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Molecular Signaling Cascades Involved in Non-melanoma Skin Carcinogenesis

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Abstract

Non-melanoma skin cancer (NMSC) is the most common cancer world-wide and the incidence continues to rise, in part due to increasing numbers in high-risk groups such as organ transplant recipients and those taking photosensitizing medications. The most significant risk factor for NMSC is ultraviolet radiation (UVR) from sunlight, specifically UVB, which is the leading cause of DNA damage, photoaging, and malignant transformation in the skin. Activation of apoptosis following UVR exposure allows the elimination of irreversibly damaged cells that may harbor oncogenic mutations. However, UVR also activates signaling cascades that promote the survival of these potentially cancerous cells, resulting in tumor initiation. Thus, the UVR-induced stress response in the skin is multi-faceted and requires coordinated activation of numerous pathways controlling DNA damage repair, inflammation, and kinase-mediated signal transduction that lead to either cell survival or cell death. This review focuses on the central signaling mechanisms that respond to UVR and the subsequent cellular changes. Given the prevalence of NMSC and the resulting health care burden, many of these pathways provide promising targets for continued study aimed at both chemoprevention and chemotherapy.

Keywords

non-melanoma skin cancer; ultraviolet radiation; DNA damage response; apoptosis; signal transduction

INTRODUCTION

There are an estimated 3.5 million people in the US who have been diagnosed with non-melanoma skin cancer (NMSC) making it the most common form of cancer in the country [1], with medical related costs reaching $650 million annually [2]. The incidence of NMSC is also high in the United Kingdom; it has been reported that 1 out of every 1000 individuals in the UK are diagnosed with the disease per year [3]. NMSC has become a major health

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DECLARATIONS OF INTEREST

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concern in both countries as the incidence of NMSC in the US has increased a remarkable 300% since 1994 [4], while the rate of NMSC occurrence in the UK appears to be rising faster than any other European country [3]. NMSC is classified into two major forms: basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). BCCs, which arise from basal cell layer, constitute the majority of all diagnosed skin cancers (80%) and are rarely life threatening or metastatic. SCCs, which can arise from hair follicle stem cells [5], account for 16% of all skin cancers and are much more dangerous; these cancers are more likely to invade and metastasize [2, 6]. Incidence of NMSC, like many cancers, is much higher in older adults, and individuals who are over 60 years of age account for nearly 80% of all NSMC cases [6, 7]. More alarmingly however, the rate of NMSC occurrence is steadily increasing in patients younger than 35 years [8]. Taken together, these facts demonstrate the importance of understanding the molecular pathways behind NMSC development in order to increase our ability to diagnose, prevent and treat NMSC.

Ultraviolet radiation (UVR) from the sun is the greatest environmental risk factor for developing NMSC [9]. There are three distinct energetic forms of UV light that are emitted from the sun: UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm). However, only UVA and UVB pierce the atmosphere and reach the general populace [10]. Although UVA can penetrate deeper into the skin, UVB is the more energetic and accounts for the majority of the biologically damaging effects from sun exposure; these effects include direct DNA damage, activation of receptor-mediated signaling pathways, and formation of reactive oxygen species (ROS) [11]. Heightened exposure to UVR, both in intensity and in duration, directly increases the risk of NMSC. Therefore, individuals who are fair-skinned (Fitzpatrick Scale- Type I and II) spend large amounts of time in the sun or on tanning beds, and/or live near the equator are more susceptible to UVB-induced skin carcinogenesis [2, 6, 7, 9, 12]. Currently, according to American Cancer Society (ACS), the best methods for protecting against the harmful effects of UVR when outdoors are to wear protective clothing, seek shade when possible, apply copious amounts of sunblock, and protect the eyes with sunglasses. However, previous medical conditions can also dramatically increase an individual’s vulnerability to NMSC in response to UVR exposure, even when following these guidelines. Patients who have had SCCs or related diseases in the past have an increased chance to acquire additional SCCs, as well as melanoma [13]. Similarly, it has been shown that patients who take immunosuppressive drugs following an organ transplant are 60–100x more likely to develop SCC than the general population [14, 15].

The molecular mechanisms behind UVR-induced skin carcinogenesis are complex. UVR, specifically UVB, is considered a complete carcinogen as it can both initiate and promote cancer. UVR is able to penetrate through the skin to affect the keratinocyte stem cells, located in the basal layer of the epidermis. DNA damage can result from UVR-irradiation, which primarily generates cyclobutane pyrimidine dimers and (6–4) photoproducts [16]. These damaged cells will undergo DNA repair, most commonly through nucleotide excision repair (NER) to correct the UVR-induced damage [17]. The importance of the DNA repair response following UVR exposure cannot be underestimated. Individuals who are born with Xeroderma pigmentosum (XP), an autosomal recessive genetic disease resulting in mutations in NER, have an extraordinary 2000-fold increased incidence of NMSC and melanoma [18–20]. If the irradiated keratinocyte cannot repair the UVR-induced DNA

damage, it will either undergo cell cycle arrest or initiate apoptosis as a regulatory defense mechanism [21]. Importantly, UVR-irradiation can also initiate tumorigenesis by inducing pro-survival pathways in keratinocytes that oppose and counteract apoptosis, thereby allowing damaged cells to survive. UVR can activate these pathways through direct DNA damage of critical target genes [22], activation of cell surface receptors [22], and/or increased inflammation and immunosuppression [23]. Many of these pathways can promote tumorigenesis through increased cell cycle progression and proliferation [2, 24]. Thus, it is important to understand how these pathways are regulated in order to develop novel drugs and therapeutic strategies to both treat NMSC, as well as act as chemopreventive agents in high-risk individuals. This review will highlight key signaling pathways that are activated in response to UVR and describe the downstream pathophysiological responses each pathway elicits.

DNA DAMAGE

DNA damage from direct UVR exposure results in the formation of cyclobutane pyrimidine dimers, (6–4) photoproducts, DNA cross-links, single strand breaks (SSB) and double strand breaks (DSB) [2]. If the DNA damage caused by the resulting adducts is not repaired, a UVR-specific permanent mutation is introduced into the genome. Specifically, a cytosine that is adjacent to a thymine or another cytosine is mutated to a thymine [25]. This mutation is commonly found in the tumor suppressor p53 gene of SCCs and historically was considered the the primary driver of NMSC initiation [26]. However, more recent studies have begun to show that, in addition to direct DNA damage, UVR-induced signal transduction can also play a central role in NMSC initiation. Due to the large number of molecular pathways that are activated in response to UVR, UVR exposure can elicit a response in keratinocytes that is simultaneously both oncogenic/pro-survival and tumor suppressive/apoptotic [27]. Alterations in any of these pathways in damaged cells can shift the balance in favor of survival or death. Among the first of these signaling mechanisms to be studied were the pathways controlled by the phosphatidylinositol 3-kinase-related kinases (PIKK kinases) ataxia telangiectasia mutated (ATM) and Rad3-related (ATR) proteins.

ATM/ATR signaling

The PIKK family of proteins are critical in maintaining and regulating DNA damage checkpoints [28]. Specifically, ATM and ATR are essential proteins activated in response to UVR-induced DNA damage [29, 30]. In addition to their role in recruiting [31] or being recruited by [32] the DNA repair machinery to the site of damage, ATM and ATR activate a downstream signaling cascade that results in either repair or apoptosis, depending on the extent of DNA damage (Figure 1A,B). In the absence of stimulation, ATM resides as an inactive dimer. Upon UVR-induced DSB, ATM undergoes autophosphorylation (at Ser^{367}, Ser^{1893}, Ser^{1981} [33], and Ser^{2996} [34]) and becomes an active monomer [35], which subsequently phosphorylates checkpoint kinase 2 (CHK2) at Thr^{68} [36]. Activated CHK2 inhibits the CDC25 phosphatases through phosphorylation at Ser^{216}, which promotes their degradation. This reduction in CDC25 activity prevents the cell from entering mitosis [37]. Additionally, ATM has been shown to phosphorylate p53 (at Ser^{9}, Ser^{15}, and Ser^{46}), resulting in increased p53 stability and accumulation [38, 39], which leads to cell cycle

arrest in G1 [40]. More recent studies have shown that in addition to direct p53 phosphorylation, ATM also phosphorylates the E3 ubiquitin-protein ligase mouse double minute 2 homolog (MDM2) at Ser^{394}. MDM2 normally targets p53 for degradation; however, phosphorylation by ATM inhibits this action, further increasing the stability of p53 [41]. By halting the cell cycle through either CDC25 degradation or p53 accumulation, ATM provides the damaged cell both time to repair the DSB and also prevents its replication [28, 42]. The presence of DSB that cannot be repaired can trigger apoptosis as a result of prolonged activation of ATM. Both ATM and subsequent CHK2 activation can phosphorylate and activate the pro-apoptotic transcription factor E2F1 at Ser^{31} and Ser^{364} respectively [43, 44]. Therefore, ATM-dependent apoptosis can occur through increased activity of both p53 and E2F1 (Figure 1A).

