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Chemoprevention of human skin cancer

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Abstract

The incidence of skin cancer has been rising in recent years with significant effects on public health. Primary prevention has proven inadequate in impacting the incidence of skin cancer, thus stimulating the development of chemopreventive strategies. The majority of skin cancer chemoprevention studies focus on occurrence of new nonmelanoma skin cancers (NMSC) in individuals with a previous NMSC, or on reduction in the number of premalignant skin lesions such as actinic keratoses (AK). Dysplastic nevi, a likely precursor of melanoma, are also potential targets for chemoprevention strategies. Premalignant lesions are especially attractive as endpoints since they are more common than frank cancer, resulting in reduced sample size, length, and cost of clinical trials. Development of new agents that affect the pathogenesis of skin cancer will be discussed, from elucidation of molecular targets to implementation of trials designed to determine the effects of chemopreventive interventions on human skin cancer.

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1. Background

1.1. Impact of skin cancer

Over 1 000 000 new skin cancers are diagnosed yearly in the US accounting for approximately 40% of all new cancer diagnoses. The incidence of skin cancer has been increasing in recent years and this increase is expected to continue as the population ages and larger amounts of ultraviolet radiation reach the earth's surface due to depletion of the ozone layer [1]. The majority (approximately 80%) of skin cancers are basal cell carcinomas (BCC), approximately 16% are squamous cell carcinomas (SCC), and approximately 4% are melanomas.

Melanoma originates from pigment-producing cells (melanocytes) within the epidermis and is by far the most likely skin cancer to metastasize and cause death. In 2000, it is estimated that there will be 47 700 new cases of melanoma and 7700 deaths [2]. Melanoma, while associated with exposure to sunlight, is thought to be related more with severe sunburns or intermittent exposure, rather than chronic exposure [3]. While vitally important to the reduction of skin cancer mortality, research on melanoma chemoprevention is still preliminary with relatively few small studies focusing on dysplastic nevi as a target for intervention [4–6]. This paucity of chemoprevention studies for melanoma is likely related to the lack of animal models. This differs from SCC for which numerous animal models exist. Recently, a number of animal models for melanoma have been developed creating the opportunity for melanoma chemoprevention studies in both animal models and humans [7,8]. Melanoma can be cured if detected early. Five year survival rates for stage I melanoma are greater than 90% [9]. However, efficacy of treatment at later stages of melanoma is very limited, increasing the need for effective chemoprevention strategies.

BCC and SCC originate from epidermal keratinocytes. BCC is a slow growing tumor that rarely metastasizes, but it can cause tremendous morbidity. In contrast, SCCs are more likely to metastasize. SCC was responsible for approximately 1200 deaths in 1998 (a number equivalent to the yearly mortality attributed to Hodgkin's lymphoma) [2,10,11]. Also, a high percentage of patients with SCC develop a second primary skin cancer within 5 years [12–14]. SCCs and BCCs occur primarily on sun-exposed areas of the body and have been strongly associated with chronic sun exposure [15]. This review will focus on chemoprevention of nonmelanoma skin cancers (NMSC).

1.2. Incidence of AK and relationship to SCC

Actinic keratoses (AK) (also known as solar or senile keratosis) is the most common precancerous dermatosis. AK is typified by atypical epithelial proliferation and appears as thickened, cornified, scaly lesions that develop due to disregulated keratinocyte maturation. AK appears to be attributable to ultraviolet (UV) radiation exposure [16]. Evidence that AKs are the premalignant precursors of SCC, but not BCC, includes shared risk factors, a histologic continuum, and the presence of similar molecular/genetic alterations in both AK and SCC [17]. Approximately 40–50% of Australians aged 40 or older have at least one AK with an average of 6–8, and this incidence increases with advancing age [17–19]. Other studies from Australia and the US have reported AKs in 11–26% of the population [17]. The rate of SCC in the population is much lower than the rate of premalignant AK demonstrating that not all AK progress to SCC [19]. Presence of AK serves as a major risk factor for increased NMSC risk. In a 5 year longitudinal study, 60% of SCCs arose from a preexisting AK while in a 10 year follow-up of AK patients there was a 6–10% rate of malignant progression to SCC or 1 per 1000 per year in individual lesions [20]. A study from Arizona in individuals with 10 or more AK reported a cumulative probability of 14% for developing an SCC within 5 years [21]. While most AKs do not progress to SCC, it is believed that AK represents SCC in situ at its earliest stages [22].

