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Ultraviolet B Regulation of Transcription Factor Families:

Roles of Nuclear Factor-kappa B (NF-κB) and Activator Protein-1 (AP-1) in UVB-Induced Skin Carcinogenesis

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Abstract

Prolonged and repeated exposure of the skin to ultraviolet light (UV) leads not only to aging of the skin but also increases the incidence of non-melanoma skin cancer (NMSC). Damage of cells induced by ultraviolet B (UVB) light both at the DNA level and molecular level initiates the activation of transcription factor pathways, which in turn regulate the expression of a number of genes termed the "UV response genes". Two such transcription factor families that are activated in this way are those of the nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) families. These two transcription factor families have been identified to be involved in the processes of cell proliferation, cell differentiation and cell survival and therefore play important roles in tumorigenesis. The study of these two transcription factor pathways and the cross-talk between them in response to UVB exposure may help with the development of new chemopreventive strategies for the prevention of UVB-induced skin carcinogenesis.

Keywords

Ultraviolet B-radiation (UVB); Nuclear Factor-kappa B (NF-κB); Activator Protein-1 (AP-1); transcription factor families; UV response genes; photochemoprevention

INTRODUCTION

The incidence of skin cancer in the US population has been increasing over the past few years and is expected to continue to rise as the population ages. This is mainly due to the depletion of the ozone layer and subsequent amplification of ultraviolet radiation (UV) that is now reaching the earth's surface [1]. Each year over 1,000,000 new cases of non-melanoma skin cancer (NMSC) are reported in the United States making up 40% of all diagnosed cancers.

Chronic exposure to sunlight is the major etiological factor for the development of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) [2]. Premature skin aging, sunburns, immunesuppression and activation of latent viruses are also well-documented events that take place after sun exposure [3]. The spectrum of UV from sunlight can be separated into three wavelengths, UVA (320-400nm), UVB (280-320nm) and UVC (200-280nm). Primarily UVA and UVB reach the earth's surface as UVC is filtered out by the ozone layer [4]. Both UVB and to a lesser extent UVA are responsible for sunlight induced cancers of the skin [5,6]. UVA accounts for 90-99% of the UV that reaches the earth's surface with UVB contributing the

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other 1-10% [7]. However, UVB has been identified to be 1,000-10,000 times more carcinogenic than UVA. Both UVB and UVA have been demonstrated to be complete carcinogens capable of initiation, promotion and progression of exposed epidermal cells leading to squamous cell carcinoma in mouse models. Using these models, UVB irradiation has been identified to initiate and promote the formation of mouse skin tumors while UVA acts primarily as a tumor-promoting agent [8,9].

UV Exposure and DNA Damage

DNA damage and mutations are hallmarks of UV exposure. DNA damage after UVA radiation is likely due to the creation of reactive oxygen species, leading to characteristic mutations of G to T transversions, as DNA does not significantly absorb UVA [10]. UVB on the other hand is absorbed by DNA and induces photoproducts such as DNA strand breaks, DNA crosslinks, DNA-protein crosslinks, DNA cyclobutane dimers, 6-4 photoproducts and cytosine photohydrates [7]. It has been shown that cyclobutane pyrimidine dimers (CPDs) contribute 80% of mutations that are caused by UVB exposure to mammalian cells [11]. These dimers when not repaired lead to signature mutations (C-T or CC-TT) upon DNA replication and are observed in the tumor suppressor gene p53 in sun-damaged skin, actinic keratoses and SCCs [12]. The role of the *p53* tumor suppressor gene is to induce cell cycle arrest allowing time for repair of these DNA lesions or to move the damaged cells towards death or apoptosis when these mutations are too severe [13]. Mutations of the *p53* gene have been identified in almost all SCC skin cancers and in 50% of all other human cancers [14]. These mutations in the *p53* gene are observed early in UV-induced skin carcinogenesis and it has been suggested that this mutation maybe a marker for "initiated" cells [15]. Once a cell has been initiated the process cannot be reversed and may occur at any time throughout life [16].

Exposures to UVB and UVA also have effects on tumor promotion through the up-regulation of gene transcription known as the "UV response". Events leading to tumor promotion through UVA exposure have been recently reviewed [17], so UVB will be emphasized throughout the rest of this paper. Proteins that are induced after UVB exposure can be classified by their functionality which include the proto-oncogene products (such as AP-1 and NF-κB family members, and p53), growth factors (interleukin 1α (IL 1α), basic fibroblast growth factor (bFGF)), target genes of transcription factor families (such as AP-1, NF-κB and p53) [18] and differentiation specific markers (keratins) [19]. It has been recently reported that the death-domain-containing protein PIDD plays a role in DNA-damage-induced NF-κB activation by amplifying sumoylation and ubiquitination of NEMO, identifying a possible mechanism for transcription factor activation after DNA damage [20]. Both AP-1 and NF-κB transcription factor families are known to play roles in cell differentiation, cell proliferation and cell survival and therefore play important roles in tumorigenesis [21-23]. Inhibition of p53-dependant transcriptional activity leads to increased UVB-induced AP-1 and NF-κB activation. The same was observed with p53 deficient cells suggesting that p53 plays an inhibitory role on the pathways that lead to AP-1 and NF-κB activation after UV exposure [24]. NF-κB and AP-1 will be discussed later within this review.