In contrast, ATR is activated by generation of SSB due to UV-induced damage and replication stress/stalling [28, 45]. Similar to ATM, ATR halts the cell cycle to allow sufficient time for DNA repair or, if the resulting DNA damage is too excessive, induce apoptosis. Interestingly, direct signaling cross-talk exists between the two kinases as activated ATR can directly phosphorylate ATM at Ser^{1981} in response to UV exposure, resulting in the activation of ATM [46]. UV-induced activation of ATR leads to the phosphorylation of checkpoint kinase 1 (CHK1) at Ser^{345} [47], which subsequently phosphorylates and inhibits CDC25 activity, halting the cell cycle in a manner similar to CHK2 [48]. CHK1 can also inhibit CDC25 activity by either the activation of Never In Mitosis A (NIMA)-Related Kinase 11 (NEK11), which phosphorylates and inhibits CDC25 [49, 50], or *via* direct inhibition of the polo-like kinase 1 (PLK-1), a known activator of CDC25 [51]. Analogous to the action of ATM, ATR increases p53 stability through phosphorylation of MDM2 at Ser^{407}. This phosphorylation reduces the MDM2-dependent nuclear export of p53, thereby increasing p53 activity [52]. Both ATM and ATR can also directly phosphorylate (at Ser^{19}) and inhibit the E3 ubiquitin ligase seven in absentia homologue 1 (SIAH1), resulting in activation of p53. In the absence of stimulation, SIAH1 promotes the degradation of the homeodomain-interacting protein kinase 2 (HIPK2) [53], thus preventing HIPK2 from activating p53 [54] (Figure 1B).

UVR-induced activation of ATR has also been linked to the cyclin-dependent kinase inhibitor $p21^{WAF1/Cip1}$ (p21) pathway, which interacts with numerous cyclins/CDKs to halt the cell cycle [55]. Reports show that ATR has UVR dosage dependent, paradoxical effects on the p21 pathway. At high doses, ATR inhibits p21, which appears counter-intuitive; however, this inhibition has been shown to promote apoptosis [56], which may be the result of excessive DNA damage. Interestingly, low dose UVR protects p21 from s-phase kinase-associated protein 2 (SKP2)-mediated degradation, leading to a delay in cell cycle progression to allow DNA repair [57]. Furthering the complexity, a recent report shows that high energy UVC exposure can lead to increased p21 mRNA stability in a CHK1-dependent fashion *via* cytoplasmic localization of the RNA binding protein HuR. In the absence of ATR activation, HuR is localized to the nucleus due to cyclin-dependent kinase 1 (CDK1)-dependent phosphorylation of HuR at Ser^{202}. Activation of ATR/CHK1 inhibits CDK1, which allows HuR to translocate to the cytoplasm and stabilize p21 mRNA [58]. While several reports show that complete inhibition of ATR can increase both tumor development and aging [59, 60], another study found that the diminished expression/function of ATR in

UVR-sensitive mice resulted in protection from UVR-induced skin carcinogenesis [61]. Therefore, when developing therapeutics that target DNA repair proteins for the treatment of NMSC, it is important to consider how different levels of activity of these proteins may affect skin tumorigenesis.

INFLAMMATION

Multiple studies have shown an association between inflammation and the promotion of various cancer types, and inflammation has been referred to as the "seventh hallmark of cancer" [62–64]. In fact, the correlation between cancer and inflammation dates back to 1863, when Rudolf Virchow discovered leukocytes associated with tumor cells [65]. Inflammatory pathways are classically activated upon skin injury *via* UVR-induced sunburn, which stimulates the dilation of blood vessels to increase blood supply to the injury site, changes in the microvascular structure that help plasma proteins enter from the blood stream, and increased migration of white blood cells to the site through the endothelium [66]. These responses create a microenvironment that aids in wound healing. However, this environment can be hijacked to help stimulate tumorigenesis [62]. Inflammation has been shown to increase keratinocyte survival, proliferation, and transformation in NMSC development and progression, as well as promoting invasion and metastasis [66]. Clinical studies have demonstrated that inflammation is involved in the progression from actinic keratosis (AK) to SCC. Additionally, chronic inflammation is consistently observed in the invasive periphery of tumor cells in patients with SCC [66]. Since inflammation is a hallmark response of sunburned skin [67], it is of obvious importance to understand the molecular pathways involved and their effect on NMSC progression. Here we will highlight two key inflammatory pathways involved in UVR-induced inflammation: the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) pathway and the signal transducer and activator of transcription 3 (STAT3) pathway.

The NFκB pathway

The NFκB pathway has been extensively studied and revealed to be crucial in mediating inflammation of the skin (reviewed in [68]). Inactive NFκB exists primarily as a cytoplasmic heterodimer of the p65 and p50 subunits, which are bound to the inhibitory protein IκB. Induction of the canonical NFκB pathway can occur either *via* direct UVR activation or by the binding of UV-induced cytokines to plasma membrane receptors. The most commonly associated receptors with NFκB activation in NMSC are Receptor Tyrosine Kinases (RTK, discussed elsewhere in this review), Tumor Necrosis Factor receptors (TNFR1, reviewed in [69]), and Toll-like receptors (TLRs), specifically TLR2, 3, & 4 [70] (Figure 2A). In general, TLR activation occurs following UVR-induced ligand binding, which recruits the I kappa B kinase (IKK) complex, consisting of IKKα and β, as well as the NFκB essential modulator (NEMO/IKKγ), to the TLR receptor, resulting in activation of IKK and subsequently, NFκB. Specifically, activation of the TLR4 induces a conformational change, which allows binding of the Myeloid Differentiation Primary Response 88 (MyD88) protein to the TLR. The IL-1 receptor-associated kinase (IRAK) family of kinases then bind to MyD88, inducing autophosphorylation and activation of IRAK [71] and recruitment of TNF receptor associated factor 6 (TRAF6) proteins. TRAF6 proteins are E3 ubiquitin ligases that interact

with ubiquitin conjugating enzymes to promote the creation of polyubiquitin chains [72]. The recruitment of the IKK complex to the newly formed ubiquitin chains at the receptor is driven by the ubiquitin-binding protein NEMO. This NEMO-dependent binding of the IKK complex to TRAF6 allows for the proximal phosphorylation and activation of IKK (at $Ser^{177\&181}$ for IKKβ and at $Ser^{176\&180}$ for IKKα) [73–75]. The activated IKK proteins in turn phosphorylate IκB at Ser^{32} and Ser^{36}, inducing ubiquitination and proteasomal degradation of the protein and releasing the inhibition of NFκB [76]. The active NFκB heterodimer translocates to the nucleus to promote the transcription of pro-inflammatory genes [77], specifically genes that control apoptosis, as well as genes that lead to the production of cytokines and interferons that regulate the immune/inflammatory response. Among these targets are TNFα, IL-1, IL-6 and IL-8 [78]. IKK can also phosphorylate the p65/50 dimer directly at Ser^{536} of p65. This phosphorylation site has been shown to further increase NFκB nuclear transcriptional activity [79, 80]. Recent reports show that the p65 subunit of NFκB is essential for skin carcinogenesis in mice. Loss of p65 prevented both SCC tumor initiation and tumor promotion [77]. Other reports show that dsRNA released from UVB-induced necrotic keratinocytes can activate TLR3, which results in the archetypal sunburned skin inflammatory response [81]. In addition, there are numerous studies that link activation of NFκB to a variety of other skin diseases (reviewed in [82]).

