SB 203580 alone or combined with the inhibition of MEK, resulted in an increase in NF- κ B DNA binding affinity at both 6 and 24 h post UVB irradiation (Fig. 2A lanes 4 and 7 vs. 2 and 5, Fig. 2B lanes 3 and 6 vs. 2 and 5); suggesting that although p38 inhibition decreases MSK1 phosphorylation at both time points, additional regulatory mechanisms may be at work. In addition, our data indicated that the inhibition of NF- κ B activation is not accompanied with the inhibition of nuclear translocation of NF- κ B (Fig. 2 vs. 5). These results indicate that although MSK1 is highly phosphorylated at 6 h post irradiation, NF- κ B has yet to translocate to nucleus and is not available for transactivation via MSK1. However, once NF- κ B has been compartmentalized to the nucleus, specific inhibition of MSK1 is able to attenuate NF- κ B binding affinity. Additionally, treatment of the cells with specific inhibitors failed to elicit significant changes in NF- κ B translocation. Combined, these observations confirm that MSK1 is responsible for enhancing the binding affinity of NF- κ B via transactivation at 24 h post irradiation.

The analysis of the upstream activators of MSK1 revealed a time-dependent requirement of p38 and ERK. Results indicated that p38 activity during the early-phase (6 h) response to UVB irradiation is a requirement as treatment with specific inhibitor SB 203580 resulted in a decrease in MSK1 phosphorylation to near basal levels (Fig. 1A lane 6 vs. 1). However, treatment with specific inhibitor PD 98059 for MEK (upstream of ERK) was unable to fully attenuate MSK1 phosphorylation (Fig. 1A lane 4 vs. 1). Combined, these results indicate that during the early-phase response signaling through p38 is paramount to MSK1 activation while signaling through ERK plays a lesser, but still required, role. This dynamic shifts during the late-phase response as inhibition of either p38 or MEK is able to decrease MSK1 phosphorylation back to basal levels (Fig. 1B lanes 4 and 6 vs. 1). Combined treatment with both inhibitors during the late-phase results in a prevention of MSK1 phosphorylation, while the early-phase phosphorylation is not completely blocked. Together, these combined observations reveal a time dependent shift in the necessity of p38 and ERK signaling to MSK1 post UVB irradiation. In order to confirm NF- κ B binding levels observed through

inhibition of p38 and MEK were indeed acting through MSK1 we employed a JB6 Cl41 cell line expressing a pCMV5-Flag-MSK1-A195/NH₂ vector for a dominant-negative N-terminal kinase dead MSK1 mutant. Although some endogenous MSK1 is most likely still present (as apparent in a slight decrease in NF- κ B binding affinity following treatment with H89), this model allowed us to analyze NF- κ B activation in a system mostly devoid of functional MSK. In contrast to the wild type cell line, treatment with either PD 98059 or SB 203580 failed to elicit a change in NF- κ B binding affinity (Fig. 4A lanes 6 and 7 vs. 5) at 24 h. These observations confirm that p38 inhibition-induced NF- κ B activation and MEK inhibition-induced NF- κ B inactivation (Fig. 2A lanes 7 and 6 vs. 5) observed in wild type cells post irradiation are dependent on proper MSK1 function. Decreased levels of NF- κ B binding following inhibition of MEK can be explained by the observed decrease in MSK1 phosphorylation (Fig. 1B lane 4 vs. 2), however, increased levels of NF- κ B binding following p38 inhibition presents an interesting scenario as decreased MSK1 phosphorylation is observed along side increased NF- κ B binding affinity. Specific inhibition of p38 could affect the activity of ERK as it has been reported that p38 can negatively regulate the function of MEK and ERK through activation of protein phosphatase 2A (31). However, results from the N-terminal dead MSK1 cell line show a MSK1 dependent desensitization to p38 inhibition-induced NF- κ B activation. As such, future research into the association of MSK1 with p38 will be necessary to elucidate the mechanism behind this activation. Combined treatment with p38 and MEK inhibitors showed an increase in NF- κ B binding at both the early- and late-phases in the N-terminal dead MSK1 cell line (Fig. 4B lane 3 vs. 2 and 6 vs. 5) similar in trend of the wild type cell line. This observation can most likely be contributed to disruption of multiple sections of the complex signaling pathways upstream of NF- κ B.

