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Activation of p38 MAP Kinase and JNK Pathways by UVA Irradiation

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Abstract

There are more than two million new cases of non-melanoma skin cancers (NMSCs) diagnosed each year in the United States. The clear etiological factor is chronic exposure to solar radiation from the sun. The wavelengths of solar light that reach the earth's surface include UVB (280–320nm) which accounts for 1–10% and UVA (320–400nm) which accounts for 90–99% of the radiation. While most of the published research has focused on the effects of UVB, little is known concerning UVA-mediated signal transduction pathways and their role in skin tumor promotion and progression giving rise to squamous cell carcinomas (SCCs). Here we have focused on UVA-mediated activation of p38 MAP kinase and c-Jun N-terminal kinase (JNK) and their roles in activator protein-1 (AP-1) mediated transcription, cyclooxygenase-2 (COX-2) and Bcl-XL expression. Since p38 MAP kinase and JNK play major roles in the expression of UVA-induced AP-1, COX-2 and Bcl-XL, pharmacological inhibitors of these kinases may be useful in the chemoprevention of SCC skin cancer.

1. Introduction

According to American Cancer Society, skin cancer is the most common cancer, accounting for about half of all cancers in the United States. Skin cancer is classified as melanoma (4% of total cases) and non-melanoma skin cancers (NMSCs, 96% of total cases). NMSCs is further divided into basal-cell carcinomas (BCCs) and squamous-cell carcinomas (SCCs), accounting for 80% and 16% of skin cancers respectively ^{1, 2}. BCC and SCC are both derived from the basal layer of the epidermis of the skin, but whereas BCCs are slow growing and rarely metastasize, SCCs can be highly invasive and can metastasize ¹.

It is estimated that there are more than two million NMSC cases in the US each year ^{3, 4}. Although typically not lethal, NMSCs are responsible for tissue deformity and substantial morbidity, particularly in high-risk populations, such as people with light skin or with organ transplantation. The direct cost for treatment of NMSCs in the United States was estimated to be \$1.5 billion in 2004 ⁵. Besides, actinic keratosis (AK) is also considered as an early stage of invasive SCC and carcinoma *in situ*. If AK is included, the direct cost would increase to more than \$2.3 billion ⁵. The incidence of NMSCs continues to rise in recent years. This trend is in sharp contrast to most other malignancies which in general are declining ^{3, 4, 6}. A plausible explanation for this trend is that exposure to ultraviolet

irradiation (UVR) from sunlight has increased, due to the gradual depletion of the ozone layer in the past decades. Thus, it is critical to better understand the mechanisms and development of NMSCs for better prevention and treatment.

The UV irradiation of sunlight is subdivided based on wavelength: UVA (320–400 nm), UVB (280–320 nm) and UVC (200–280 nm). As UVC is shielded by the ozone layer, only UVA (90–99%) and UVB (1–10%) wavelengths can reach the earth's surface. Although UVA has less energy than UVB, it constitutes the vast majority of UV irradiation from sunlight and penetrates deeper in human skin than UVB ⁷.

Mounting evidence has clearly shown that UVA irradiation causes DNA lesions through both direct and indirect mechanisms. It is believed that the primary genotoxic effect of UVA results from generation of reactive oxygen species (ROS), including superoxide radical, hydrogen peroxide and hydroxyl radical, with singlet oxygen as the major ROS species. The ROS in turn, causes oxidative damage of macromolecules including DNA and cellular structures, e.g. gap junctional intercellular communication (GJIC) in normal human keratinocytes ⁸. However, a study published by Mouret et al challenged the above notion ⁹. They found that in UVA exposed human skin, the predominant DNA lesion is cyclobutane pyrimidine dimers (CPDs), which are products of direct photosensitization. Overall, UVA is able to cause genomic instability, i.e. mutations of oncogenes and tumor suppressors. For example, p53 is the most extensively studied example for UVA induced gene mutation. Huang et al. demonstrated that the predominant mutation induced by UVA is A:T>C:G transversions in p53 gene in EHS (engineered human skin) ¹⁰. p53 loss-of-function/ mutations have been associated with 60% of cancers. Likewise, another tumor suppressor, PTEN (phosphatase and tensin homologue deleted on chromosome 10) was also found to be downregulated in HaCaT cells treated with chronic UVA exposure ¹¹ and in heterozygous PTEN knockout mice ¹². The carcinogenicity of UVA has been demonstrated in cultured cells which acquired resistance to apoptosis and became transformed upon chronic exposure to low dose of UVA ¹¹. Importantly, various mouse models have clearly demonstrated that UVA alone can induce papillomas and SCC *in vivo* ^{12–14}. These results highlighted the notion that UVA is also a complete carcinogen as is UVB.