The STAT3 pathway

The STAT family of transcription factors are induced in response to growth factors and cytokines. This family of proteins regulates factors that can affect both tumorigenesis and the tumor microenvironment [83]. STAT3 has been shown to play an important role in survival and proliferation of keratinocytes exposed to UVB. Targeted overexpression of STAT3 in the basal epidermis (K14-STAT3) accelerates skin tumor formation in mice exposed to UVB, while targeted deletion of STAT3 in the same cells confers resistance [84]. Interestingly, altering STAT3 activity within basal keratinocytes has profound effects on hair follicle stem cells and skin progenitor cells [85], which are implicated as the origin cells in NMSCs. Constitutively active STAT3 has been found in both mouse and human SCCs, and plays a role in promoting skin inflammation during tumor development [86]. In the canonical STAT3 pathway (Figure 2B), UVR exposure activates the epidermal growth factor receptor (EGFR), a cell surface RTK, which phosphorylates the Janus-associated-kinase (Jak). This allows the recruitment of STAT3 to the receptor complex through binding of the Src Homology 2 (SH2) domain of STAT3 to phosphorylated Jak [87]. Following recruitment, STAT3 is phosphorylated at Tyr^{705}, which induces dimerization of STAT3 and allows it to bind to DNA and increase transcription of pro-inflammatory genes [88]. Specifically, STAT3 promotes the transcription of numerous genes that are related to inflammation (ie. interleukins), tumor promotion (c-Myc and c-Fos), cell survival (Bcl-2, Cyclin D1 and Survivin), and metastasis (Twist and Zeb1) [89, 90]. Additionally, studies in mice have shown that UVR-induced reactive oxygen species (ROS) activates protein kinase c epsilon (PKCε), which directly phosphorylates STAT3 at Ser^{727} [91, 92]. While phosphorylation of STAT3 at Tyr^{705} is required to activate STAT3 transcriptional activity, studies show that dual phosphorylation of STAT3 at Tyr^{705} and Ser^{727} further increases the activity of STAT3-dependent transcription [91, 93]. Non-canonical STAT3 pathways have also been linked to tumorigenesis of various cancers. It is important to note that while little

is currently known of the non-canonical STAT3 pathways in UVR-induced skin carcinogenesis, these pathways are being actively investigated [94, 95].

OXIDATIVE STRESS

UVR exposure activates multiple stress response pathways in keratinocytes. Oxidative stress, defined as unbalanced ROS production and antioxidant defenses, has been linked to the initiation of numerous cancers, including NMSC [96]. Antioxidants have shown clinical efficacy to both prevent and treat cancer [97]. However, recent studies show that prolonged increases in ROS production within cancer cells have tumor suppressive roles and initiate apoptotic pathways; therefore, administering antioxidative therapies may actually harm a patient depending on the stage of their cancer [98]. Importantly, UVR exposure transiently increases ROS production within the skin both immediately following UVR-irritation, as well as in a second response up to 3 hours later [99]. Depending on the activated molecular pathway, UVR-induced oxidative stress has been linked to multiple skin conditions including aging, inflammation and skin cancer. There is an inherent defense network present in keratinocytes to combat ROS accumulation; however, following UVB exposure, ROS production can be increased to such high levels that these antioxidative defenses are overwhelmed [100]. Tumor development can result from a combination of increased ROS production and reduced antioxidant defense mechanisms in the skin, which leads to DNA damage and activating mutations [101, 102]. Interestingly, differences in the accumulation of antioxidant enzymes such as the superoxide dismutases and catalase were observed in patient biopsies of both non-melanoma and melanoma tumors. NMSC had strikingly lower levels of these enzymes than their melanoma counterparts, suggesting that chronic UVR exposure associated with NMSC development leads to weakened antioxidant defense response during tumorigenesis [103]. This review describes two critical pathways that are activated in response to UVR-induced oxidative stress NMSC development: the p38 mitogen-activated protein kinases (p38) pathway and c-Jun N-terminal kinases (JNK) pathway.

p38 signaling

The pathophysiological effect of UVR-induced activation of both p38 and JNK in keratinocytes is controversial, as reports show that these stress response proteins can elicit both pro- and anti-survival mechanisms [104]. However, given the bimodal role that oxidative stress plays in cancer, this seemingly contradictory response is not surprising. p38 is a member of the mitogen-activated protein kinase (MAPK) family of proteins and responds to a variety of cellular stress stimuli, including oxidative stress (Figure 3A). The dual specificity mitogen-activated protein kinase kinase (MKK) 3 and 6 phosphorylate p38 at Thr^{180} and Tyr^{182} within the activation loop of the protein. Once active, p38 phosphorylates both cytoplasmic and nuclear targets [105]. ROS-dependent activation of p38 in keratinocytes has also been linked with activation of apoptosis signal-regulating kinase 1 (ASK1), a member of the MAP Kinase Kinase Kinase (MAP3K) family of proteins that is upstream of both p38 and MKK3/6. Under oxidative stress, activation of ASK1 occurs following disassociation from the inhibitory thiol-disulphide oxidoreductase thioredoxin-1 (Trx1) protein [106]. Other studies reveal that cells lacking specific mitogen-activated

protein kinase phosphatases (MKP), phosphatases that target MAPKs, have increased p38 activation following UVR exposure. Importantly, increased ROS production has been shown to inhibit MKPs, which triggers increased p38 activation in response to elevated oxidative stress [107, 108]. It is also important to note that UVR can activate the p38 pathway independent of oxidative stress. Specifically, UVR-induced DNA damage can elicit a p38 response and can regulate the cell cycle through activation of MAPKAPK-2 (MK2) [109, 110].

Recent studies have demonstrated that inhibition of p38 increases cell survival in murine SCC *in vivo* [111, 112]. In agreement with this, activation of p38 in response to UVR promotes apoptosis in keratinocytes by phosphorylating p53 at Ser^{33} and Ser^{46}, which increases the apoptotic activity of p53 [113]. Other reports indicate that UVR-induced p38 activation induces NOXA-dependent apoptosis through increased protein expression of hypoxia-inducible factor 1-alpha (HIF-1α) in a p53-independent manner [114]. In contrast, it has been shown that p38 activation increases UVR-induced survival of keratinocytes though upregulation of the cancer associated genes Bcl-XL and COX-2 [104]. Activation of p38 can also directly phosphorylate glycogen synthase kinase 3 beta (GSK3β) at Thr^{43} and Thr ^{390} (Ser^{389} in mice), which promotes β-catenin dependent growth and proliferation [115]. Moreover, transgenic mice expressing a dominant negative p38 protein are resistant to skin carcinogenesis upon exposure to chronic UVR-irradiation, perhaps due to a reduction in p38-dependent hyperproliferation [116].

JNK signaling

JNK, like p38, is a member of the MAPK family that is activated upon phosphorylation following oxidative stress by upstream MKKs (MKK4 and 7) at Thr^{183} and Tyr^{185} (Figure 3B). MKK4 and 7, like MKK3 and 6, are activated in response to ROS production through either increased ASK-1 activity or reduced MKP activity [117]. JNK activation occurs in human keratinocytes as quickly as 5 minutes following UVR exposure, with maximal activity peaking at 30 min post-UVR. This correlates perfectly with UVR-induced p38 kinase activity [118]. A major target of JNK is the transcription factor activator protein-1 (AP-1). AP-1 exists as either a homodimer containing two c-Jun or c-Fos proteins, or a heterodimer containing one of each. AP-1 is known primarily as an oncogenic transcription factor as it is involved in promoting cell proliferation, specifically through transcription of cell cycle regulator genes (such as cyclin D1, cyclin A, cyclin E, p53, p21, $p16^{Ink4a}$ and $p19^{ARF}$). Additionally, AP-1 can elicit a cell survival response through crosstalk with NFκB [119]. Once activated, JNK can phosphorylate c-Jun at Ser^{63} and Ser^{73} [120] and this phosphorylation increases AP-1 activity in response to UVR exposure [121]. Bowden and colleagues showed that pharmacological inhibition of JNK in human keratinocytes sensitizes cells to UVR-induced apoptosis *in vitro* [122], which correlates with a study by the same group showing that *in vivo* inhibition of AP-1 protected transgenic mice expressing a dominant negative c-jun from UVB-induced SCC [123]. Additionally, pharmacological inhibition of JNK inhibited the growth of xenograft studies using human head and neck SCCs in mice [124]. However, similar to p38, JNK activation can induce a pro-apoptotic response. Exposure of lung adenocarcinoma ASTC-a-1 cells to high dose UVB results in JNK-dependent nuclear localization of the pro-apoptotic factor FOXO3a (discussed

elsewhere in this review) [125], but this localization does not occur with lower doses of UVB. This low vs high dosage effect may be similar to the response described above for ATR. Specifically, UVR-dependent JNK activation is initially protective; however, when the damage induced by UVR-irradiation reaches a threshold level, the JNK-mediated signaling mechanisms within the cell activate apoptosis. It has also been shown in HEK293T cells that UVR-induced JNK activation can induce mitochondrial-derived intrinsic apoptosis through inhibition of Bcl-2 directly, or indirectly though activation of BIM (Bcl-2-like protein 11) [126]. However, this effect has not yet been studied in keratinocytes.