1.3. Clinical and histologic features of AK and SCC

Risk factors for AK and SCC include both host and environmental factors. These factors include aging, fair skin pigmentation, compromised immune function, presence of genetic disorders of DNA repair, and exposure to UV through occupation or recreation. Skin types that burn easily and tan poorly (Types I and II) are at greatest risk of developing AKs and those at higher elevations are at increased risk [15,23]. AKs also have the potential for spontaneous regression with reduction in UV exposure and reappearance if UV exposure is resumed. Eventually lesions will become permanent [23]. Clinically, AKs appear as red, scaly, non-substantive papules on chronically sun-exposed areas such as the face, ears, and dorsal surfaces of forearms and hands [24]. Histologically, AKs are characterized by dysplasia of keratinocytes with loss of cellular polarity and nuclear atypia. The diagnosis of SCC is made when these atypical cells pass through the basement membrane, invading into the underlying der-

mis. AK is cytologically indistinguishable from SCC [25]. A new classification system has been proposed for AK by Cockerell et al. [22]. This new system uses the term, keratinocytic intraepithelial neoplasia (KIN), and is very similar to the system used to describe cervical intraepithelial neoplasia (CIN). Sites adjacent to AKs contain histologic alterations that include nuclear heterogeneity, variation in cell size, and loss of polarity, suggesting extensive preneoplastic alterations of sun-damaged skin. Alterations in sites adjacent to AK may also be a result of paracine factors secreted by the AK itself. These findings promote the concept that there is a field of cancerization or field effect due to chronic exposure to sunlight [26].

2. Sunlight as a carcinogen

2.1. Ultraviolet (UV)-induced DNA damage

Sunlight can be divided into three categories, depending on the wavelength, UVC (200–280 nm); UVB (280–320 nm); and UVA (320–400 nm). The UV radiation that reaches the earth's surface is composed of 1–10% UVB and 90–99% UVA, with UVC being filtered by the ozone layer [27]. Shorter wavelength UVB is thought to be primarily responsible for the DNA damage associated with UV exposure. UV is a complete carcinogen that can induce all phases of skin carcinogenesis (initiation, promotion, and progression). This damage can include formation of cyclobutane dimers, 6-4 photoproducts, cytosine photohydrates, DNA adducts, DNA strand breaks, DNA strand crosslinks, and DNA-protein crosslinks [27,28]. The most common UV-induced DNA photoproducts are seen at adjacent pyrimidines (cytosine or thymine) as cyclobutane dimers or 6-4 photoproducts [29]. Cyclobutane dimers, if not repaired, lead to the signature mutation of UV irradiation (CC to TT and C to T). This type of mutation is common in skin cancers but not in other internal epithelial cancers [30]. UVA also can have detrimental effects on cells due to formation of reactive oxygen species and may play a role in the promotion phase of UV-induced skin carcinogenesis [27].

2.2. p53 gene response to UV-induced DNA damage

The importance of the tumor suppressor gene, p53, in epidermal cells is illustrated by its biological function. p53 is essential in maintaining genomic integrity by blocking DNA replication in response to DNA damage from exposure to agents like UV light. Cells with extensive damage are blocked from entering the cell cycle and instead undergo apoptosis or cell death [31,32].

p53 is present at low levels in normal cells but in response to DNA damage, the half-life of p53 increases post-translationally from minutes to hours. Exposure of normal epidermal keratinocytes to UV light results in a transient increase in p53 protein levels and resultant arrest in the G1 phase of the cell cycle. This increase in p53 expression is related to the dose and wavelength of UV irradiation [33–36]. UVA irradiation induces p53 in the basal layer while UVB induces p53 in all layers. UVC induces p53 in the granular layer and stratum spinosum [33]. Mutation of the p53 gene often produces a stable protein that can be detected by immunohistochemical methods [37].

2.3. p53 mutations in UV-induced carcinogenesis

Mutations in p53 have been identified in chronically sun-damaged skin, AK, and SCC [38–45]. The high incidence of p53 mutations early in UV-induced carcinogenesis strongly supports an important role for the p53 gene in the development of SCC. Furthermore, most p53 mutations are consistent with UV as the causative agent (i.e. CC to TT and C to T substitutions at dipyrimidine sites) [38–45]. The role of p53 in early skin cancer pathogenesis appears unique as p53 mutation in the majority of other cancer types is a late event. As an example, in the development of colon cancer, p53 mutations occur during the transition of a large precancerous adenoma to a carcinoma [31,32].