2. Signaling Pathways Activated by UVA in Human Kertinocytes

2.1 Activation of p38 MAP Kinase

2.1.1 Expression of p38 in Skin—Key events in UV mediated skin carcinogenesis are related to cell survival and/or apoptosis, critical steps for cells bearing mutations to survive and later on spread the mutation through clonal expansion ^{15–17}. Our laboratory has been focusing on UVA mediated cell survival as well as apoptosis. In dissecting the mechanisms of UVA-mediated skin carcinogenesis, our laboratory has identified that activation of MAP kinase pathways, i.e. p38 MAP kinase and JNK (c-Jun N-terminal kinase) pathway, as key events in UVA responses. These two pathways are known to be activated by cellular stresses, through typical modules consisting of three tiers of kinases: MAPK kinase kinases (MKKKs), MAPK kinases (MKKs) and MAPKs ^{18, 19}.

p38 MAP kinase is activated by the upstream dual specific MKKs, primarily MKK3 and MKK6, which phosphorylate a conserved Thr–X–Tyr motif in the activation loop (T180 and Y182 in p38 α)^{18, 19}. Upon activation, p38 migrates into the nucleus and phosphorylates its downstream targets including, MAPKAP2 (MK2), MNK1 and 2, ATF1/2/6, Elk-1, GADD153, p53, and MSK1/2, which are involved in stress response and cell survival¹⁹.

There are four p38 isoforms of p38 identified up to date: p38 α (MAPK14), p38 β (MAPK11), p38 γ (MAPK13) and p38 δ (MAPK12), with p38 α is the most dominant isoform (commonly referred to as p38). Although these isoforms are homologous, they differ in several aspects: tissue specific expression, substrates, sensitivity to pyridinyl imidazole inhibitors (e.g. SB203580) and functions. For example, the expression of p38 α and p38 β isoforms is ubiquitous while p38 δ isoform is also expressed in many tissues. p38 γ , however, is mainly expressed in skeletal muscles^{19, 20}.

In skin, only the p38 α , p38 β and p38 δ isoforms are detected while p38 γ is absent²¹. It has been concluded that p38 α and p38 β are important for stress response and inflammation in skin and p38 δ plays a critical role in the differentiation of keratinocytes²².

2.1.2 ROS Activates p38 MAP Kinase

2.1.2.1 Activation of p38 by ASK1 Kinase: Considerable published data demonstrated that ROS production leads to activation of p38 MAP kinase^{8, 23-26}. There is evidence indicating that ROS induces p38 MAP kinase activation through ASK1 (apoptosis signal regulated kinase-1), a MAPK kinase kinase (MKKK)²⁶. This protein kinase is activated by oligomerization and phosphorylation. In untreated cells, thiol-disulphide oxidoreductase thioredoxin-1 (Trx1) covalently binds to ASK1 and inhibits its activity. However, upon H₂O₂ treatment, ASK1 is rapidly activated through dissociation from Trx1 and multimerization by forming interchain disulfide bonds²⁷.

2.1.2.2 Activation of p38 through Inhibition of Phosphatases: Activity of MAP kinases depends upon the balance between upstream kinases and phosphatases²⁰. For example, MEF cells lack DUSP1/MKP1 showed sustained activation of p38 and JNK upon 30 J/m² UVC irradiation²⁸. There are ten MKPs (mitogen-activated protein kinase phosphatases) that have been shown to counteract MAPK activities by dephosphorylating their phosphothreonine and phosphotyrosine residues within the Thr-X-Tyr activation motif at the same time²⁹. For this reason, these MKPs are classified as DUSPs (dual-specificity phosphatases) that belong to the PTP (protein tyrosine phosphatase) family^{29, 30}. Each of these MKPs has relative specificity for its substrates. For instance, MKP1 (DUSP1) has a substrate preference as follows: JNK > p38 >> ERK1/2²⁹. MKPs also have different subcellular distribution, e.g. MKP1 (DUSP1) and MKP2 (DUSP4) are primarily nuclear while MKP3 (DUSP6) is cytoplasmic³⁰.