Single UVB Exposure Events in the SKH-1 Hairless Mouse Model

A number of *in vivo* models have been utilized to determine the molecular events that take place after UVB exposure to the skin. One of these models is the SKH-1 hairless mouse, which is the most common and highly sensitive model for photocarcinogenesis. Single exposure effects on SKH-1 hairless mice provide insight into the early damaging and signaling events after UVB radiation. Exposure of SKH-1 mice to a single dose of 180mJ/cm^2 UVB results in reddening of the skin (erythema) 3 hours post exposure, 85% of epidermal cells being positive for thymine dimers (1 hour post UVB), and 76% showing DNA strand breaks (1 hour post UVB). Increases in wild-type p53 and p21(WAF1/CIP1) proteins are also observed after 1-2

hours and the levels of both these proteins reach maximal levels 8-12 hours post UVB exposure [25]. Work from this laboratory has also identified rapid activation of the p38 and PI3K pathways after a single exposure of UVB in SKH-1 mouse skin [26]. Both of these pathways are known to be involved in the activation of the transcription factor AP-1, a known skin tumor promoter that will be discussed further in later sections of this review. Increased expression of COX-2 in response to UVB was also observed after this single exposure, a response previously seen in human skin and cultured keratinocytes [27]. Other groups have examined alterations in the cell cycle machinery after a minimal erythemic dose delivered to the skin showing that most cells of the epidermis entered S phase around 24 hours post exposure [28]. Expression of cyclin D1, A and E increased 12 hours post UVB exposure, as did cdk-2 but not levels of cdk-4 or cdk-6.

Chronic UVB Exposure Events in the SKH-1 Hairless Mouse Model

Chronic UVB exposure of SKH-1 mice allows for the analysis of proteins that are up regulated within both the skin and squamous cell carcinomas of these animals. Expression of p53, p21, bax and E2F-1 plus the presence of epidermal thickening and sunburn cells have been shown to be induced in chronically exposed skin, while bcl-2 protein expression decreases [29]. Others have observed increases in COX-2 protein expression after UVB exposure and over-expression of COX-2 in squamous cell carcinomas in both humans and mice [30]. Expression of cell cycle markers cyclin D1 and cyclin A have also been examined suggesting that expression of p53 and cyclin D1 together correlate with the development of skin tumors [31]. Over-expression of cyclin D1 has been observed in squamous carcinoma and papillomas but not in normal or hyperproliferative skin while decreased expression leads to reduced skin carcinogenesis [32]. Kim *et al*. [31] reported that the magnitude of cyclin D1 over-expression correlates with skin tumor progression, and the onset of cyclin D1 accumulation coincides with the sudden increase in the number of tumors per animal. Considering the persistent expression of cyclin D1 during UVB-induced murine skin carcinogenesis, cyclin D1 might contribute to the neoplastic process by providing a growth advantage during the early stages of tumor promotion. Experiments from this laboratory utilizing an SKH-1 mouse model expressing a dominant negative *c-jun* (TAM67) in the epidermis showed significantly decreased levels of cyclin D1 in SCCs from these transgenic mice, when compared to non-transgenic littermates, but did not affect basal levels of cyclin D1 expression [33]. Expression of antisense cyclin D1 *in vitro* and *ex vivo* has been observed to increase apoptosis and tumor shrinkage in human squamous carcinomas suggesting that observations seen in murine experiments may correlate with human skin cancer development [34].

A recent report has added further insight into the multiple molecular events that take place as normal skin develops into SCCs after UV exposure. Through the utilization of serial analysis of gene expression (SAGE) a number of unique genes were identified that have not been previously associated with skin carcinogenesis [35]. This collaborative group identified changes in expression of 200 genes when comparing SCCs to normal SKH-1 mouse skin. A number of genes identified in SCCs were also observed to be increased by an acute exposure of UV including those of keratins 6a, 6b and 16, small proline-rich proteins and S100 calcium binding protein A9. Those with decreased expression included keratin 1 and annexin A8. Studies such as these not only identify pathways that are regulated by exposure to UV but allow for the identification of early biomarkers for the onset of UV-induced carcinomas.

As mentioned above, two transcription factor families that are activated after UVB exposure of mammalian cells are AP-1 and NF-κB. Both pathways are known to play roles in cell proliferation, cell survival and cell differentiation and are therefore involved in the process of tumorigenesis. These two transcription factor families will now be discussed further.