As discussed earlier, mutation of p53 often leads to a stabilization of the protein which can then be measured by immunohistochemistry. This p53 protein overexpression has been observed throughout the sequence of UV-induced skin carcinogenesis [42,44,46–55]. Of particular interest, normal-appearing skin adjacent to AK, SCC, and BCC has been shown to contain focal areas of p53 protein overexpression [47,49,51,54,56].

Although mutation of the proto-oncogene, ras, is considered to be the initiating event in classical chemically-induced rodent skin models, it does not appear to be as important as p53 mutations in UV-induced skin carcinogenesis [57]. Early studies suggested a role for Harvey (H)-ras mutations in UV-induced human skin carcinogenesis [58–60] in contrast to later studies where the incidence of H-ras mutations was low [61,62].

2.4. UV-induced alterations in second messenger systems

UV irradiation induces many different cellular responses. Erythema (sunburn) and an inflammatory response occur in both UV-irradiated rodent and human skin [63]. UVB-induced inflammation is characterized by an accumulation of mononuclear and polynuclear cells within the dermis and induction of vascular endothelial adhesion molecules [64]. This UVB-induced

inflammation of epidermis results in production of cytokines, such as interleukin-1α (IL-1α), IL-6, IL-8, and tumor necrosis factor α (TNFα). Genes for these cytokines contain nuclear factor κB (NFκB) binding sites in their promoters, implicating NFκB in their transcriptional regulation [65]. NFκB, a pro-inflammatory and redox-regulated transcription factor, is one of the primary targets of UV light in human skin [66,67].

UV radiation can initiate carcinogenesis through DNA mutations and chromosomal alterations. However, at least some of the promoting effects of UV involve alterations in mitogen-activated protein kinase (MAPK) signal transduction pathways. These MAPK pathways affect the regulation of transcription factors, and consequently the control of genes involved in cell proliferation, differentiation, and tumorigenesis. MAP kinases are terminal enzymes in a three enzyme cascade that act sequentially within a single pathway or across pathways to produce a series of phosphorylation reactions and activation of individual cascade members.

Induction of the Activator Protein-1 (AP-1) transcription factor plays a critical role in skin tumor promotion in both chemical and UVB-induced skin carcinogenesis models. This induction of AP-1 occurs via stimulation of MAPK pathways in UVB-irradiated mouse and human keratinocytes as well as in mouse and human epidermis in vivo [68–71]. Dissection of the MAPK signal transduction pathway that leads to induction of AP-1, demonstrates that UVB can induce the MAPK cascade through activation of acidic sphingomyelinases followed by ceramide activation of atypical PKCs as illustrated in Fig. 1. Atypical PKCs then phosphorylate and activate MAPK/extracellular signal-regulated kinase (ERK)-kinase or MEK [72]. The pathway continues with phosphorylation and activation of ERK by MEK, followed by activation of ternary complex factor (TCF). Moreover, in mouse keratinocytes, UVB-induced translocation of the atypical PKC isoform PKCζ (cytosol to membrane), activation of MAPK family members, and AP-1 transcriptional activation occur via activation of ERKs, but not JNKs or p38 [72].

This pathway through MEK and ERK ultimately results in transcription of c-Fos, an important member of the AP-1 family of proteins. UVB-induced c-Fos expression is an early epigenetic event and occurs through the binding of the transcription factors TCF and serum response factor (SRF) to specific sites within the DNA referred to as the serum response element (SRE). These signal transduction cascades can lead to production of AP-1 and secondary cellular responses such as cell proliferation and apoptosis [73–75]. In human skin, UVB induces the activity of all three major MAPKs; JNK, ERK, and p38. The UVB-induced signalling pathways that lead to activation of JNK and p38 are less well understood [73].

UVB signaling pathways involving other PKC isoforms are currently being explored. PKCs, depending on the isoform involved, can influence cell growth or proliferation, differentiation, apoptosis, and gene expression [76,77]. In mouse epidermal cancer cells, UVB induces translocation and activation of the PKC isoforms ε and δ, both of which can mediate UVB-induced signal transduction and apoptosis through activation of ERK and JNK (Fig. 1) [78].

Other effects of UVB include activation of the epidermal growth factor receptor (EGFR), ERK, and p38 signaling pathways through generation of reactive oxygen species (ROS) [79]. Hydrogen peroxide (H_2O_2), induced by UVB, can act as a second messenger resulting in phosphorylation and activation of EGFR [80].