The combined results from this study reveal a novel and dynamic pathway for the late-phase activation of NF- κ B in response to UVB irradiation. In our proposed model UVB irradiation stimulates the activation of MSK1 via phosphorylation from ERK and p38. During the early-phase response, activation of MSK1 is heavily dependent on the function of p38, as opposed to the late-phase in which p38 and ERK work in a concerted effort to phosphorylate MSK1. Although MSK1 is highly phosphorylated during the early-phase, it is not until UVB has stimulated the translocation of NF- κ B into the nucleus of the cell during the late-phase that MSK1 is able to transactivate NF- κ B into a fully active form (Fig. 6).

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References

1. Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene*. 1999; 18(49):6853–66. [PubMed: 10602461]
2. Dolcet X, Llobet D, Pallares J, Matias-Guiu X. NF-kB in development and progression of human cancer. *Virchows Arch*. 2005; 446(5):475–82. [PubMed: 15856292]
3. Chen G, Hardy K, Bunting K, Daley S, Ma L, Shannon MF. Regulation of the IL-21 gene by the NF-kappaB transcription factor c-Rel. *J Immunol*. 2010; 185(4):2350–9. [PubMed: 20639489]

4. Dev A, Iyer S, Razani B, Cheng G. NF-kappaB and innate immunity. *Curr Top Microbiol Immunol*. 2011; 349:115–43. [PubMed: 20848362]
5. Wang YJ, Lu J, Wu DM, Zheng ZH, Zheng YL, Wang XH, et al. Ursolic acid attenuates lipopolysaccharide-induced cognitive deficits in mouse brain through suppressing p38/NF-kappaB mediated inflammatory pathways. *Neurobiol Learn Mem*. 2011; 96(2):156–65. [PubMed: 21496491]
6. Cervantes CF, Bergqvist S, Kjaergaard M, Kroon G, Sue SC, Dyson HJ, et al. The RelA nuclear localization signal folds upon binding to IkappaBalpha. *J Mol Biol*. 2011; 405(3):754–64. [PubMed: 21094161]
7. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. *Annu Rev Immunol*. 2000; 18:621–63. [PubMed: 10837071]
8. Ganchi PA, Sun SC, Greene WC, Ballard DW. I kappa B/MAD-3 masks the nuclear localization signal of NF-kappa B p65 and requires the transactivation domain to inhibit NF-kappa B p65 DNA binding. *Mol Biol Cell*. 1992; 3(12):1339–52. [PubMed: 1493333]
9. Hacker H, Karin M. Regulation and function of IKK and IKK-related kinases. *Sci STKE*. 2006; 2006(357):re13. [PubMed: 17047224]
10. Hatakeyama S, Kitagawa M, Nakayama K, Shirane M, Matsumoto M, Hattori K, et al. Ubiquitin-dependent degradation of IkappaBalpha is mediated by a ubiquitin ligase Skp1/Cul 1/F-box protein FWD1. *Proc Natl Acad Sci U S A*. 1999; 96(7):3859–63. [PubMed: 10097128]
11. Fagerlund R, Melen K, Cao X, Julkunen I. NF-kappaB p52, RelB and c-Rel are transported into the nucleus via a subset of importin alpha molecules. *Cell Signal*. 2008; 20(8):1442–51. [PubMed: 18462924]
12. Laszlo CF, Wu S. Mechanism of UV-induced IkappaBalpha-independent activation of NF-kappaB. *Photochem Photobiol*. 2008; 84(6):1564–8. [PubMed: 18627520]
13. Vermeulen L, De Wilde G, Van Damme P, Vanden Berghe W, Haegeman G. Transcriptional activation of the NF-kappaB p65 subunit by mitogen- and stress-activated protein kinase-1 (MSK1). *EMBO J*. 2003; 22(6):1313–24. [PubMed: 12628924]
14. Deak M, Clifton AD, Lucocq LM, Alessi DR. Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. *EMBO J*. 1998; 17(15):4426–41. [PubMed: 9687510]
15. Jinlian L, Yingbin Z, Chunbo W. p38 MAPK in regulating cellular responses to ultraviolet radiation. *J Biomed Sci*. 2007; 14(3):303–12. [PubMed: 17334833]