RTK ACTIVATION

As mentioned above, activation of various signaling pathways in the skin can occur as quickly as 5 minutes following exposure to UVR with peak activity occurring between 30 minutes to 1 hour on average [127–129]. The RTKs belong to an important family of receptors that are rapidly activated in response to UVR and have been linked to NMSC [24, 130, 131]. Key RTKs that are induced in response to UVR include the insulin receptor (IR), the insulin like growth factor 1 receptor (IGF-1-R), and EGFR [132]. Activation occurs when two transmembrane RTK subunits dimerize, typically in response to ligand binding, which induces autophosphorylation of their cytosolic kinase domains. While UVR-induced autocrine activation of RTKs has been previously described [133–135], the near immediate activation of RTKs upon exposure to UVR may be independent of ligand binding. Studies have shown that RTK activation can be propagated through UVR-induced ROS production [136, 137], while other reports suggest the possibility that activation occurs through cell surface clustering of RTKs in response to UVR to promote autophosphorylation and subsequent activation [137, 138]. RTK activation in keratinocytes predominantly initiates an anti-apoptotic and cell proliferative response, which are critical processes for both wound repair and growth of new skin [139]. However, aberrant UVB-induced activation of RTK pathways can lead to both NMSC initiation and promotion [7]. It is for this reason that a variety of drugs are being developed to target and inhibit RTKs directly, as well as inhibiting the downstream components of the RTK signaling cascade (see for example [140, 141]). This review discusses the two best-studied NMSC-related RTK pathways: the phosphoinositide 3-kinase (PI3K)/mechanistic (mammalian) target of rapamycin (mTOR) pathway and the rapidly accelerated fibrosarcoma kinase (RAF)/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinases (ERK) pathway.

PI3K/mTOR signaling

The serine/threonine kinase mTOR is a PIKK kinase that is part of an incompletely defined signaling network that responds to diverse nutrients, growth factors and cellular stressors [142–144], including UVR [129, 145, 146]. mTOR exists in two distinct signaling complexes: the rapamycin-sensitive mTOR complex 1 (mTORC1) and the rapamycin-resistant mTORC2 [143], both of which are important in NMSC development in response to UVB exposure. mTOR was identified as a therapeutic target in several cancer types, as upregulated mTOR signaling is frequently found in cancers [142–144]. Additionally, mutations in two critical upstream proteins of the mTOR pathway, PI3K and phosphatase and tensin homolog (PTEN), are commonly found in NMSC [147, 148]. Reports show that

increased levels of both mTORC1 and mTORC2 activity are present during progression from normal keratinocytes to pre-cancerous actinic keratosis, and culminating in NMSC development [149]. Activation of mTORC1-dependent pathways (Figure 4A) occurs following upstream activation of Protein Kinase B (AKT/PKB) *via* the PI3K pathway. Following autophosphorylation of the UVR-inducible RTKs (EGFR, IGF-R-1, and IR), PI3K, a heterodimer that consists of the p85 regulator subunit and the p110 kinase subunit, is recruited to the receptor through binding of the SH2 domain on p85 to the phosphorylated RTK. The p85 subunit is subsequently phosphorylated at Tyr^{458}, activating the heterodimer. This allows PI3K to phosphorylate the phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate phosphatidylinositol (3,4,5)- trisphosphate (PIP_3) on the plasma membrane. PIP_3 recruits both phosphoinositide-dependent kinase-1 (PDK1) and AKT to the receptor complex, where PDK1 can activate and phosphorylate AKT at Thr^{308}. AKT is a central regulator of numerous cellular processes including cell survival, metabolism, protein translation, and angiogenesis [150]. Three AKT isoforms have been described, of which AKT1 and 2 have been strongly implicated in tumorigenesis; however, studies suggest that melanoma development may depended on AKT3 [151]. The tumor suppressor PTEN negatively regulates PI3K-dependent signaling though PIP3 dephosphorylation and conversion to PIP_2. Phosphorylation by PDK1 activates AKT, which subsequently phosphorylates and inhibits the mTORC1 negative regulator Tuberous Sclerosis Complex 1/2 (TSC1/2). This inhibition allows the ras homolog enriched in brain (RHEB) GTPase to bind to and activate mTORC1 [152]. mTORC1 activation leads to cell growth and proliferation primarily through the downstream phosphorylation (at Thr^{389}) and activation of ribosomal protein S6 kinase beta-1 (S6K1), which controls ribosome biogenesis, as well as activation of cap-dependent translation *via* the phosphorylation (at Thr^{37}, Thr^{46}, Ser^{65}, and Thr^{70}) and subsequent degradation of the eukaryotic translation initiation factor 4E (eIF4E) inhibitory 4E-binding protein 1 (4E-BP1) [153]. This mTORC1-dependent signaling cascade has been described in detail in multiple reviews [142–144, 154–156].

Activation of mTORC1 plays a causal role in tumor promotion [157], and the mTORC1 specific inhibitor rapamycin (Sirolimus) is approved as a therapeutic in multiple cancer types [141, 158–160]. Rapamycin allosterically inhibits mTORC1 by binding to the FK-binding protein 12 (FKBP12) in the cytosol, which can then directly bind to the mTOR kinase subunit of mTORC1 and block its function [161]. Notably, rapamycin has no direct inhibitory effect on the activity of mTORC2 and, depending on length of exposure and tissue type, the indirect effects of rapamycin treatment on mTORC2 can vary [162]. DiGiovanni and colleagues showed that mTORC1 inhibition with topical rapamycin blocks dermal inflammation that is linked with the hyperproliferative response to tumor promotion in mice [163]. In addition, organ-transplant patients who took rapamycin as an immunosuppressive drug had a significant reduction in acquired NMSC when compared to patients taking cyclosporine A [164]. Our lab and others have demonstrated that mTORC1 is required for keratinocyte hyperproliferation in response to tumor promotion in murine skin carcinogenesis models [129, 165]. There is controversy regarding the role of mTORC1 in NMSC tumor initiation. Studies in other cancer models demonstrate that treatment with rapamycin can either increase or decrease cell survival [166–170]. Studies from our lab showed that treatment with rapamycin or genetic knockdown of the essential mTORC1

scaffolding protein Raptor does not affect cell viability or apoptosis following exposure to UVB in spontaneously immortalized human keratinocytes (HaCaT cells) or mouse primary keratinocytes [129, 145]. In contrast, a recent report by Bowden and colleagues showed that rapamycin treatment did increase UVR-induced keratinocyte cell death in mice [165]. The differing results seen in these studies are likely due to the differences in both model systems and UVR-dosages used. For example, our *in vitro* studies used UVB dosages between 20–35 mJ/cm^2, whereas the Bowden group used 640 mJ/cm^2 UVB for acute *in vivo* exposure experiments.

Studies linking mTORC2 to tumor initiation have suggested that this less studied of the mTOR complexes is also a viable target for therapeutic intervention [171]. Using mouse models with stage-specific deletion of Rictor in the basal layer of the epidermis, our recent work has established an essential role for mTORC2-controlled pathways in both skin tumor development and maintenance [145]. These results suggest that inhibition of mTORC2 signaling may be an effective strategy for both treatment and prevention of NMSC. Though the mechanism of mTORC2 signaling is only partially defined (Figure 4A), recent reports show that the activation of mTORC2 occurs following PIP_3 phosphorylation by PI3K [172]. The pleckstrin homology (PH) domain of the mTORC2 scaffolding/inhibitory protein SIN1 is recruited to the plasma membrane, where it binds to PIP_3. Binding of SIN1 to PIP_3 induces a conformational change in mTORC2 and uncovers the active kinase site of mTOR [173]. The first role described for mTORC2 was the control of actin cytoskeleton rearrangement [174]; however, induction of mTORC2 also activates both the serum-and glucocorticoid-induced protein kinase SGK and AKT, both of which are involved in controlling apoptosis in response to various cellular stresses and have been implicated as potential therapeutic targets in cancer [171]. Following its initial binding of mTORC2, PIP_3 recruits the PH domain of AKT to mTORC2, which permits the phosphorylation of AKT at the hydrophobic motif site Ser^{473} by mTORC2 [173]. Recent studies have revealed that the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) can also regulate mTORC2 activation. In response to UVR-induced DNA damage, DNA-PKcs exits the nucleus and binds to SIN1. This interaction promotes mTORC2-mediated phosphorylation of AKT at Ser^{473} [175]. This phosphorylation, in addition to the PDK1-dependent Thr^{308} phosphorylation, increases the substrate specificity of AKT for downstream targets. While phosphorylation of AKT at Thr^{308} is sufficient to induce mTORC1-specific activation [154], studies suggest that AKT must be phosphorylated at both Thr^{308} and Ser^{473} in order to control downstream cell survival pathways, including regulation and inhibition of pro-apoptotic proteins including BAD (Bcl-2-associated death promoter), BAX (Bcl-2-associated X), GSK-3β, ASK1, Caspase-9, and FOXO3a [150, 176].