UVB irradiation also induces phosphorylation of p53 at serine 15 resulting in stabilization of p53 protein. This specific phosphorylation of p53 interferes with the binding of MDM2, a negative regulator of p53 transcription. ERKs and p38 kinase appear to have a direct role in phosphorylation of p53 [81,82].

Another effect of UV radiation in skin is increased prostanoid production and upregulation of cyclooxygenase-2 (COX-2) [83]. Expression of COX-2 and prostaglandin E_2 (PGE_2) are increased in precancerous

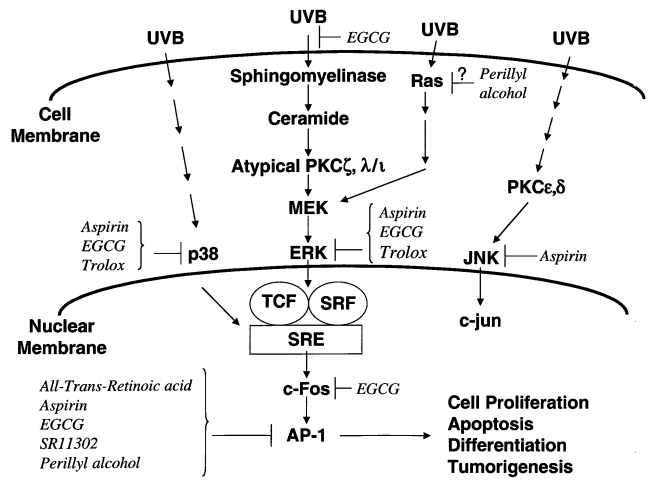

Fig. 1. UVB-induced MAPK Signaling Pathways. UVB has been shown to signal through the MAPK cascade and includes ERK, JNK, and p38. In this simplified model, UVB-irradiation activates acidic sphingomyelinases which in turn activates ceramide followed by activation of atypical PKCs. Atypical PKCs (PKC-ζ or PKC-λ/ι) phosphorylate and activate MEK, followed by ERK. ERK phosphorylates and activates TCF which then binds with SRF to an SRE site within the promoter of specific genes. Activation of this pathway results in c-Fos expression followed by AP-1 activation. UVB also activates PKCε and δ followed by activation of JNK and c-Jun. Another affect of UVB is activation of the p38 pathway. UVB can also induce signalling through the Ras pathway. The ultimate result of these signal transduction cascades is a cellular response such as proliferation, differentiation, apoptosis or tumorigenesis. The postulated molecular targets of the chemoprevention agents EGCG, perillyl alcohol, aspirin, and retinoids are shown.

AK and in NMSCs [84,85]. COX-2 expression in normal epidermis is low and restricted to regions of differentiated epidermis. In contrast, overexpression of COX-2 in mouse and human skin carcinogenesis contributes to the development of skin cancer [86].

COX exists as two isoforms, COX-1 is constitutively expressed while COX-2 is inducible by a number of stimuli including growth factors and cytokines. COX-2 is thought to work primarily through biosynthesis of prostaglandins to modulate cell proliferation, tumor growth, angiogenesis, and immune responsiveness [87–90].

3. Skin cancer chemoprevention

3.1. Cancer chemoprevention and use of surrogate endpoint biomarkers (SEBs)

Cancer chemoprevention strategies, if effective, could prevent or delay the occurrence of cancer in high-risk populations, such as those with premalignant lesions or previously resected cancer, using dietary or chemical interventions. Since carcinogenesis takes many years to decades, clinical trials using cancer incidence as an endpoint require large sample-sizes, long follow-up, and are very costly. The rationale for the use of SEBs is the circumvention of these issues by using smaller sample sizes and studies of shorter duration [91]. In this setting, an SEB can be a biologic event that takes place between an exposure and the subsequent development of cancer. It may be a discreet event such as formation of a premalignant lesion, or a measurement such as cell proliferation or quantification of apoptosis [92,93].

Useful SEBs can serve as predictors to identify subjects at risk of developing cancer and/or subjects in whom an intervention could be effective [94]. Ideally, an SEB is associated closely with the carcinogenic pathway, modulated by chemopreventive agents, and must be measured reliably in easily accessible biologic samples [91,95]. SEBs can be broadly categorized in two ways (1) markers with biologic relevance to the carcinogenic pathway, such as premalignant lesions or measures of proliferation or apoptosis; or (2) markers that demonstrate the effect of a particular agent. This can be demonstrated by the suppression of polyamine levels in tissue treated with the polyamine synthesis inhibitor, difluoromethylornithine (DFMO) [95].