ACTIVATOR PROTEIN-1 (AP-1)

The activator protein-1 (AP-1) transcription factor family is made up of homodimers or heterodimers of Jun or heterodimers of Jun-Fos proteins. There are three members of the Jun protein family, namely c-Jun, JunB and JunD while the Fos family is composed of c-Fos, FosB, Fra-1 and Fra-2. Both Fos and Jun family members contain basic region leucine zipper (bZIP) domains allowing them to bind to other proteins that contain a bZIP domain. Investigations have identified interactions between c-Jun and ATF-2 (a bZIP family protein) that lead to a mutual regulation of these two proteins[36]. Activation and subsequent binding of AP-1 protein dimers to specific DNA sequences bring about cell proliferation and survival effects by regulating the expression and function of a number of genes such as collagenase-1, c-Jun, cyclin D1, p21, p19 and p16 [22,37]. Evidence for the role of AP-1 in skin tumor promotion and progression have been identified through utilizing the JB6 cell model [38], with later investigations demonstrating that constitutively elevated levels of AP-1 are present in malignant but not benign mouse tumors [39]. For skin tumor promotion and progression, it would appear that repeated transient activations of AP-1 are required [38].

The utilization of a dominant negative c-Jun (TAM67) under the control of the human keratin-14 (K14) promoter, which directs expression of TAM67 to the basal cells of the epidermis in transgenic mice models, has allowed further understanding of the role of AP-1 in skin cancer development. Transgenic mice expressing TAM67 have demonstrated that functional AP-1 is required for phorbol ester induced tumor promotion in multistage mouse skin carcinogenesis [40] and for okadaic acid induced skin tumor promotion [41]. Experiments from this laboratory using SKH-1 hairless mice that express K14-TAM67 have identified a functional role for AP-1 in UVB-induced skin carcinogenesis [33] with a decrease in tumor size being correlated with the expression of cyclin D1. Taken together, these investigations suggest that the specific signaling pathways that are inhibited by the expression of a dominant negative c-Jun are involved in both chemical and UVB-induced skin carcinogenesis.

Studies from this laboratory in human keratinocytes have identified that the AP-1 family protein heterodimer of c-Fos and JunD binds to an AP-1 cis element in response to UVB exposure [42]. Expression of c-Fos protein is induced in response to UVB exposure where levels of JunD remain constant. Several signaling cascades have been identified to be important for c-fos transcription. These include the mitogen activating protein kinases (MAPKs) such as c-Jun amino terminal kinases (JNKs), extracellular signal related kinases (ERKs) and p38 MAP kinases. Activation of both p38 and ERK are involved in c-fos expression in human keratinocytes after UVB irradiation [43] and are therefore potential targets for chemopreventive agents. Inhibition of the p38 and ERK cascades in a squamous cell carcinoma cell line has been shown to inhibit UVA and UVB-induced MMP-1 and MMP-10 expression suggesting a role for AP-1 in their regulation [44]. Similar results have also been identified *in vivo* in human skin where a low dose of UVB upregulates AP-1 and NF-κB with the induction of metaloproteinase proteins also being observed [45]. Involvement of these signaling cascades in AP-1 activation has recently been published by Zenz and Wagner [46] as well as Bowden [47] and therefore will not be discussed further.

Recent investigations from this laboratory targeting the p38 and phosphatidylinositol 3-kinase (PI3K) pathways, through the topical application of pharmacological inhibitors before UVB exposure, have identified that these two pathways are involved in the activation of COX-2 and AP-1 in the skin of SKH-1 hairless mice [26]. The activation of the p38 and PI3K pathways were observed to be rapid, both starting within 30 minutes post UVB exposure. Through the utilization of an SKH-1 hairless luciferase reporter mouse model, SB202190 or LY294002 topical application before UVB exposure was seen to significantly inhibit UVB-induced AP-1 transactivation 48 hours post UVB exposure, as was expression of COX-2 protein. Other

investigators also examined the activation of p38 MAPK in response to UVB-induced inflammation and identified, through the use of a pharmacological inhibitor (SB242235), the inhibition of COX-2 and p38 MAPK signaling. This group also identified the inhibition of IL-6 and KC (murine IL-8) after a 30-minute oral gavage treatment of SB242235 before UVB exposure [48]. Taken together these findings identify the p38 MAPK and PI3K pathways as playing major roles in UVB-induced AP-1 activation and COX-2 expression which have both been established to be involved in UVB-induced skin carcinogenesis. Long-term UVB studies are therefore essential to identify the potential use of p38 and PI3K inhibitors as chemopreventive agents.

NUCLEAR FACTOR-KAPPA B (NF-κB)

The mammalian Rel/NF-κB transcription factor family is made up of 5 family members which function as hetero- or homodimeric transcription factors. Family members belong to two classes; those of the Rel proteins include RelA (p65), RelB and c-Rel and those of the NF-κB family that include NF-κB1 (p105/p50) and NF-κB2 (p100/p52). Members of the Rel family contain a Rel homology domain at the N-terminus allowing for dimerization and DNA binding, and a C-terminal transcription-modulating domain. NF-κB family members also contain the Rel homology domain but have a series of ankyrin repeats at the C-terminus [49]. The Rel homology domain is responsible for DNA binding, dimerization and also contains a nuclear localization sequence (NLS). The family members function as hetero- or homodimeric transcription factors with the abundant dimer pairing being that of p65/p50 proteins. These dimers are retained as an inactive complex in the cytoplasm of the cell bound to a family of inhibitor proteins, the IκBs. Members of the Rel/NF-κB family have been shown to regulate genes that are involved in immunity, inflammation, cell cycle, apoptosis and oncogenesis [49,50]. Expression of the inhibitor protein IκBα is also under the control of NF-κB leading to a tightly regulated negative feedback loop. A number of investigators have suggested a role for the transcription factor NF-κB in the initiation, development and promotion of carcinomas of the skin (reviewed by Bell *et al*. [51]).