The transcription factor FOXO3a has been identified as a critical component in the apoptotic pathway of several cancer types [177–185]. FOXO3a is a member of the FOX (Forkhead Box) superfamily of transcription factors that control diverse cellular functions [182]. The subclass FOX"O" proteins chiefly regulate oxidative stress, cell cycle progression, and apoptosis [183, 184, 186]. FOXO3a directly targets and increases transcription of both extrinsic (*Trail and FasL)* and intrinsic (*BIM and PUMA)* apoptotic genes [183], which are implicated in UVR-induced apoptosis [187–189]. While there are a variety of cellular elements that post-translationally modify FOXO3a, AKT and ERK are considered two of its

primary negative regulators [186, 190, 191]. Upon phosphorylation of AKT at Thr^{308} and Ser^{473}, AKT translocates into the nucleus and phosphorylates FOXO3a at three sites: Thr^{32}, Ser^{256} and Ser^{319} [192] (Figure 4A). Previous studies show that AKT-mediated phosphorylation of FOXO3a alone does not reduce its activity. Instead, the 14-3-3ζ chaperone binds to this evolutionally conserved motif and reduces the affinity of FOXO3a for DNA by masking the DNA binding domain [193]. In addition, 14-3-3ζ binding masks two nuclear localization sequences on the FOXO3a [194], which along with two already exposed nuclear export signals, induces FOXO3a cytoplasmic localization and sequestration [195]. Our lab has recently demonstrated that knockdown of either rictor or mSIN1, two key structural components of mTORC2, sensitizes keratinocytes to UVB-induced apoptosis, and that this effect is dependent on AKT-mediated FOXO3a regulation [127]. Upon exposure to UVR, FOXO3a is shuttled out of the nucleus and sequestered into the cytoplasm. However, disruption of mTORC2/AKT signaling inhibits this process and an increased accumulation of FOXO3a is observed in the nucleus. The increased sensitivity to UVR-induced apoptosis following inhibition of mTORC2 signaling is rescued with simultaneous knockdown of FOXO3a, suggesting this process is dependent on the activity of FOXO3a [127].

Raf/MEK/ERK signaling

Activation of RAF/MEK/ERK signaling also occurs downstream of EGFR in response to UVR exposure [196, 197], and this pathway has been identified as a chemoprevention target in NMSC [2, 198]. Following EGFR activation (Figure 4B), the growth factor receptor-bound protein 2 (GRB2) is recruited to the receptor, resulting in binding to the guanine nucleotide exchange factor Son of Sevenless (SOS). This enables SOS to liberate the bound GDP on the RAS GTPase. Once GDP is released, RAS quickly binds a free GTP and induces RAF activation [199]. There are three isoforms of RAF that are involved in signaling to the MEK/ERK pathway: A-RAF, B-RAF, and C-RAF (RAF1). Mutations in B-RAF in particular are linked with development of melanoma and are reviewed in [200]. All three RAF isoforms can bind to and phosphorylate MEK1 and 2 (at Ser^{217} and Ser^{221}). This phosphorylation activates MEK, which in turn phosphorylates (at Thr^{202} and Tyr^{204}) and activates ERK (p44/42 MAPK) 1 and 2 [201]. Once activated, ERK regulates numerous downstream targets involved in cancer that control cell growth and survival including c-Jun, c-Myc, IKK, and FOXO3a [202]. Interestingly, studies show that certain patients who were successfully treated for BCCs through the use of smoothened (Smo) inhibitors, inhibitors that block sonic hedgehog (SHH) signaling, later develop SCCs at the same site. It was determined that following Smo inhibition, the regressed BCC cells switch from aberrant SHH activation to increased RAS/MEK/ERK signaling, which promotes the development of SCC [203].

Similar to AKT, ERK can phosphorylate FOXO3a (at Ser^{294}, Ser^{344}, and Ser^{425}) in the nucleus, which triggers its exit into the cytoplasm (Figure 4B). However, contrary to mTORC2/AKT/14-3-3ζ regulation, FOXO3a is targeted for MDM2-mediated cytoplasmic degradation in response to the ERK-specific phosphorylation [204]. In our recent study, we noticed a UVR-induced increase in total FOXO3a abundance in HaCaT cells with intact mTORC2 signaling that was not present following inhibition of mTORC2 [127], suggesting a possible degradation of FOXO3a. Several studies suggest that 14-3-3ζ binding acts as a

"protective barrier" for FOXO3a, which guards FOXO3a from PP2A-mediated dephosphorylation and subsequent degradation triggered by other signaling pathways, including the RAF/MEK/ERK pathway [195, 205]. Therefore, we simultaneously inhibited both of these pathways in HaCaT cells and showed an increase in nuclear FOXO3a protein both before and immediately after exposure to UVR. These results correlated with a further increased sensitivity to UVR-induced apoptosis compared to inhibition of either pathway alone, which was also dependent on FOXO3a [127]. It has been demonstrated that dual-targeting of the mTORC2/AKT and RAF/MEK/ERK pathways in advanced cancers produces better efficacy in patients than inhibition of either pathway alone [206]. In addition, studies in colon and pancreatic cancer models also show that dual inhibition of these pathways specifically increases the tumor suppressor activity of FOXO3a [185, 207, 208]. These results reflect the extensive cross-talk that occurs between the PI3K/mTOR and the RAF/MEK/ERK pathways (see [209] for review). Given these results, combination therapy with a TOR kinase inhibitor and MEK inhibitor for NMSC prevention warrants further investigation.

APOPTOSIS

A common denominator relevant to each reviewed signaling pathway is the ability to inhibit and/or induce caspase-dependent apoptosis upon exposure to UVR. As discussed above, increased cell survival due to inhibition of apoptosis is a key contributor to UVR-induced initiation of NMSC; therefore, an understanding of the signaling cascades that result in apoptosis initiation will provide insight into NMSC development. Caspase-dependent apoptosis is described as either extrinsic, death receptor (DR)-dependent apoptosis or intrinsic apoptosis initiated at the mitochondria [210]. Initiation of extrinsic apoptosis (Figure 5) requires ligand binding and activation of plasma membrane DRs, which are members of the tumor necrosis factor super family of receptors (TNFR) [84]. Three examples of DRs that have been linked to in skin carcinogenesis are TNF-R1, the Fas/APO1/CD95 receptor (FasR), and the TNF-related apoptosis-inducing ligand receptors 1 and 2 (TRAIL-R1/DR4 and TRAIL-R2/DR5) [89, 203], each with a specific extracellular ligand reflected in the receptor name (i.e. TRAIL binds to TRAIL-R). Negative regulators of DRs, referred to as decoy death receptors (DcR), have parallel extracellular domains that allow them to act as ligand sinks; however, only the active DRs have the fully functional intracellular death domain (DD) needed to induce apoptosis [81]. TNFR1 signaling is reviewed in detail in [69]. Briefly, FasR and TRAIL-R1/2 trimerize upon ligand binding, which allows the recruitment and homotypic binding of fas-associated protein with death domain (FADD) to the death domain binding sites on the intracellular domain of the DR. This binding allows FADD to recruit the pro-apoptotic proteins procaspase-8 and -10 into a complex referred to as the death-inducing signaling complex (DISC) [211]. Following recruitment by FADD, pro-caspase 8 is ubiquitinated *via* a complex consisting of the cullin3 ubiquitin E3 ligase (CUL3) and RING-box protein 1 (RBX1). The resulting ubiquitination permits the binding of pro-caspase 8 to ubiquitin-binding protein p62/sequestosome-1 (p62), which promotes aggregation and homodimerization of pro-caspase 8 allowing auto-proteolytic cleavage of pro-caspase 8 into its active form [212]. This process is inhibited by

the cellular FLICE-like inhibitory protein (c-FLIP), which binds to FADD and prevents the dimerization and subsequent activation of caspase-8.

Once cleaved, the caspase-8 dimer causes the downstream activation of pro-caspase-3, -6, and -7. Activation of these caspases, primarily caspase-3, induces cell death through the cleavage of DNA, as well as various proteins involved in cell maintenance and survival. In this context, cleavage of poly (ADP-ribose) polymerase (PARP) is often considered an endpoint marker of caspase-dependent apoptosis [213]. Therapeutics that target DR activation may be an effective treatment for NMSC, as *in vivo* studies have shown that activation of these receptors prevent skin tumor development in a mouse model [214]. In addition, targeting of either TRAIL-R or FasR has been shown to be an effective therapy against several types of cancers [215], although data on NMSC are not currently available.