Compelling evidence has shown that PTPs are subject to inactivation by ROS^{28,31-33}. PTP1B is well characterized for this mechanism. It turned out that the cysteine at the catalytic center is nucleophilic and easily oxidized to form cysteine sulfenic acid intermediates, (Cys-SOH) and sulphenyl-amide, leading to significant change of

conformation and loss of substrate binding capacity^{31, 33}. The activity can be restored by reducing agents or scavenging ROS by Trx-dependent reductases³².

Since the PTP family members are all cysteine-based³¹, ROS may inactivate the whole family through a similar mechanism. Indeed, inhibition of MKP1, MKP3, MKP5 and MKP7 was reported in mouse fibroblast cells treated with H₂O₂³². Gross et al showed that UVC (2 kJ/m²) caused a 7-fold decrease in PTP activity in TRMP cells³⁴. Similarly, Xu et al found that 50 mJ/cm² of UVC causes significant oxidation of RPTP- κ (receptor-type protein tyrosine phosphatase κ), leading to a 60% reduction of its activity in primary human keratinocytes. Moreover, this inhibition can be reversed by the reducing agent, DTT³⁵.

2.1.3 Activation of p38 by DNA Damage Pathway—As mentioned earlier, UVA causes DNA damage both directly and indirectly. In recent years, it is also found that p38 MAP kinase is implicated in DNA damage response caused by UV or DNA damaging drugs, e.g. cisplatin and doxorubicin³⁶⁻³⁸. In these reports, DNA lesions trigger activation ATM (Ataxia-telangiectasia mutated) and ATR (ataxia-telangiectasia) kinases which inactivate Cdc25 to halt the cell cycle progress and to initiate DNA damage repair mechanisms. ATM activates Chk2 and ATR activates Chk1 through phosphorylation. Chk1 and Chk2 in turn halt the cell cycle progress through inactivating Cdc25 phosphatase family^{38, 39}. Interestingly, p38MAPK/MK2 activation in response to UVC was shown to be independent of ATM and ATR, suggesting that UV likely causes cellular lesions other than DNA damage that result in p38MAPK activation^{37, 38}. The role that p38 MAP kinase plays in DNA damage response is dependent on MAPKAP kinase -2 (MK2), since knockdown MK2 by siRNA eliminated the checkpoint function conferred by p38³⁶. MK2 knockdown also decreased cell survival rate when the cells were challenged with low to moderate doses of UVC (5-50J/m²)³⁶, consistent with the anti-apoptotic role played by p38. Moreover, in the absence of p53, cells depend on p38MAPK/MK2 for cell-cycle arrest and survival after DNA damage. In p53^{-/-} MEF cells, MK2 knockdown by shRNA led to checkpoint defect and mitotic catastrophe³⁷. This finding is important for skin carcinogenesis since loss of p53 is an early event associated with tumor initiation.

2.2 MK2 Is a Critical Player Downstream of p38 in Skin

Recently, MK2 has been clearly demonstrated to play a critical role in skin carcinogenesis in mice treated with DMBA/TPA⁴⁰. The onset of papillomas was 8 weeks after the start of TPA treatment in the wild-type C57BL/6 mice, whereas papillomas were not found in the MK2 K/O mice until 12 weeks. At week 18, 93% of the wild type mice developed papillomas with an average of 5.4 per mouse. In contrast, only 7% of the K/O mice developed one papilloma, indicating that MK2 is required for the development of skin tumor⁴⁰.