Numerous stimuli have been identified to activate NF-κB including cytokines, lipopolysaccharide (LPS), UV light and physical stress (reviewed in [52]). Three pathways have been identified for the activation of NF-κB allowing for its translocation from the cytoplasm to the nucleus where it binds to specific sequences on target gene DNA. In the “traditional” pathway, stimuli such as pro-inflammatory cytokines activate the IκB kinase (IKK) complex, which in turn phosphorylates the inhibitor protein IκBα at serines 32 and 36. This phosphorylation leads to polyubiquitination of lysines 21 and 22 targeting the IκBα protein for subsequent 26S proteosomal degradation. Degradation of IκBα exposes the NLS of the NF-κB proteins allowing for translocation of the protein dimer to the nucleus where they bind to DNA and affect gene expression [52]. Two other pathways of NF-κB activation that have been identified; those of tyrosine phosphorylation-dependant activation of NF-κB, and a pathway that effects NF-κB2/p100 dimerization with RelB in the cytoplasm [53,54]. However, the role of these latter two pathways in UVB activation of NF-κB has not been published.

Previous work in this laboratory has identified that exposure of a mouse keratinocyte cell line (308 cells) to a dose of 250J/m^2 UVB causes a biphasic NF-κB DNA binding response [55]. Continuing work from the Bowden laboratory has identified that the early UVB-induced NF-κB DNA binding response is not through degradation of the inhibitor protein IκBα whereas the later activation event is, as measured by western blotting techniques (unpublished data). Publicationsfrom the Peyron group identified a pathway that leads to NF-κB activation, but not with associated IκBα degradation, through phosphorylation of tyrosine groups on IκBα [53,56]. Preliminary experiments (unpublished data) have identified that transient transfections of IκBα tyrosine mutants at Tyr42 and Tyr305 (kind gifts from Dr. Peyron) can inhibit UVB-

induced transactivation of NF-κB by up to 50%, the same inhibition seen with transient expression of an IκBαM (Ser32 and Ser36) mutant. Further examination of these events is ongoing but these findings suggest that UVB-induced NF-κB activation maybe through two distinct pathways, those of tyrosine phosphorylation and later serine phosphorylation of IκBα.

A number of recent publications have questioned the role of NF-κB in skin carcinogenesis. Several of these publications have pointed to the involvement of NF-κB in the initiation, development and promotion of skin cancer. Use of a SENCAR mouse model has identified that expression of NF-κB in normal epidermis is localized to the cytoplasm of basal cells. However, after a two-stage carcinogenesis protocol, levels of p50 and p52 were elevated and expression of IκBα was reduced in squamous cell carcinomas and papillomas [57]. The best-characterized subunit of NF-κB, p65 has been identified to be necessary for transformation of a mouse epidermal tumor promoter resistant cell line. The difference between susceptible (P+) or resistant (P-) JB6 cells to tumor promoter-induced neoplastic transformation was not due to altered NF-κB protein levels or degradation of IκBα but to an inability of the P- cells to activate p65 [58]. Recently published work suggests a link between α-catenin and NF-κB [59] identifying that reduced α-catenin, activated NF-κB and inflammation are common features in human squamous cell carcinomas. Evidence suggests that inflammation may be a prerequisite of tumorigenesis, and as hypothesized in one review, NF-κB may be a crucial mediator of inflammation-induced tumor growth and progression [60].

Use of NF-κB decoy oligonucleotides have further identified that UV activation of NF-κB and its downstream target genes lead to inflammatory events, a critical process for the development of sunburn in the skin [61]. Inhibition of the expression of a number of UVB-induced proinflammatory cytokines including interleukin-1 and -6 were observed in this system. Continuation of this work identified that topical application of NF-κB decoys to hairless rat skin inhibited UV-induced sunburn cell formation but not erythema [62]. Parallel experiments in a mouse keratinocyte cell line identified that the down regulation of NF-κB caused enhanced UV-induced apoptosis suggesting a mechanism for the observations *in vivo* [62].

Transgenic mouse models have provided insight into the role of the NF-κB pathway in skin development and physiology. Most of these models have sought to inhibit NF-κB expression and activation in the skin through dominant-negative forms of the inhibitor protein IκBα linked to specific keratin promoters. Transgenic mice created by Seitz *et al*. expressing an IκBα mutant under the control of the K14 promoter displayed an absence of NF-κB subunit expression in the suprabasal layers with all mice developing hyperplasia 4 days after birth. These mice exhibited growth retardation, inhibition of normal hair formation and died 5-7 days after birth [63]. Expression of p50 under the control of K14 led to epidermal hypoplasia with the epidermis being made up of as little as two viable cell layers with mice dieing within 5 days of birth. As suggested by the authors of this study, functional NF-κB appears necessary for growth inhibition in stratified epithelium. Further work by this group identified NF-κB's role in protecting normal epithelial cells from TNFα and Fas induced apoptosis suggesting a role for NF-κB in the regulation of homeostasis in stratified epithelium [64].