Classically, intrinsic apoptosis (Figure 5) is activated in response to cellular stressors such as DNA damage or hypoxia, resulting in mitochondrial outer membrane (MOM) permeabilization (MOMP) and subsequent release of cytochrome C into the cytosol [176, 216]. Specifically, MOMP is generated following the formation of pores in the MOM by the proteins BAX and BAK (Bcl-2 homologous antagonist) though the specific mechanism is still the subject of investigation [217]. In the absence of UVR-induced cellular stress, the activities of BAX and BAK are inhibited by the anti-apoptotic Bcl-2 (B-cell lymphoma 2) proteins, including Bcl-2, Bcl-xL (Bcl-extra-large), and Bcl-w (Bcl-2-like protein 2) [218]. Following UVR-irradiation, the BH3-only family of proteins, which include BID (BH3 interacting-domain death agonist), BIM, BAD, PUMA (p53 up regulator of apoptosis), and NOXA, bind to and inhibit the anti-apoptotic Bcl-2 proteins [219]. BID and BIM can also directly activate BAX and BAK, resulting in pore formation on the MOM [220]. UVR-induced activation of the BH3-family of proteins results from increased activity of pro-apoptotic transcription factors such as FOXO3a, HIF-1α, and p53 [218, 221]. In contrast, UVR-inducible signal transduction pathways can also reduce the activity of the BH3-only proteins through direct protein inhibition (i.e. AKT phosphorylation of BAD), as well as indirect inhibition of upstream pro-apoptotic transcription factors (i.e. inhibition of p53) [24, 27].

Following its release into the cytosol, cytochrome C and the apoptotic protease activating factor 1 (APAF-1) form a complex referred to as the ‘apoptosome’. This complex recruits and activates caspase 9, which in turn activates caspase-3 and -7 to induce cell death [222]. Cross-talk between the extrinsic and intrinsic pathway exists in keratinocytes primarily through caspase-8 [223, 224]. In addition to activating caspase-3, -6, and -7, caspase-8 can cleave BID to generate a truncated form (tBID), which is able to both inhibit the Bcl-2 proteins and promote BAX/BAM activation and subsequent mitochondrial mediated cell death [216] (Figure 5). Thus, because UVR-irradiation can initiate intrinsic apoptosis through DR activation as well as through direct mechanisms such as DNA damage, oxidative stress and TLR/RTK activation, this apoptotic pathway is viewed as a more viable therapeutic target in the prevention and treatment of NMSC. Therapies designed to either inhibit the anti-apoptotic Bcl-2 proteins or mimic the pro-apoptotic BH3-only family are currently being tested as a means of treating both melanoma and NMSC [219, 225].

CONCLUSIONS

Recognizing the complexity of UVR-induced signal transduction is critical to our understanding of NMSC tumorigenesis. Depending on the intensity and duration of UVR irradiation, as well as the level of cellular damage (i.e. DNA/oxidative damage) that results from exposure to UVR, each activated signaling pathway can elicit varying responses that can be either tumor suppressive, oncogenic, or a mixture of both. As with most physiological mechanisms, there is a great deal of redundancy that exists within the cell, which allows extensive regulation and cross-talk between pathways. As a result, UVR exposure does not activate unique pathways in isolation, but rather elicits an intricate stress response that may become pathologic. Furthermore, genetic variation between individuals, in addition to genetic heterogeneity in tumors that develop, can play an important role in evaluating whether a targeted treatment will be beneficial, ineffective, or possibly even harmful. Thus, continued intensive study of the signaling pathways that respond to UVR will help lead to the development of more effective novel therapeutics in both the prevention and treatment of NMSC.

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Abbreviations used

NMSC	non-melanoma skin cancer
BCC	basal cell carcinoma
SCC	squamous cell carcinoma
UVR	Ultraviolet radiation
ROS	reactive oxygen species
XP	Xeroderma pigmentosum
SSB	single strand breaks
DSB	double strand breaks
PIKK	phosphatidylinositol 3-kinase-related kinases
ATM	ataxia telangiectasia mutated protein
ATR	ataxia telangiectasia and Rad3-related protein
CHK	checkpoint kinase
MDM2	mouse double minute 2 homolog

NIMA	Never In Mitosis A
NEK11	NIMA-Related Kinase 11
PLK-1	polo-like kinase 1
SIAH1	seven in absentia homologue 1
HIPK2	homeodomain-interacting protein kinase 2
SKP2	s-phase kinase-associated protein 2
CDK1	cyclin-dependent kinase 1
AK	actinic keratosis
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
STAT3	signal transducer and activator of transcription 3
RTK	Receptor Tyrosine Kinase
TNFR1	Tumor Necrosis Factor receptors
TLR	Toll-like receptors
IKK	I kappa B kinase
NEMO	NFκB essential modulator
MyD88	Myeloid Differentiation Primary Response 88
IRAK	IL-1 receptor-associated kinase
TRAF6	TNF receptor associated factor 6
EGFR	epidermal growth factor receptor
Jak	Janus-associated-kinase
SH2	Src Homology 2
PKCε	protein kinase c epsilon
JNK	c-Jun N-terminal kinases
MAPK	mitogen-activated protein kinase
MKK	mitogen-activated protein kinase kinase
ASK1	apoptosis signal-regulating kinase 1
MAP3K	MAP kinase kinase kinase
Trx1	thiol-disulphide oxidoreductase thioredoxin-1
MKP	mitogen-activated protein kinase phosphatase

HIF-1α	hypoxia-inducible factor 1-alpha
GSK3β	glycogen synthase kinase 3 beta
AP-1	activator protein-1
FOXO3a	Forkhead box O3a
Bcl-2	B-cell lymphoma 2
BIM	Bcl-2-like protein 11
IR	insulin receptor
IGF-1-R	insulin like growth factor 1 receptor
PI3K	phosphoinositide 3-kinase
mTOR	mechanistic (mammalian) target of rapamycin
RAF	rapidly accelerated fibrosarcoma kinase
MEK	mitogen-activated protein kinase kinase
ERK	extracellular signal-regulated kinases
PTEN	phosphatase and tensin homolog
AKT/PKB	Protein Kinase B
PIP_2	phospholipid phosphatidylinositol 4,5-bisphosphate
PIP_3	phosphatidylinositol (3,4,5)-trisphosphate
PDK1	phosphoinositide-dependent kinase-1
TSC	Tuberous Sclerosis Complex
RHEB	ras homolog enriched in brain
S6K1	S6 kinase beta-1
eIF4E	eukaryotic translation initiation factor 4E
4E-BP1	4E-binding protein 1
FKBP12	FK-binding protein 12
PH	pleckstrin homology
DNA-PKcs	DNA-dependent protein kinase catalytic subunit
BAD	Bcl-2-associated death promoter
BAX	Bcl-2-associated X
GRB2	growth factor receptor-bound protein 2

SOS	Son of Sevenless
Smo	smoothened
SHH	sonic hedgehog
DR	death receptor
TNFR	tumor necrosis factor receptor
FasR	Fas/APO1/CD95 receptor
TRAIL	TNF-related apoptosis-inducing ligand receptor
DcR	decoy death receptors
DD	death domain
FADD	fas-associated protein with death domain
DISC	death-inducing signaling complex
CUL3	cullin3 ubiquitin E3 ligase
RBX1	RING-box protein 1
c-FLIP	cellular FLICE-like inhibitory protein
PARP	poly (ADP-ribose) polymerase
MOMP	mitochondrial outer membrane permeabilization
BAK	Bcl-2 homologous antagonist
Bcl-xL	Bcl-extra-large
Bcl-w	Bcl-2-like protein 2
BID	BH3 interacting-domain death agonist
PUMA	p53 up regulator of apoptosis
APAF-1	apoptotic protease activating factor 1
tBID	truncated BID

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Summary Statement

The intracellular signaling response that leads to non-melanoma skin cancer (NMSC) is remarkably complex. This review summarizes key pathways activated by exposure to UV irradiation, the most important risk factor for NMSC, and describes their deregulation in NMSC development.