We and others have demonstrated that UVA irradiation activates p38 MAP kinase in multiple cell types treated with different UVA dose levels. It has been found that p38 MAP kinase is activated in JB6 cells (mouse epidermal) with UVA at 160 kJ/m²⁴¹. Similarly, activation of p38 reaches maximum within 15-30 minutes in human skin fibroblasts irradiated with 300 kJ/m² UVA⁴². In immortalized human keratinocytes (HaCaT), our

laboratory also observed that p38 is activated within 5 minutes with the peak between 30 – 45 minutes in response to 250 kJ/m² UVA^{43, 44}. In synergizing with arsenite, dose levels as low as 10 kJ/m² UVA can boost p38 activation dramatically in HaCaT cells⁴⁵.

2.3 Anti-apoptotic Role of p38 Activation

Activated p38 MAP kinase has a broad range of substrates that are implicated in multiple biological processes including protein and mRNA degradation, cytoskeleton organization, endocytosis, cell migration^{19, 20}. It is also implicated in apoptosis with either pro- or anti-apoptotic effect. For example, UVB promotes apoptosis in human keratinocytes by inducing Bax translocation to the mitochondria and cytochrome c release. However, this apoptotic response can be blocked by inhibition of p38 MAPK with either dominant negative p38 or SB203580 in HaCaT cells⁴⁶. Furthermore, p38 is also critical to induce Noxa, a BH-3 only pro-apoptotic molecule which is indispensable for UVB induced apoptosis in NHK cells⁴⁷. Consistently, inhibition of p38 with SB202190 significantly reduces sunburn cells and subcorneal pustules in the mouse skin with acute UVB irradiation⁴⁸. These data indicate that p38 plays a pro-apoptotic role in keratinocytes.

Our laboratory demonstrated that p38 plays an anti-apoptotic function in HaCaT cells, because inhibition of p38 with 2 μ M of SB202190 leads to extensive apoptosis in UVA irradiated cells⁴⁹. These cells showed typical apoptotic features, e.g. condensed chromatin, nuclear fragmentation and apoptotic body formation, release of cytochrome c, and cleavages of caspases⁴⁹. Possible explanations for these controversial data may include differential responses to UVA and UVB, and dependence upon p53 since HaCaT cells bear a nonfunctional version of p53^{7, 16}.

2.3.1 p38 Activation Increases Bcl-XL Expression through Stabilizing mRNA—

Our laboratory set out to unravel the mechanism(s) by which p38 enhances the survival of HaCaT irradiated with UVA. Consistent with the data published by others, we found that two cancer related genes, COX-2 and Bcl-XL, were upregulated in UVA irradiated HaCaT cells^{44, 50, 51, 49, 52}. Notably, expression of both genes is dependent upon activation of p38, since inhibition of p38 with SB202190 abolishes their induction in UVA irradiated HaCaT cells^{44, 49}.

We further explored the mechanism by which UVA upregulates Bcl-XL gene expression through p38 MAP kinase. It turns out that p38, upon activation, stabilizes Bcl-XL mRNA^{49, 52}. Bcl-XL mRNA contains a relatively long 3'-UTR, which possesses three canonical ARE (AU-rich) elements. The ARE elements can recruit RNA binding proteins that will either stabilize or destabilize mRNA molecules depending upon cellular context⁵³. We also demonstrated that UVA irradiation increases the reporter activity in cells transiently transfected with the pRL-TK harboring Bcl-XL 3'-UTR downstream of Renilla luciferase⁴⁹. The induction of the reporter activity was abolished when the cells were cotransfected with a dominant-negative p38 MAP kinase. Our findings strongly support the notion that UVA stabilization of Bcl-XL mRNA depends upon the 3'-UTR and is regulated by p38 MAP kinase⁴⁹.