A second group who expressed mutant IκBα in the epidermis of mice under the control of a K5 promoter observed similar results as Seitz *et al*. but noted increased basal numbers of apoptotic cells and most surprisingly the spontaneous appearance of SCCs. Exposure of these mice to UVB led to increased UV-induced apoptosis suggesting that NF-κB plays a protective role within the skin in regard to programmed cell death [65]. Later work by this group demonstrated that the spontaneous skin tumors that appeared in these NF-κB deficient transgenic mice developed independently of Ha-ras or p53 gene mutations, hallmark signs of initiated epidermal cells [66].

Investigations such as these in transgenic animals bring up questions with regard to the use of chemopreventive agents for the down-regulation of NF-κB in the epidermis. Dajee *et al*. furthered these concerns by observing that the blocking of NF-κB activation in human epidermal cells expressing oncogenic Ras leads to the generation of malignant human epidermal tissue that resemble SCCs [67]. However, a number of questions should be asked about these findings. In both the transgenic mouse models and the genetically blocked NF-κB human epidermis model, expression of NF-κB is completely blocked, a phenomenon difficult to replicate with treatment of the epidermis with chemopreventive agents. The inability to completely block specific UVB-induced pathways has been observed in this laboratory after topical application of pharmacological inhibitors to the skin of SKH-1 mice [26]. Also, it is well documented that knocking out the expression of NF-κB family members leads to embryonic or neonatal lethality [63,68-70] possibly due to the need for NF-κB in neonatal development. Investigations looking into the functions of NF-κB in adult mouse skin have shown that deleting RelA (p65) leads to epidermal hyperplasia but is not accompanied by abnormal differentiation, inflammation or apoptosis [71], suggesting that NF-κB in adult skin has a different role than observed in neonatal and developing epidermis. Support for this comes from recent investigations identifying that RelA and c-rel control development of the epidermis during embryogenesis but regulate homeostasis of the epidermis in adult skin [72]. With such contradicting data on the role of NF-κB in the epidermis, further investigation in both normal and initiated skin needs to be undertaken.

It is also possible that by inhibiting the NF-κB pathway, other pathways are being down and/or up-regulated in the epidermis. Numerous agents that are inhibitors of NF-κB are also observed to inhibit AP-1 suggesting cross-talk between transcription factor families. This cross-talk may occur through direct protein-protein interactions or through interactions between active signaling pathways and will be discussed below.

CROSS-TALK BETWEEN AP-1 AND NF-κB

Investigations have shown that both AP-1 and NF-κB are required for maintaining a transformed phenotype whereas inhibition of either family suppresses the transformation response in human keratinocytes, transgenic mice and JB6 cells [23,73]. Physical interactions between AP-1 and NF-κB have been reported and are known to take place via interactions between the bZIP and Rel homology domains. These interactions lead to enhanced DNA binding and physiological function [74]. More recent investigations into these physical interactions utilizing the bimolecular fluorescence complementation (BIFC) assay have also confirmed that interactions between p50 and p65 with Fos and Jun proteins are mediated by the bZIP domains [75]. However, these interactions did not require DNA binding by Fos or Jun or an intact leucine zipper dimerization domain. Co-transfection of either p50 or p65 with Jun was also observed to inhibit the transcriptional activity of a collagenase-1 reporter. Other investigators have identified that overexpression of Jun-D in rat hepatocytes leads to a threefold induction of NF-κB transactivation [76].

The dominant negative c-Jun (TAM67) has been a useful tool for examining the interactions between AP-1 and NF-κB. Studies have identified that TAM67 is able to physically interact with p65 within the nucleus of keratinocytes leading to reduced expression of NF-κB target genes and also suppressing the tumor phenotype in these cells [77]. A recent publication from this laboratory has identified that TAM67 is able to physically interact with the transcription factors CREB, ATF-1 and ATF-2 [78] where further work identified that TAM67 was also able to physically interact with p50 and two inhibitor proteins of NF-κB, namely IκBα and IκBβ[55]. Activation of UVB-induced AP-1 and NF-κB transactivation are inhibited by the expression of TAM67 or a phosphorylation deficient c-Jun in mouse keratinocyte 308 cells, but it appears that the inhibition of these two transcription factor families are through two

different components of the jun bZIP domain. It was observed that expression of the complete bZIP domain inhibited both UVB-induced NF-κB and AP-1 transactivation whereas expression of the leucine zipper inhibited only NF-κB. From these studies it could be suggested that mimetics of the two components of the bZIP domain would be useful for the specific inhibition of two pathways that are known to be involved in NMSCs [55]. These studies agree with results from pancreatic cancer cell lines where expression of a leucine zipper protein inhibits NF-κB transactivation in response to TNFα or TPA but has no effect on AP-1 reporter gene expression [79].