Premalignant AKs are used as SEBs in skin cancer chemoprevention trials because they lie directly in the pathway to cancer. An intervention may reverse or delay the progression of these premalignant lesions to cancer [94]. For example, a recent study of retinol in high-risk subjects prevented the development of cutaneous SCC in subjects with numerous AK [21]. In addition to the AKs themselves, specific genetic alterations (mutation of p53) and phenotypic characteristics of AKs, (cell proliferation or apoptosis) can be used as SEBs to demonstrate the effect of a chemopreventive agent. Listed in Table 1 are some of the targets for UV-induced human skin cancer chemoprevention trials.

Table 1
Targets of human skin cancer chemoprevention trials

Clinical or histologic endpoints
BCC or SCC incidence and multiplicity
AK incidence and multiplicity
Histological phenotypic endpoints
Histological characterization (semi-quantitative)
Cellular proliferation
Apoptosis (cell death)
Molecular targets
p53 mutation—frequency and type of mutation
p53 protein expression
AP-1 expression and/or specific components of the MAP kinase pathway
Retinoid receptors expression
Polyamine pathway—levels of specific polyamines
Arachidonic acid pathway—Cyclooxygenase-2 (COX-2)
Expression and activity of COX enzymes
Prostaglandin synthesis

Clinical endpoints such as tumor incidence and number are obvious targets for chemoprevention studies. However, the addition of measures that reflect basic cellular events (histologic change, cell proliferation and death or apoptosis) and molecular targets (nuclear retinoid receptors, p53 mutation/overexpression) or pathways (MAP kinase/AP-1 pathways, arachadonic acid/cyclooxygenase pathways, polyamine synthesis) should be included. These endpoints could function as SEBs in chemoprevention studies and aid in determining the mechanism(s) by which agents act to alter the carcinogenesis process.

3.2. Chemopreventive agents and molecular targets

The ideal chemoprevention agent, in addition to inhibiting skin cancer, would have minimal toxicity for use in healthy populations and would differentially affect premalignant or malignant cells leaving normal cells unaffected [96]. Candidate agents could be selected based on epidemiologic evidence and activity in, in vitro and in vivo models of UV-induced skin carcinogenesis. With the identification of genetic alterations, molecular pathways, and cellular targets critical to the carcinogenesis process it will be important to use this information to develop agents with rational mechanisms of action. Reliance on data from epidemiology or animal studies alone may result in trials with agents that are not useful in chemoprevention. β-carotene is a good example of a putative chemoprevention agent that lacked sufficient experimental evidence prior to initia-

tion of clinical trials [97]. Epidemiologic studies supporting a role for β-carotene as a chemopreventive agent were not confirmed by animal or mechanistic studies. β-carotene may have been a marker for other micronutrients in fruits and vegetables [97].

Due to the multistep nature of UV-induced skin cancer and the fact that progression from precancer to cancer can take many years to decades, the best opportunity for intervention may be in the promotion phase of carcinogenesis rather than treating the resultant cancer [97]. As examples of molecular targets involved in skin cancer promotion the UV-induced MAPK signal transduction pathway is illustrated, in Fig. 1. Indicated in Fig. 1 are chemoprevention agents and points in the UVB signal transduction pathway where these agents appear to be acting. Elucidation of these and other pathways is key to determining the mechanism of action of chemopreventive agents.