16. Risco A, del Fresno C, Mambol A, Alsina-Beauchamp D, MacKenzie KF, Yang HT, et al. p38gamma and p38delta kinases regulate the Toll-like receptor 4 (TLR4)-induced cytokine production by controlling ERK1/2 protein kinase pathway activation. *Proc Natl Acad Sci U S A*. 2012; 109(28):11200–5. [PubMed: 22733747]
17. Gong X, Ming X, Deng P, Jiang Y. Mechanisms regulating the nuclear translocation of p38 MAP kinase. *J Cell Biochem*. 2010; 110(6):1420–9. [PubMed: 20506250]
18. Lidke DS, Huang F, Post JN, Rieger B, Wilsbacher J, Thomas JL, et al. ERK nuclear translocation is dimerization-independent but controlled by the rate of phosphorylation. *J Biol Chem*. 2010; 285(5):3092–102. [PubMed: 19920141]
19. Roux PP, Blenis J. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiol Mol Biol Rev*. 2004; 68(2):320–44. [PubMed: 15187187]
20. Jones SW, Erikson E, Blenis J, Maller JL, Erikson RL. A Xenopus ribosomal protein S6 kinase has two apparent kinase domains that are each similar to distinct protein kinases. *Proc Natl Acad Sci U S A*. 1988; 85(10):3377–81. [PubMed: 3368449]
21. McCoy CE, Campbell DG, Deak M, Bloomberg GB, Arthur JS. MSK1 activity is controlled by multiple phosphorylation sites. *Biochem J*. 2005; 387(Pt 2):507–17. [PubMed: 15568999]
22. Soloaga A, Thomson S, Wiggin GR, Rampersaud N, Dyson MH, Hazzalin CA, et al. MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14. *EMBO J*. 2003; 22(11):2788–97. [PubMed: 12773393]
23. Kaiser M, Wiggin GR, Lightfoot K, Arthur JS, Macdonald A. MSK regulate TCR-induced CREB phosphorylation but not immediate early gene transcription. *Eur J Immunol*. 2007; 37(9):2583–95. [PubMed: 17668895]

24. Darragh J, Soloaga A, Beardmore VA, Wingate AD, Wiggin GR, Pegg M, et al. MSKs are required for the transcription of the nuclear orphan receptors Nur77, Nurr1 and Nor1 downstream of MAPK signalling. *Biochem J.* 2005; 390(Pt 3):749–59. [PubMed: 15910281]
25. Yan M, Xu Q, Zhang P, Zhou XJ, Zhang ZY, Chen WT. Correlation of NF-kappaB signal pathway with tumor metastasis of human head and neck squamous cell carcinoma. *BMC Cancer.* 2010; 10:437. [PubMed: 20716363]
26. McNulty SE, del Rosario R, Cen D, Meyskens FL Jr, Yang S. Comparative expression of NFkappaB proteins in melanocytes of normal skin vs. benign intradermal naevus and human metastatic melanoma biopsies. *Pigment Cell Res.* 2004; 17(2):173–80. [PubMed: 15016307]
27. Adhami VM, Afaq F, Ahmad N. Suppression of ultraviolet B exposure-mediated activation of NF-kappaB in normal human keratinocytes by resveratrol. *Neoplasia.* 2003; 5(1):74–82. [PubMed: 12659672]
28. Afaq F, Adhami VM, Ahmad N, Mukhtar H. Inhibition of ultraviolet B-mediated activation of nuclear factor kappaB in normal human epidermal keratinocytes by green tea Constituent (–)-epigallocatechin-3-gallate. *Oncogene.* 2003; 22(7):1035–44. [PubMed: 12592390]
29. Afaq F, Ahmad N, Mukhtar H. Suppression of UVB-induced phosphorylation of mitogen-activated protein kinases and nuclear factor kappa B by green tea polyphenol in SKH-1 hairless mice. *Oncogene.* 2003; 22(58):9254–64. [PubMed: 14681684]
30. Reagan-Shaw S, Mukhtar H, Ahmad N. Resveratrol imparts photoprotection of normal cells and enhances the efficacy of radiation therapy in cancer cells. *Photochem Photobiol.* 2008; 84(2):415–21. [PubMed: 18221451]
31. Li SP, Junttila MR, Han J, Kahari VM, Westermarck J. p38 Mitogen-activated protein kinase pathway suppresses cell survival by inducing dephosphorylation of mitogen-activated protein/extracellular signal-regulated kinase kinase1,2. *Cancer Res.* 2003; 63(13):3473–7. [PubMed: 12839928]