There is also evidence in the literature that NF-κB regulation can be influenced by signaling cascades that are involved in the activation of AP-1. The phosphorylation of p65 at S536 after TNFα exposure in the JB6 mouse model appears to be regulated by levels of ERK. These investigators showed that low levels of ERK led to decreased levels of IKKβ resulting in insufficient phosphorylation of p65 at S536 [80]. Other investigations in JB6 cells utilizing the tumor promoting effects of arsenic have found that inhibition of ERKs or JNKs cause dramatic decreases in NF-κB activity [81]. In mouse skin, the PI3K/Akt pathway has been implicated in the transactivation of NF-κB after exposure to diesel exhaust particles [82], whereas constitutive activation of Akt in melanoma has been linked to enhanced NF-κB nuclear localization and transactivation [83]. A suggested mechanism for Akt's influence on NF-κB activation is through Akt's physical and cooperative association with Cot (Tpl-2), a serine/threonine kinase able to activate MAPK signaling pathways that target AP-1 [84].

Although these kinase-NF-κB interactions have not been described in UVB-induced systems, most appear to be activated via cytokines that are up-regulated in response to cellular stress. Activation of specific signaling pathways and their interactions with NF-κB as well as dual inhibition of AP-1 and NF-κB target genes seem promising strategies for cancer prevention and maybe cancer treatment.

Agents such as chlorogenic acid and emodin have been recently identified to inhibit both NF-κB and AP-1 transcription factor families and decrease the phosphorylation of a number of signaling cascades. Treatment of JB6 cells with chlorogenic acid inhibits UVB-induced AP-1 and NF-κB transactivation, and decreases the phosphorylation of JNK, p38 and MAPK kinase 4, while stimulating the enzymatic activities of glutathione S-transferase and NAD(P)H:quinone oxidoreductase [85]. Inhibition of AP-1 and NF-κB by emodin, an active component of the herb *Rheum palmatum* inhibits TPA induced invasion of human squamous cell carcinoma cells *in vitro* through the decreased expression of MMP-9. The phosphorylation of ERK and JNK were also suppressed but no effects were observed on p38 kinase phosphorylation [86].

The application of compounds such as those mentioned above whose mechanism of action are through multiple transcription factor and signaling cascade targets have the potential to be chemopreventive agents for photocarcino-genesis.

CHEMOPREVENTION

Prevention of skin cancer such as avoidance of excessive sun exposure, wearing protective clothing, the application of sunscreens and blocks, and application of UV blocking films to automobile and house windows have helped to lower the risk of skin cancer but are still not completely effective [3,87]. Education, of course, is still the key but with application of sunscreens, people are spending more time in the sun due to their belief of the added protection. Studies have reported mixed findings when looking at the ability of sunscreens to protect against NMSCs with some reports indicating that due to the increased exposure time, increases in the incidence of malignant melanoma are being observed [88-90]. Therefore, new-targeted approaches are needed for the chemoprevention of skin cancer. Chemoprevention, as defined by the Oxford English Dictionary, is the prevention of a disease, especially cancer, by the

administration of drugs, dietary supplements, plant extracts etc. Potential agents for chemoprevention should of course be easy to apply or administer, have few side effects and be specific for the molecular target of choice. Possible mechanisms for their action would be to hinder events that lead to initiation by such mechanisms as scavenging DNA-reactive electrophiles and free radicals, enhancing activation of detoxifying enzymes and altering certain DNA repair mechanisms. Inhibiting processes of promotion and progression by scavenging of reactive oxygen species, manipulating genes that are involved in cell proliferation, differentiation and apoptosis and by decreasing inflammation [91] should also be considered. Due to the recent publication of excellent reviews on photochemoprevention and UVB light signaling [47,92], select studies will be mentioned here.

Early investigations using topical applications of Aspirin have shown the inhibition of UVB-induced AP-1 transactivation in AP-1 luciferase reporter mice [93]. Chronic UVB exposure protocols using topical treatment of aspirin or sodium salicylate furthered these findings and reported a delayed UVB-induced tumor formation in the treated groups when compared to vehicle control SKH-1 mice [94]. Perillyl alcohol topical pretreatment has also been observed to inhibit tumor incidence and multiplicity, average tumor size, and the average tumor burden/mouse while inhibiting UVB-induced AP-1 transactivation [95].

More recent investigations have identified a number of pharmacological and natural agents that inhibit the expression of UVB-induced AP-1. Treatment of primary mouse keratinocytes before exposure to UVB with all-trans-retinoic acid or 9-cis-retinoic acid inhibited AP-1 activity by more than 50% [96]. Treatment of the human keratinocyte cell line HaCaT with curcumin was observed to not only inhibit UVB-induced AP-1 DNA binding activity but also to inhibit mRNA and protein expression of COX-2, and the activation of p38 MAPK and JNK [97]. Parthenolide, a sesquiterpene lactone from the plant Chrysanthemum parthenium (a well known plant in natural medicine) when fed to mice was able to inhibit the onset of UVB-induced papilloma incidence, significantly reduce the number of papillomas per mouse and reduce the tumor size when compared to the UVB-only group. Further experiments in JB6 cells suggest that the actions of this agent are through decreased AP-1 DNA binding and activation after UVB exposure, and through the inhibition of JNK and p38 kinase activation which in this study led to UVB-induced apoptosis of the JB6 cells [98].