Experimentally, green and black tea compounds have a number of mechanisms to explain their skin cancer chemoprevention activities. Green and black tea polyphenols and black tea theaflavins inhibit UVB-induced activation of AP-1, MAP kinase and ERK; moreover, they inhibit enhanced expression of c-Fos in human keratinocytes [98–101]. Epigallocatechin-3-gallate (EGCG), the green tea polyphenol proposed to have the highest antioxidant activity [102], inhibits autophosphorylation of the EGFR and suppresses cell proliferation [103]. In breast cancer cells, EGCG inhibits inducible nitric oxide synthase (iNOS) through a downregulation of NFκB in response to EGF [104]. EGCG differentially induces apoptosis and cell cycle arrest in epidermoid carcinoma cells, with no affect on normal cells [105,106]. Furthermore, green and black tea polyphenols inhibit TPA-induced upregulation of the aradidonic acid pathway and biosynthesis of eicosinoids, PKC, ornithine decarboxylase (ODC) and polyamines, COX-1 and 2, the MAPK pathway, AP-1, NFκB, c-Jun, c-Fos, c-Myc, p53, Bcl-2, p21 and p27 [106–109]. EGCG protects against UV-induced inflammation while topical administration of green tea polyphenols to both mouse and human skin inhibits the pro-inflammatory cytokines, IL-1α and TNFα. Additionally, EGCG suppresses UVB-induced erythema and infiltration of leukocytes, depletion of Langerhan cells (CD-1), increased PGE_2, and increased numbers of apoptotic cells [63,110]. In contrast, the caffeine component of tea has antitumor activity in UVB-induced mouse skin cancer models, but does not affect AP-1 activity suggesting that caffeine works via a different mechanism than tea polyphenols [101]. Tea components are of interest due to the epidemiology showing an association between tea consumption and decreased risk of many cancer types. Tea is one of the most widely consumed beverages world-wide [102,106,111]. Green and black tea have antitumor activity in both chemically and UV-induced skin carcinogenesis models [112,113]. The antitumor activity of tea remains even when administered after UVB exposure, indicating that the biological activity goes beyond a simple antioxidant or sunscreen effect [101,110,112,113].

DFMO, an inhibitor of ornithine decarboxylase (ODC), results in a reduction of polyamine synthesis and inhibition of both chemically and UVB-induced skin tumorigenesis in rodent models. Upregulation of polyamines in the promotion phase of chemically and UVB-induced skin carcinogenesis models is proposed as a critical factor in skin cancer development [114–122]. Polyamines are ubiquitous polycations that are essential for normal cellular proliferation, differentiation, and apoptosis [123]. ODC and polyamines are upregulated in rodent and human premalignant and malignant tumors [124]. Oral administration of DFMO inhibits the development of UVB-induced mouse skin tumors and furthermore, causes regression of established UV-induced tumors when DFMO is administered after tumors are formed [120,121]. Mechanisms for DFMO may also include reduction in the rate of tumor cell growth [120,125,126], inhibition of tumor invasion through downregulation of metalloproteases [127], suppression of angiogenesis [128], and altered DNA repair following DNA damaging agents like UVB radiation [129,130].

Perillyl alcohol, a monoterpene derived from citrus peel, has antitumor activity in a number of tumor types that include UV-induced skin carcinogenesis [131,132]. As an inhibitor of Ras farnesylation, perillyl alcohol prevents attachment of Ras to the cell membrane thereby inhibiting Ras signalling pathways. This disruption of Ras can affect downstream signalling pathways that would likely include inhibition of AP-1 transcriptional activity [132,133]. Perillyl alcohol has activity in the initiation phase of carcinogenesis though modulation of phase II drug metabolizing enzymes that are important in the bioactivation of carcinogenic compounds. Moreover, perillyl alcohol functions later in carcinogenesis via induction of apoptosis and/or an inhibition of proteins that require isoprenylation for activity, such as Ras [134].

Nonsteroidal anti-inflammatory drugs (NSAIDs), like aspirin and salicylates, inhibit COX activity rather than expression, producing a reduction of growth stimulatory eicosinoids (i.e. prostaglandins). Prostaglandins are involved in the promotion phase of both chemically and UV-induced skin carcinogenesis animal models [83,116]. Unfortunately, NSAIDs that inhibit both COX-1 and COX-2 are associated with significant toxicity due to loss of COX-1, limiting their usefulness as chemopreventive agents. In contrast, selective COX-2 inhibitors have reduced toxicity profiles making them better candidates for chemoprevention [86]. Oral administration of celecoxib, a selective COX-2 inhibitor,

decreases the incidence and number of UVB-induced mouse skin cancers and decreases COX-2 activity in skin tumors. This upregulation of COX-2 in tumors suggests that the arachidonic acid/COX-2/eicosioind pathway plays an important role in photocarcinogenesis [83,116]. COX-2 inhibitors are effective in both the early and late stages of UVB-induced skin carcinogenesis [83]. Aspirin, a nonselective NSAID, inhibits UVB-induced AP-1 activation though inhibition of MAP kinase signaling that includes suppression of JNK, ERK, and p38 activity [135,136]. Similarly, treatment of squamous cancer cells with the COX-2 inhibitors, flosulide or NS398, inhibits COX-2 activity, decreases PGE_2, and inhibits cell growth [86]. NSAID suppression of PGE_2 in turn inhibits UVB-induced inflammation and immune response [137]. NSAIDs also have COX and PG-independent mechanisms as demonstrated in colorectal carcinogenesis. Sulindac sulfone, an NSAID metabolite with no PG activity, still has antitumor activity, inhibits cell growth, and induces apoptosis in colon cancer cells [138]. Angiogenesis, another important component of tumor development, is also inhibited by NSAIDs [139].