Published evidence has shown that mRNA 3'-UTRs confer stability through their interactions with different RNA binding proteins and microRNAs^{54, 55}. Therefore, we tried to identify possible RNA binding proteins that may stabilize Bcl-XL mRNA in UVA irradiated HaCaT cells with a proteomic approach. One such protein identified was nucleolin, a ubiquitously expressed protein participating in numerous biological functions, e.g. ribosomal biogenesis, cell cycle regulation and apoptosis⁵⁶. Nucleolin has been implicated in stabilizing multiple mRNAs including p53, IL-2, GADD45 and Bcl-2⁵⁷⁻⁵⁹. We demonstrated that nucleolin specifically binds to the Bcl-XL 3'-UTR and stabilizes Bcl-XL mRNA⁵². Moreover, nucleolin interacts with PABP (poly(A) binding protein) through its RGG (Arginine-Glycine-Glycine) motif. Although the mechanism of how nucleolin stabilizes Bcl-XL mRNA is not clear, it is possible that p38 directly phosphorylates nucleolin⁶⁰, changing its subcellular localization or binding properties. Consequently, nucleolin may stabilize Bcl-XL mRNA through its interaction with PABP to protect the poly(A) tail and to facilitate the mRNA molecule to form a closed-loop structure⁵².

2.3.2 Activation of p38 Upregulates Cox-2—COX-2 is another cancer-related gene upregulated by p38 MAP kinase. We found that p38 also increases COX-2 mRNA abundance through a similar stabilization mechanism discussed above⁴⁴. Several lines of evidence suggest that p38 stabilizes COX-2 mRNA in a 3'-UTR dependent manner upon UVA irradiation. p38 MAP kinase has been shown to play a role stabilizing various mRNA molecules through regulating RNA binding proteins and altering their binding to 3'-UTRs in other cell types⁶¹. COX-2 mRNA possess a very long 3'-UTR with multiple ARE elements which confer a stabilization effect in a p38 dependent manner⁶². Consistently, we found that inhibition of p38 by SB202190 also reduces the activity of COX-2 3'-UTR reporters in UVA treated HaCaT cells⁴⁴.

It is possible that mRNA stabilization is dependent upon activation of MAPKAP kinase-2 (MK2)⁶², which confers p38 biological functions and plays a critical role in regulating stability of numerous mRNAs⁶³. Indeed, phosphorylation of MK2 was observed in UVA irradiated HaCaT cells in our previous studies^{43, 44}. Inhibition of p38 by either dominant negative p38 or SB202190 completely blocked MK2 phosphorylation in UVA treated HaCaT cells^{43, 44}. Alternatively, COX-2 mRNA may be stabilized in UVA irradiated cells by binding of HuR, a conserved protein that stabilizes a number of mRNAs⁶⁴. We and others found that COX-2 mRNA was stabilized through HuR binding to the 3'-UTR using UVB irradiated HaCaT cells^{65, 66}. HuR is a nucleo-cytoplasm shuttling protein primarily localized in the nucleus. Upon phosphorylation by p38 at T118, it translocates into the cytoplasm and stabilizes its mRNA targets^{67, 68}. Since UVA irradiation activates p38, it is likely that HuR cytoplasmic localization is also enhanced in UVA irradiated HaCaT cells.

2.4 p38 MAP Kinase and Skin Carcinogenesis

The implication of p38 MAP kinase in skin carcinogenesis has been supported by multiple animal and clinical studies. For example, since p38 α knockout mice are nonviable due to placenta defects and abnormal vascular development^{69, 70}, our laboratory constructed skin-specific transgenic mice in SKH1 background expressing dominant negative p38 α to overcome this problem⁷¹. We found that tumorigenesis was significantly inhibited in these

mice ⁷¹. Importantly, aberrant activation of p38 α has been regarded as a biomarker for skin carcinogenesis ^{72, 73}.

In contrast to p38 α , knockout mice of p38 β ⁷⁴, p38 γ ⁷⁵ and p38 δ ⁷⁶ isoforms are viable and normal. Currently, there is no report regarding skin carcinogenesis in p38 β KO mice. While displaying normal skin phenotype, the p38 δ knockout mice in C57BL/6 background had lower tumor incidence, multiplicity, and size in comparison with the wild type in a typical TPA/DMBA two-stage carcinogenesis assay ⁷⁶, indicating that p38 δ is indispensable for skin carcinogenesis. Consistently, p38 δ activity is increased in human primary cutaneous SCCs ⁷⁷ and HNSCC tumors ⁷⁸.