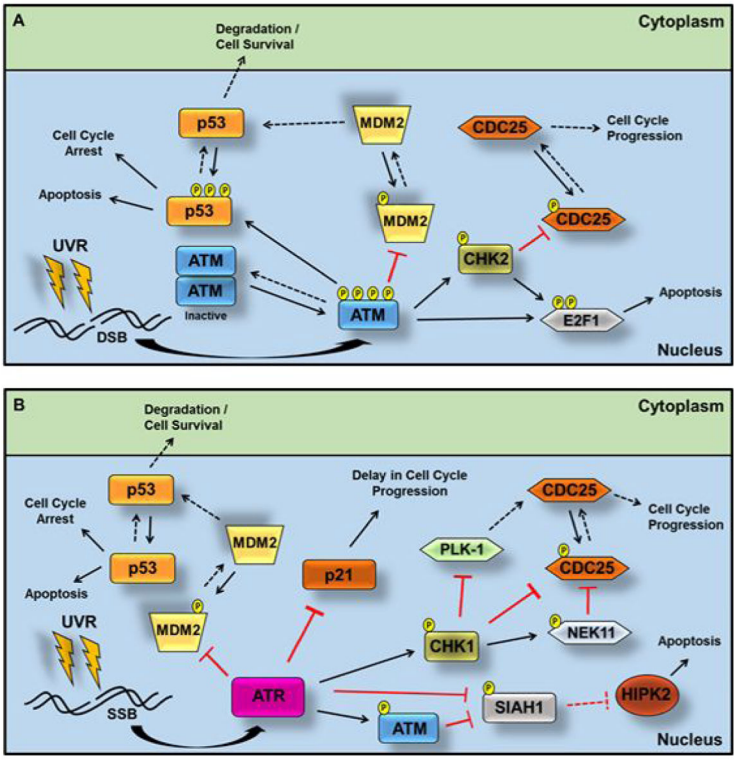

Figure 1. ATM/ATR-dependent cell cycle arrest or apoptosis in response to UVR

(*A.*) UVR-induced DSB trigger autophosphorylation of ATM at Ser^{367}, Ser^{1893}, Ser^{1981} and Ser^{2996}. The active ATM monomer phosphorylates CHK2 at Thr^{68}, which leads to inhibition of cell cycle progression through an inhibitory phosphorylation of CDC25 at Ser^{216}. ATM can either directly phosphorylates p53 at Ser^{9}, Ser^{15}, and Ser^{46} or indirectly regulate p53 through phosphorylation (at Ser^{394}) and inhibition of MDM2. Both of these events lead to cell cycle arrest or, with prolonged ATM activation, apoptosis. ATM can also phosphorylate CHK2 (at Ser^{364}) and the transcription factor E2F1 (at Ser^{31}), both of which ultimately lead to apoptosis. **(*B.*)** UVR-induced SSB lead to ATR activation and increased p53 stability through an inhibitory phosphorylation of MDM2 at Ser^{407}. Direct phosphorylation (at Ser^{19}) of the E3 ubiquitin ligase SIAH1 by ATR results in SIAH1 inhibition, which allows HIPK2 to activate p53. SIAH1 inhibition can also occur through ATR-dependent phosphorylation of ATM at Ser^{1981}. Phosphorylation of CHK1 at Ser^{345} by ATR can lead to cell cycle arrest by several routes: CHK1 can directly phosphorylate CDC25 or indirectly inhibit CDC25 through NEK11 or PLK-1. Excessive DNA damage caused by high-dose UVB can also lead to promotion of apoptosis through p21 inhibition (see text for details). Solid lines indicate UVR-induced events, while dotted lines represent outcomes in the absence of UVR.

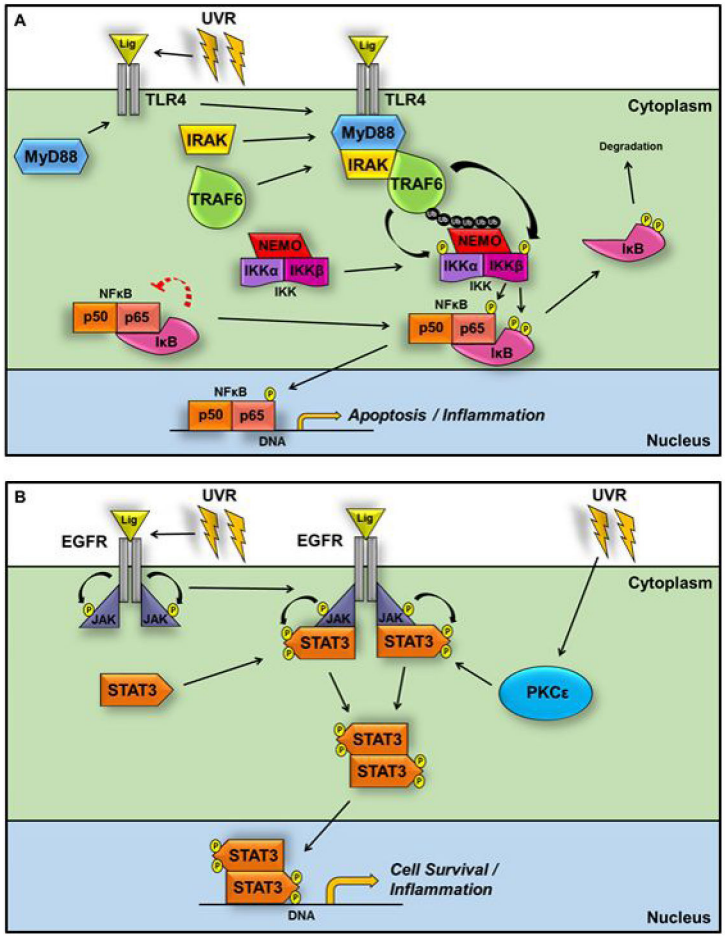

Figure 2. The inflammatory response to UVR acts through TLR4-dependent NFκB signaling and EGFR-dependent activation of STAT3

UVR-induced sunburn activates an inflammatory response that includes pathways dependent on NFκB and STAT3. **(*A.*)** Ligand binding recruits MyD88 to TLR4. IRAK binding to MyD88 causes autophosphorylation and activation of IRAK and recruits the TRAF6 E3 ubiquitin ligases. NEMO-dependent binding of the IKK complex to TRAF6 allows phosphorylation and activation of IKK (at $Ser^{177\&181}$ for IKKβ and at $Ser^{176\&180}$ for IKKα). Activated IKK phosphorylates IκB at Ser^{32} and Ser^{36} to induce ubiquitination and proteasomal degradation of IκB and release of the active NFκB heterodimer, which translocates to the nucleus to promote the transcription of pro-inflammatory genes. IKK can also phosphorylate the p65/50 dimer directly at Ser^{536} of p65. **(*B.*)** Activation of EGFR in response to UVR leads to the phosphorylation of Jak, which allows the recruitment of STAT3 to the receptor complex through binding of its SH2 domain to Jak. STAT3 is subsequently phosphorylated at Tyr^{705}, allowing dimerization and increased transcription of pro-inflammatory genes. Activation of PKCε by ROS results in direct phosphorylation of STAT3 at Ser^{727}, further increasing STAT3-dependent transcription.

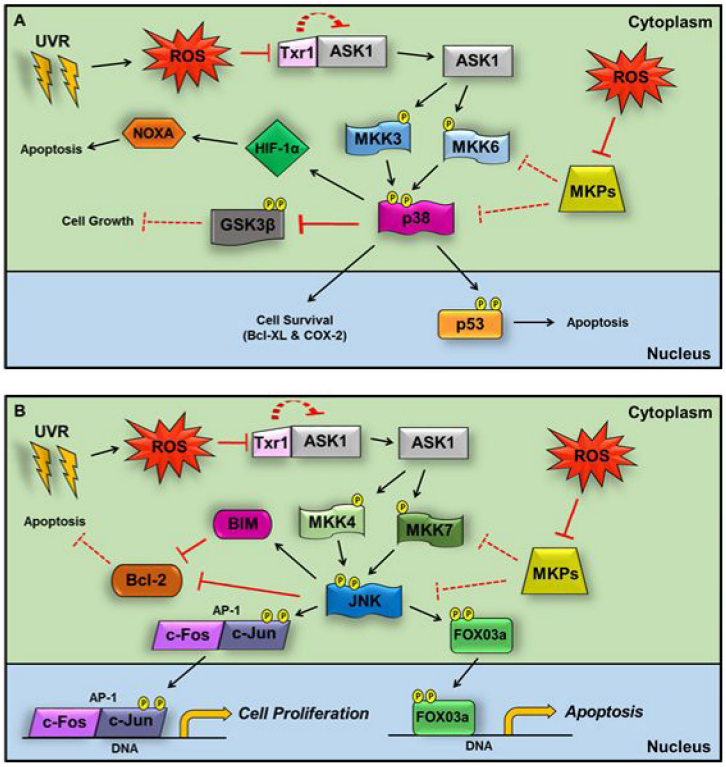

Figure 3. UVR-induced reactive oxygen species activate p38- and JNK-dependent signaling