Agents other than NSAIDs can affect COX and PG synthesis including retinoic acid and antioxidants [140–142]. COX-2, PGE_2, and cell growth are inhibited in squamous carcinoma cells treated with the antioxidant pyrrolidinethiocarbamate (PDTC) and 9-*cis*-retinoic acid [143]. Retinoids may modulate COX-2 expression through transrepression of AP-1 while the mechanism by which antioxidants affect the COX-2 pathway remains to be determined [86]. Other arachidonic acid metabolites formed via pathways, like the lipoxygenase pathway, are likely to have roles in UV-induced skin carcinogenesis and are potential targets for skin cancer chemoprevention [144].

Vitamin E (α-tocopherol) is a lipophilic antioxidant and free radical scavenger [145,146]. Topical application of the antioxidants α-tocopherol and vitamin C (ascorbic acid) delays UVB-induced mouse skin cancers, decreases UVB-induced erythema, suppresses pro-inflammatory cytokines (IL-α and IL-6), and reduces DNA synthesis [121,146–151]. Trolox or 2-carboxy-2,5,7,8-tetramethyl-6-hydroxychroman, a water soluble α-tocopherol analog, added prior to UV exposure inhibits both basal and UVB-induced intracellular H_2O_2 generation in keratinocytes. The affect of trolox is concentration and pretreatment time-dependent. Lower concentrations and short pretreatment inhibits ERKs while in contrast, higher concentrations and longer pretreatment results in suppression of p38 and enhanced apoptosis [79].

Retinoids, the most studied chemoprevention agents, are essential in a number of processes such as cell proliferation, cell differentiation, and apoptosis and during embryogenesis [152]. Retinoids have antitumor activity against skin cancers models as well as other models of carcinogenesis [153–155]. Epidemiology studies show an association between vitamin A and a decreased risk of cancer, particularly those originating from epithelium [154,156]. Mechanistically, retinoids function through activation of the nuclear receptors; retinoic acid receptors (RAR) and retinoid x receptors (RXR),each of which has three isoforms (α, β, γ). These receptors bind specific DNA sequences called retinoic acid response elements (RARE) and retinoid x response elements (RXRE) that are present in retinoid responsive genes. RARs form heterodimers with RXRs, while RXRs homodimerize and heterodimerize with other members of the steroid superfamily including RARs, thyroid hormone receptors, vitamin D receptors, peroxisome proliferator-activated receptors (PPARs), and orphan receptors [liver X receptor (LXR) and farnesoid X-activated receptor (FXR)]. Retinoids with RXR-selectivity are referred to as rexinoids [157]. Regulation of retinoid receptors is highly complex with variation in tissue expression, ligand specificity, and the ability to regulate other signalling pathways (i.e. AP-1) [158,159]. Retinoids with selective receptor activity are currently being developed. Retinoids are affective when administered systemically or topically, before and after carcinogen exposure, and the affects are reversible when retinoids are discontinued [153,154].

Potential mechanisms for the chemopreventive properties of retinoids include activation of nuclear retinoid receptors and transrepression of AP-1, growth arrest, and induction of apoptosis and differentiation [152,160,161]. Topical application of a synthetic AP-1 specific retinoid (SR11302) or *trans*-retinoic acid, a retinoid that has both AP-1 and RARE affects, suppresses TPA-induced papilloma formation in mice, inhibits AP-1 transactivation, and inhibits transformation of mouse epidermal cells in vitro [162]. In contrast, a retinoid with selective RARE transactivating activity (SR11235) did not reduce papilloma number or inhibit AP-1 transactivation. These experiments provide strong evidence that the antitumor activity of retinoids occurs via AP-1 transrepression [162,163]. Furthermore, an RXRα-selective retinoid (SR11235) inhibits RARE-dependent gene expression but did not significantly inhibit AP-1 transactivation or neoplastic transformation in vitro [162–164]. Additionally, retinoic acids suppress both TPA and EGR-mediated expression of COX-2 in squamous carcinoma cells [140,141]. Inhibition of COX-2 may be mediated through AP-1 and/or cyclic AMP response element (CRE) dependent pathways [165].