Many investigators are looking to natural products such as extracts from fruits, vegetables and herbs as agents for chemoprevention as they are tolerated well by the body even at high concentrations [99]. Investigations with green tea, the most commonly consumed drink in the world after water, have been shown to have chemopreventive properties in UVB-induced skin cancer models leading to lower tumor numbers per mouse and lower percentage of animals with tumors [100,101]. A search of the literature for the past year (2005) using the keywords “Tea” and “UVB” sheds more light on the chemopreventive properties of green tea. Interestingly, publications from the Conney group report that oral administration of green tea or caffeine inhibited UVB-induced formation of mutant p53 positive patches (putative early cellular markers of the beginning of tumor formation) by approximately 40%. Administration of caffeine or green tea after UVB treatment also enhanced the disappearance of these patches allowing the authors to suggest that the chemopreventive affects of green tea and caffeine maybe through the promotion of apoptosis [102]. Further research from the same laboratory observed that green tea and caffeine administered during chronic UVB exposure are able to change the mutation profile of the p53 gene in mutant p53 positive epidermal patches, while treatment after the cessation of UVB eradicated patches containing homozygous p53 mutations [103]. Topical applications of green tea polyphenol (GTP) before multiple UVB exposures to the skin of SKH-1 mice have been observed to inhibit the activation of NF-κB, activation of IKKα and reduce the phosphorylation and subsequent degradation of IκBα. Modulations in the MAPKs were also observed after treatment with GTP and together these inhibitory effects in

these pathways resulted in decreases in UVB-induced skin thickness, skin edema and infiltration of leukocytes [104]. Others report that green tea polyphenols are able to activate cytotoxic T-cells and inhibit angiogenesis in UVB-induced tumors [105] while others have shown their ability to inhibit UVB-induced STAT1 (ser727), ERKs, JNKs, PDK1 and p90RSK2 phosphorylation [106].

Epigallocatechin-3-gallate (EGCG), one of the major components of green tea is also able to inhibit UVB-induced nitric oxide, NF-κB activation and the expression of IL-6 [107,108]. Treatment of cultured human keratinocytes (HaCaT) with EGCG, a major polyphenolic constituent in green tea, before UVB exposure decreases NF-κB translocation to the nucleus and decreases UVB-induced iNOS mRNA and NO generation. Numerous studies have linked the expression of iNOS to the activation of NF-κB as iNOS contains NF-κB binding sites within its promoter [108].

Vitamin D analogs have also been studied for their abilities to inhibit UV-induced skin cancer. Recent work has identified that an active vitamin D analogue (1,25-dihydroxyvitamin D_3) is able to protect primary keratinocytes against the induction of cyclobutane pyrimidine dimers caused by exposure to UVB [109]. The authors suggest that these chemopreventive events may be due to the anti-proliferative ability of these vitamin D analogs. Two other recent publications from the same group have also identified that analogs of vitamin D are able to inhibit UVB-induced apoptosis, mitochondrial cytochrome c release, the production of IL-6, and also inhibit JUN kinase activity [110,111].

Others have also studied reducing the oxidative stress after UVB exposure through inducing the phase II enzymes by treatment of HCL14 cells with sulforaphane (SF), a naturally occurring antioxidant found in broccoli. Pretreatment with SF was able to dose-dependently reduce UVB-induced AP-1 activation but further investigations showed that this effect was not by induction of phase II enzymes. Addition of SF directly to nuclear extracts was able to inhibit AP-1 DNA binding as identified by EMSA suggesting that SF plays a role in directly inhibiting AP-1 DNA binding activity [112].

Resveratrol (*trans*-3,4′,5-trihydroxystilbene) which is found at high levels in red wine and grapes has strong anti-inflammatory and antiproliferative properties. Its ability to inhibit skin thickness and edema, and to down regulate the enzyme activities of cyclooxygenase and ornithine decarboxylase in the skin of SKH-1 mice after a single dose of UVB radiation suggest that this compound may be a potential chemopreventive agent [113]. Prior application of resveratrol to the skin of SKH-1 hairless mice, before multiple UVB exposures was observed to decrease cell cycle proteins, namely cyclin D1 and 2, cdk-2, 4 and 6 while stimulating further the expression of the cell cycle inhibitor WAF1/p21 and p53. Furthermore, inhibition of UVB-induced mitogen-activated protein kinase kinase and the 42 kDa isotype of mitogen-activated protein kinase (MAPK) were also significantly decreased [114]. In chronic UVB exposure experiments, resveratrol inhibited tumor onset and incidence with pretreatment or post-treatment, up-regulated Survivin and phospho-Survivin, and down-regulated Smac/DIABLO protein expression within the skin, suggesting that resveratrol induces its chemopreventive abilities by enhancing apoptosis [115]. Another agent, proanthocyanidins, which is extracted from the seed of the grape has also shown photochemopreventive properties when given through diet to SKH-1 mice, reducing UVB-induced tumor incidence, tumor multiplicity and tumor size [116].