UVR-induced activation of p38 and JNK in keratinocytes can elicit both pro- and anti-survival mechanisms. **(A.)** In the presence of ROS, ASK1 is activated by dissociation from Trx1. ASK1 phosphorylates MKK 3 and 6, which activate p38 by phosphorylation at Thr^{180} and Tyr^{182}. ROS production also inhibits MKPs, which results in increased p38 activation. Once activated, p38 can act on multiple targets. It can promote apoptosis by phosphorylating p53 at Ser^{33} and Ser^{46} or induce NOXA-dependent apoptosis through increased protein expression of HIF-1α. Conversely, p38 can increase cell survival by upregulating Bcl-XL and COX-2 or direct phosphorylation of GSK3β at Thr^{43} and Thr ^{390}. ***(B.)*** Analogous to p38, after phosphorylation by ASK1, MKK4 and 7 activate JNK by phosphorylating Thr^{183} and Tyr^{185}. Reduced MKP activity can also activate JNK. JNK targets include the AP1 transcription factor, which stimulates cell proliferation in the presence of UVB as a result of JNK phosphorylation of c-Jun at Ser^{63} and Ser^{73}. Conversely, JNK can respond to excessive damage by activating apoptosis. High dose UVB can induce JNK-dependent nuclear localization of the pro-apoptotic factor FOXO3a, and JNK can inhibit the pro-survival Bcl-2 either directly or indirectly though activation of BIM.

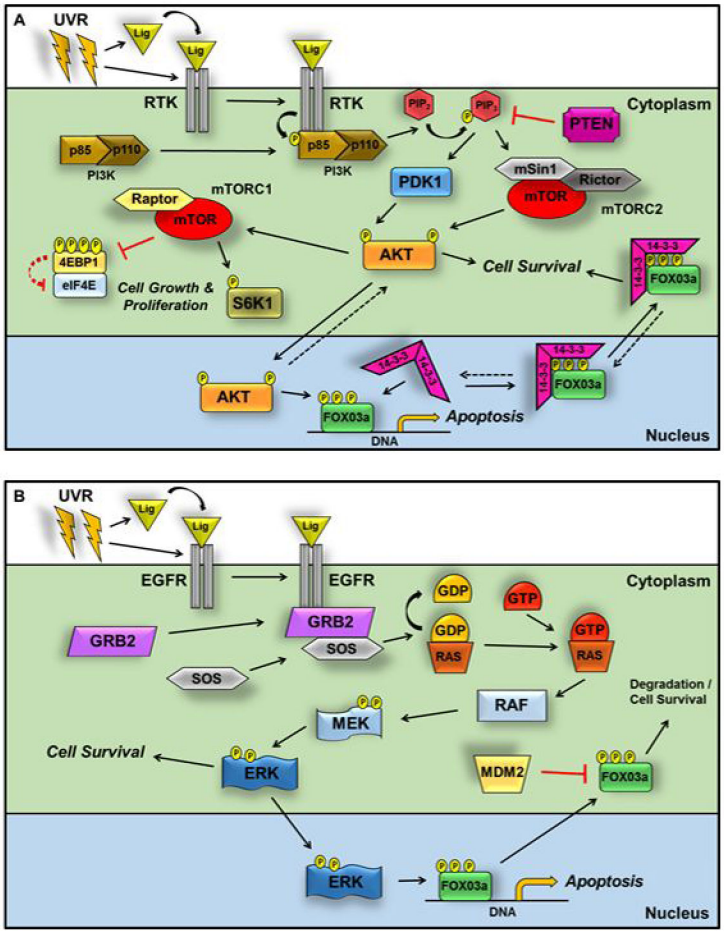

Figure 4. RTK activation by UVR results in an anti-apoptotic and cell proliferative response through PI3K/mTOR and Raf/MEK/ERK-dependent pathways

(*A.*) Autophosphorylation of multiple UVR-inducible RTKs activates the PI3K heterodimer by recruitment to the receptor and binding of the p85 subunit SH2 domain. Phosphorylation of p85 at Tyr^{458} activates the heterodimer, and PI3K phosphorylates PIP_2 to generate PIP_3. PIP_3 recruits PDK1 and AKT to the receptor complex, where PDK1 activates AKT by phosphorylation of Thr^{308}. Once phosphorylated, AKT leads to mTORC1 activation (see text for details), which regulates proliferation and ribosome biogenesis by phosphorylation of S6K1 at Thr^{389} and cap-dependent translation by phosphorylation (at Thr^{37}, Thr^{46}, Ser^{65}, and Thr^{70}) and degradation of 4EBP1. PTEN negatively regulates PI3K-dependent signaling though PIP_3 dephosphorylation and conversion to PIP_2. To activate mTORC2, the PH domain of SIN1 binds to PIP_3 to uncover the active kinase site of mTOR. PIP_3 recruits AKT to mTORC2, and mTORC2 phosphorylates AKT at the hydrophobic motif site Ser^{473}. Dual phosphorylation of AKT at Thr^{308} and Ser^{473} controls cell survival pathways by inhibition of pro-apoptotic proteins such as the FOXO3a transcription factor. AKT-dependent phosphorylation of FOXO3a at Thr^{32}, Ser^{256} and Ser^{319} causes association of the 14-3-3ζ chaperone, which binds to this motif and sequesters FOXO3a in the cytoplasm. **(*B.*)** When Raf/MEK/ERK signaling is stimulated, GRB2 and SOS are recruited to the activated EGFR. Following exchange of GDP for GTP, the Ras GTPase induces RAF activation, which

phosphorylates MEK1 and 2 at Ser^{217} and Ser^{221}, respectively. ERK is activated by MEK-dependent phosphorylation at Thr^{202} and Tyr^{204} and regulates numerous downstream targets (see text for details), including FOXO3a. Phosphorylation of FOXO3a at Ser^{294}, Ser^{344}, and Ser^{425} triggers exit of FOXO3a into the cytoplasm in a 14-3-3-independent manner. In the absence of its association with 14-3-3, FOXO3a is targeted for MDM2-mediated cytoplasmic degradation.

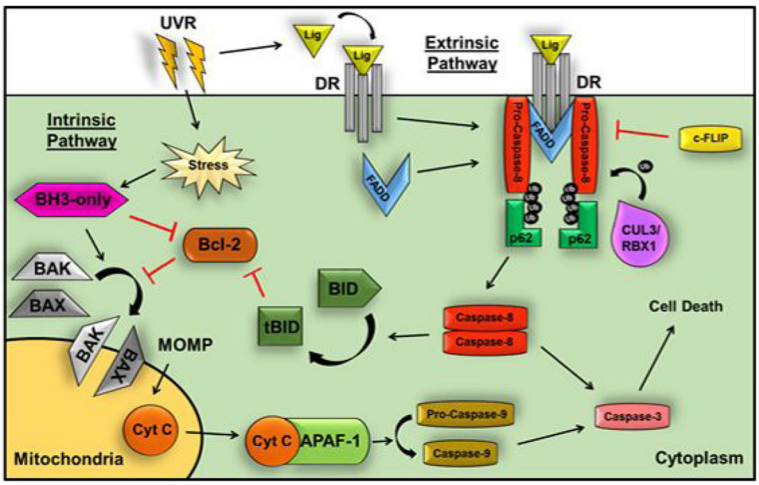

Figure 5. UVR can activate both intrinsic and extrinsic apoptosis

Extrinsic apoptosis is initiated by ligand binding to DRs such as FasR and TRAIL-R1/2, which trimerize and recruit FADD to intracellular death domain binding sites. FADD then recruits the pro-caspase-8 dimer to form the DISC. Ubiquitination of pro-caspase-8 by CUL3/RBX1 allows binding of p62 and cleavage of pro-caspase-8 into its active form. The caspase-8 dimer causes the downstream activation of pro-caspase-3 to induce cell death. This process is inhibited by c-FLIP binding to FADD, which prevents caspase-8 dimerization and activation. Intrinsic apoptosis is activated by UVR in response to both DNA damage and hypoxia. In the absence of UVR, the activities of BAX and BAK are inhibited by Bcl-2 family proteins, whereas UVR exposure causes the BH3-only proteins to bind to and inhibit Bcl-2, allowing BAX and BAK to generate mitochondrial outer membrane permeabilization (MOMP) and subsequent release of cytochrome C into the cytosol. In the cytosol, cytochrome C binds APAF-1 to recruit and activate pro-caspase-9, which initiates caspase-3-dependent cell death. Caspase-8 can also cleave BID to generate tBID, which promotes mitochondrial-mediated cell death, thus linking the intrinsic and extrinsic pathways.