Figure 1.

Expression levels of MSK and phosphorylated MSK following treatment with UV and specific inhibitors. A) JB6 CL41 cells were pretreated for 1 h with or without the inhibitors PD98050 (50 μ M) or SB203580 (10 μ M) and irradiated with 50 mJ/cm² UVB. Whole cells lysates were collected after 6 h and analyzed via western blot. B) Identical to A with the exception of lysates being collected at 24 h. C) Cells were pretreated with both PD98050 (50 μ M) and SB203580 (10 μ M) and lysates collected at 6 and 24 h post irradiation.

Figure 2.

Electromobility shift assay for NF- κ B binding activity. A) JB6 CL41 cells were pretreated for 1 h with the inhibitors PD98059 (50 μ M) or SB203580 (10 μ M) and irradiated with 50 mJ/cm² UVB. Nuclear lysates were collected after 6 or 24 h and incubated with radiolabeled NF- κ B consensus sequence. Samples were then ran on a non-denaturing gel and visualized with exposure to radiosensitive film. B) Identical to A with the exception of samples being pretreated with a combination of PD98059 (50 μ M) and SB203580 (10 μ M) or H89 (10 μ M).

Figure 3.

EMSA Supershift assay for NF- κ B binding activity. JB6 CL41 cells were irradiated with 50 mJ/cm² UVB. Nuclear lysates were collected after 6 or 24 h. Samples for supershift analysis were incubated with p50 or p65 antibody before being incubated with radiolabeled NF- κ B consensus sequence. A competition test was run on two samples by adding 20 ng unlabeled probe to the incubation mixture. Samples were then ran on a non-denaturing gel and visualized with exposure to radiosensitive film

Figure 4.

Electromobility shift assay for NF- κ B binding activity. A) Stably transfected JB6 CL41 cells containing a pCMV5-Flag-MSK1-A195/NH₂ vector for a dominant-negative N-terminal kinase dead MSK1 mutant were pretreated for 1 h with the inhibitors PD98059 (50 μ M) or SB203580 (10 μ M) and irradiated with 50 mJ/cm² UVB. Nuclear lysates were collected after 6 or 24 h and incubated with radiolabeled NF- κ B consensus sequence. Samples were then ran on a non-denaturing gel and visualized with exposure to radiosensitive film. B) Identical to A with the exception of samples being pretreated with a combination of PD98059 (50 μ M) and SB203580 (10 μ M) or H89 (10 μ M).

Figure 5.

Immunohistochemical staining of NF- κ B translocation. Cells were grown on glass cover slips and pretreated for 1 h with the inhibitors PD98059 (50 μ M), SB203580 (10 μ M), H89 (10 μ M) or a combination and irradiated with 50 mJ/cm² UVB then fixed after 6 and 24 h. Fixed cells were incubated with primary antibody for p65 then FITC-conjugated secondary antibody and visualized via fluorescence microscopy.

Figure 6.
Proposed model of MSK-1-mediated NF-κB activation following UVB irradiation.