Silibinin, a naturally occurring flavonoid agent used as a dietary supplement in the US, has also been examined for its chemopreventive affects on UVB-induced skin carcinogenesis. Topical or dietary application before chronic exposure to UVB led to decreased tumor multiplicity, decreased tumor volume per mouse and tumor volume per tumor. Analysis of

tumors versus normal SKH-1 skin showed that Silibinin phosphorylated extracellular protein kinase 1 / 2 (ERK), stress-activated protein kinase/c-Jun NH2-terminal kinase 1 / 2, and p38 mitogen-activated protein kinase while inhibiting Akt phosphorylation. Immunohistochemical and western blot analyses determined that the actions of Silibinin were by inhibition of DNA synthesis, cell proliferation, cell cycle progression and induction of apoptosis [117]. Follow-up experiments from the same group identified that Silibinin increases the number of p53 positive cells after UVB and decreases cell proliferation, apoptotic cells and sunburn cells caused by UVB exposure. In the chronic UVB model inhibition of UVB-induced phosphorylation of MAPKs and AKT were also noted [118]. Differences in the ability of Silibinin to inhibit UVB-induced phosphorylation of MAPK/p38 between these two experiments may be due to differences in the samples examined. The first publication examines phosphorylation of the MAPK/p38 in UVB-induced skin tumor samples while the second examines chronic UVB exposed skin samples (5 day exposure). Taken together the results from these two publications may suggest a differential role for MAPK/p38 in early verses late UVB exposure events. Continuation of this work with constant feeding of Silibinin before a single dose of UVB have shown that this agent is able to decrease UVB-induced thymine dimers, PCNA stained cells, apoptotic sunburn cells and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) while increasing p53 and p21/cip1-positive cell populations in the epidermis suggesting roles for this agent in DNA repair, reducing hyperproliferation or inhibiting apoptosis [119].

A variety of berries and their extracts have also been examined for their chemopreventive properties. Observations with blackberry extracts show an ability to inhibit UVB-induced AP-1 transactivation, phosphorylation of ERKs and JNKs but not p38 kinase in JB6 cells. These extracts were also able to decrease TPA-induced neoplastic transformation of JB6 P+ cells. A suggested role for this inhibitory effect is the antioxidant properties of blackberry extracts leading to reduced reactive oxygen species [120]. Cyanidin-3-glucoside, a compound found in blackberries, inhibits UVB-induced AP-1 and NF-κB transactivation and the expression of COX-2 and TNFα in pretreated JB6 cells. The actions of this compound appear to be through the down-regulation of MAPK activity possibly through its ability to scavenge free oxygen radicals [121]. Extracts from the Lingonberry, a berry closely related to a partridgeberry, are able to inhibit UVB-induced activation of AP-1 and NF-κB in JB6 P+ cells after pretreatment. These extracts also block the UVB-induced phosphorylation of ERK1 and 2, p38 and MEK 1/2 but not JNK [122]. The same authors have more recently tested extracts from strawberries on the same cell line resulting in the observations of an inhibition of UVB-induced AP-1 and NF-κB transactivation and blocking of the phosphorylation of ERKs and JNK kinases. This group suggest that the activities of these extracts may be due to their antioxidant properties and their high concentrations of superoxide dismutase (SOD), glutathione peroxidase (GSH-POD), ascorbate peroxidase (AsA-POD) and guaiacol peroxidase (G-POD), the levels of which are comparable to those found in blackberries, and could therefore increase the cell's ability to scavenge free radicals [123].

FUTURE DIRECTIONS

The incidence of skin cancers due to sun exposure continues to rise each year. Education pertaining to the harmful effects of prolonged sun exposure and the need to protect the skin with sunscreens, blocks or clothing are not having an impact on the increase in incidence. There is a need to rethink strategies to prevent this continued rise in skin cancer and the identification of potential chemopreventive agents that can be added to sunscreens or given as an oral supplement are attractive. These supplementary agents should be site specific, well tolerated and precise for target genes and activation cascades that have been discussed in this review.

To be able to achieve such a task, the consequences of activating or inactivating these individual pathways and cascades after exposure to UVB need to be carefully dissected. The use of transgenic mouse models expressing specific genes under the control of a repressor, which can be "expressed" before an exposure, would allow for individual pathway targeting and identification of the downstream gene modulations. As seen with NF-κB transgenic mice, expression of inhibitory proteins during neonatal stages of development can lead to unexpected outcomes. These observations need to be further verified in adult skin or in a system that is representative of initiated skin so that a deeper understanding of AP-1 and NF-κB mechanisms can be elucidated. The same can be stated for the UVB-induced signaling cascades, such as PI3K and p38 kinase, so that more target-specific inhibitors can be identified and placed into studies to examine their chemopreventive potential.

Other approaches to protect the skin from solar radiation should also be investigated. The availability of small molecule inhibitors selective for the inhibition of a specific target protein or kinase needs to be studied further, again allowing for pathway-specific target identification. The idea of utilizing small peptide mimetics for inhibition of specific pathways is also an attractive idea for chemoprevention, as is the creation of pro-drugs allowing for longer residency time of the agent within the skin after topical application. Through collaborative research and further identification of UVB-induced targets, the creation and identification of natural and pharmacological photochemopreventive agents will allow for the transition of these compounds from the laboratory to the clinic.

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