2.5 Activation of JNK

JNK is also activated in response to various stress signals including ultraviolet irradiation ⁷⁹. The JNK family is comprised of three members JNK1-3, encoded by MAPK8-10 genes, respectively ²⁰. JNK1 and 2 are ubiquitously expressed whereas JNK3 is primarily expressed in brain ⁷⁹. The upstream kinases for JNKs are mainly MKK4 and MKK7⁸⁰. The JNK family plays a critical role in proliferation, survival and differentiation ⁷⁹. However, opposite results were observed dependent on the nature of stimuli, cellular context and duration of extracellular signals ^{20, 79}.

In a previous study, we found that 250 kJ/m² UVA irradiation induces JNK phosphorylation in 5 minutes and reaches maximum in 30 minutes in HaCaT cells, a similar pattern as p38 activation ⁸¹. Inhibition of JNK with SP600125 leads to a decrease of c-Jun phosphorylation in a dose-dependent manner in UVA irradiated HaCaT cells ⁸¹. At 125 nM, c-Jun phosphorylation was significantly reduced. Moreover, phosphorylation of c-Jun induced by UVA was only inhibited by JNK specific inhibitor SP600125, but not p38 inhibitor SB202190 ⁸¹, indicating that activation of JNK is not dependent on p38 ⁸¹. The importance of JNK in skin carcinogenesis was later verified by Dong's group who showed that JNK2 is required for UVA-mediated skin carcinogenesis since knockout of JNK2 in SKH-1 hairless mice developed significantly fewer papillomas than the wild type control after DMBA treatment and 18 weeks of UVA exposure ⁸². Interesting, knockout of JNK1 enhances formation of papillomas ⁸², indicating that JNK1 and JNK2 function distinctly in the cell as illustrated in an early study ⁸³.

2.6 Activation of AP-1

The JNK family has been shown to phosphorylate and upregulate the transcription factor AP-1 (activator protein 1) ⁸⁴, which functions as a dimer and recognizes TPA-responsive elements (TREs consensus sequence 5'-TGAG/CTCA-3') ⁸⁵. AP-1 is made up of structurally and functionally related protein super families, i.e. Jun protein family (c-Jun, JunB and JunD), Fos protein family (c-Fos, FosB, Fra-1 and Fra-2), ATF (ATFa, ATF-2 and ATF-3), MAF (musculoaponeurotic fibrosarcoma) and JDP (JDP-1 and JDP-2) families ^{84, 86}. Depending upon the cellular context, these family members can interact with each other through their Leucine-zipper domains and form various homodimers or heterodimers to regulate their target genes, promoting cell survival, proliferation and therefore malignant transformation ^{1, 86}.

As a primary target of JNK, c-Jun is upregulated in both its abundance and phosphorylation in UVA irradiated HaCaT cells^{81, 87}. Specifically, c-Jun abundance and phosphorylation starts to rise at 15 minutes and reaches maximum at 4 hours after treatment of 250 kJ/m² UVA. Similarly, we found that expression of other AP-1 components is also elevated in UVA irradiated HaCaT cells, e.g. c-fos, Fra-1 and Fra-2. Subsequently, EMSA experiments showed that binding of the AP-1 probe with the lysates extracted from UVA irradiated HaCaT cells is also enhanced⁸⁷. To examine if increase of c-Jun abundance and phosphorylation leads to AP-1 activation, we used the HCL14 cell line in which stably expresses luciferase reporter driven by AP-1 binding site⁸⁸. We found that UVA increases AP-1 activity in HCL14 cells in both dose and time-dependent manner, with a peak activity at 250 kJ/m² and at 4 hours, the same pattern as c-Jun protein abundance⁸⁷. We further demonstrated that JNK activation is required for cell survival in UVA irradiated HaCaT cells, since inhibition of UVA-induced JNK activity with 125 nM SP600125 in both the immortalized keratinocyte cell line HaCaT and primary human keratinocytes resulted in the cleavage of caspase 8, 9, 3 and PARP. As a result, these cells showed massive apoptotic responses as determined by morphology and flow cytometry⁴³. Our data showed that p38 MAP kinase was also required for AP-1 activity in UVA response⁸¹, since inhibition of p38 by SB202190 decreased UVA induced AP-1 activity dramatically, suggesting that both pathways are required for activation of AP-1⁸¹.

Carcinogenesis is divided into three independent stages: initiation, promotion, and progression with tumor promotion/progress as the rate-limiting steps¹. It has also been well established that AP-1 plays a causative role in skin carcinogenesis, specifically in tumor promotion and/or progression steps both *in vitro* and *in vivo*⁸⁹⁻⁹². As a result, inhibition of AP-1 by TAM67 (dominant negative version of c-Jun with a deletion of its transactivation domain) blocked skin carcinogenesis induced by the complete carcinogen, UVB light⁹³. However, at this time we do not have similar direct evidence that AP-1 plays a functional role in UVA induced skin carcinogenesis.

3. Summary

UVA is the predominant irradiation from sunlight and causes DNA lesions and photodamage of other macromolecules. It is able to penetrate skin deeper into the basal layer of the skin where actively dividing keratinocytes reside. As a result, UVA induced DNA lesions predominately accumulate in these dividing cells which are potentially initiated cells for skin tumors¹⁰. The causative effect of UVA in skin carcinogenesis has been well established in numerous studies. For example, chronic exposure of low dose of UVA causes malignant transformation of HaCaT cells in culture¹¹ and induces skin carcinogenesis in different mouse models as well as human skin^{13, 14, 94, 95}.

To summarize, our laboratory has delineated p38 MAP kinase and JNK pathways in the UVA response in human keratinocytes (Fig 1). UVA irradiation activates these pathways leading to resistance to apoptosis. Using dominant negative p38 α transgenic mice, we showed that UVB induced skin carcinogenesis was significantly reduced in comparison to the wild type SKH-1 hairless mice⁷¹. This result suggests the potential of p38 as a chemoprevention target in UVB and perhaps also UVA induced skin carcinogenesis.

Although no data are currently available, we anticipate that pharmacologic inhibition of p38 and/or JNK would be able to inhibit UVA induced non-melanoma skin tumors (i.e. SCCs). Consistently, knockout of MK2, downstream target of p38, also significantly promotes resistance to skin carcinogenesis induced by DMBA/TPA in mice ⁴⁰. These data provided the rationale for targeting p38MAP kinase pathway for chemoprevention of NMSC.

Studies on UVA induced biological effects are important for at risk population(s), i.e. people with light skin and frequent exposure to sunlight, or those frequently using tanning booths (primarily UVA). In recent years, these studies have become increasingly important for organ transplantation patients who take thiopurine derived drugs, such as azathiopurine. The incidence of skin cancer, primarily SCC, has increased (50-200 fold) in these patients ⁹⁶. It turned out that the product of thiopurine, 6-thioguanine (6-TG) nucleotides, incorporates into DNA and absorbs UVA primarily at 340nm, yielding singlet oxygen (¹O₂) in cultured human cells ⁹⁷. Recently, it has been shown that UVA covalently crosslinks 6-TG in DNA and nuclear proteins, e.g. DNA repair and replication factors, blocking DNA damage replication repair mechanisms ⁹⁸.

UVA can also be an important environmental hazard for some regions with arsenite contamination of drinking water, since UVA and arsenite are synergistic in inducing skin cancers in SKH1 hairless mice ^{99, 100}. These regions include several US southwestern states (California, Nevada and Utah) and some developing countries, such as India, Bangladesh, Mexico, Chile and China ¹⁰¹. Given the fact that approximately 40 – 100 million people living in these regions are still consuming arsenite contaminated water¹⁰¹, further study is warranted to better understand UVA responses and its interaction with arsenite.

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Fig 1. Scheme of UVA signaling in human keratinocytes

UVA from sunlight can produce DNA damage through direct photodamage or generation of reactive oxygen species (ROS). Both DNA lesions and ROS subsequent contribute to activation of p38 MAP kinase or JNK through the MAP kinase module (boxed and shaded). Activated p38 MAP kinase phosphorylates its substrates, including MK2, which is critical for stabilization of numerous mRNA molecules, e.g. COX-2 and Bcl-XL. Meanwhile, activation of JNK promotes AP-1 activity. As a result, both p38 and JNK activation render cells more resistant to apoptosis. This critical step may lead to accumulation DNA damage and consequently, carcinogenesis. Solid arrows indicate direct interaction; Dashed arrows denote multiple steps.