PPARs are another target for chemoprevention agent development. Multiple isoforms of PPARs have been identified including PPAR-α1, -α2, -α3, δ (also referred to as PPAR-β), and PPAR-γ and are expressed in a tissue-specific manner. Like retinoid receptors, PPARs

belong to the steroid receptor superfamily, form heterodimers with RXRs, and bind peroxisome proliferator response elements (PPRE). PPARs control lipid homeostasis and ligands for PPARs include fatty acids (linoleic acid and arachidonic acid), and eicosinoids [166,167]. PPARs likely play a role in many types of cancer development as shown recently in colorectal cancer. NSAIDs inhibit PPAR-δ activity counteracting the effect of alterations in the adenomatous polyposis coli (APC), a key gene in colorectal carcinogenesis [168,169]. Furthermore, COX-2 expression is induced in a PPAR-δ dependent manner in colon cancer cells [170].

Cultured keratinocytes express all three PPAR isoforms, while rodent epidermis expresses PPAR-α and δ [171,172]. The PPAR-α activator, 4-chloro-6-(2, 3-xylidino)-2-pyrimidinylthioacetic acid (WY-14,643) applied topically, is active in chemically-induced skin tumors in mice, while PPAR-δ and PPAR-γ activators had no affect [173]. PPAR-α ligands induce keratinocyte differentiation with induction of structural proteins, inhibit cell proliferation, and induce apoptosis in vitro and in vivo [174,175]. The AP-1 pathway may also be involved in at least some of the responses to PPARs [176].

Vitamin D receptors, which also belong to the steroid hormone receptor superfamily, are essential in calcium homeostasis, are antiproliferative, pro-differentiating, and can modulate immune function making them potential skin cancer chemopreventive agents [177]. 1α-25-Dihydroxyvitamin D_3 (calcitriol) is active in chemically-induced mouse skin cancer models, but has severe toxicity due to calcium effects [178,179]. Vitamin D analogs or deltanoids [177] have been developed to reduce the toxicity associated with calcitriol [180]. A number of topically-applied deltanoids have antitumor activity in a chemically-induced skin carcinogenesis model but have not yet been tested in UVB-induced skin models [177].

A number of other potential chemopreventive agents have been primarily studied in chemically-induced skin carcinogenesis and in vitro models. Resveratrol (3,5,49-trihydroxystibene), a pytoatexin present in grapes, berries, and peanuts, has a number of potential mechanisms that include activity as an antioxidant, antimutagen, induction of phase II drug metabolizing enzymes, inhibition of COX-1, COX-2, as well as hydroperoxidase activity, and suppression of chemically-induced tumorigenesis in a mouse skin model [181,182]. In vitro, resveratrol inhibits TPA- and EGFR-induced transformation, while pre-treatment of mouse skin with reservatrol inhibits TPA-induced measures of oxidative stress (H_2O_2, glutathione levels, oxidized glutathione reductase, superoxide dismutase) and upregulation of c-Fos and TGF-β1 [184]. Reservatrol activates the MAP kinases, ERK, JNK, and p38 kinase and induces apoptosis by a p53-dependent mechanism. Activation of p53 requires serine 15 phosphorylation is ERK- and p38-dependent [81,183]. Additionally, topical application of a polyphenolic fraction of grape seeds had anti-tumor activity in a chemically-induced mouse skin model and inhibited TPA-induced upregulation of PKC and ODC activity [185–187]. These studies suggest that reservatrol may be affective in UVB-induced skin carcinogenesis models.

Isothiocyanates are present in cruciferous vegetables and have activity in carcinogen-induced animal models of lung, esophagus, and mammary gland [188–191]. Functions of isothiocyanates include modification of carcinogen metabolism and induction of apoptosis [192]. Phenethyl isothiocynate (PEITC) suppresses neoplastic transformation and induces apoptosis in a p53-dependent manner in TPA- and EGF-induced mouse epidermal cells [192]. Isothiocyanates will need to be tested in UVB-induced skin carcinogenesis models to determine their efficacy as skin chemopreventive agents.

Another chemopreventive target is the phosphatidylinositol-3 kinase (PI-3 K) signaling pathway. TPA, EGF and insulin induce PI-3 K and AP-1 activation as well as malignant transformation in mouse epidermal cells, while inositol hexaphosphate blocks these effects [193].

As discussed previously, mutation of the tumor suppressor gene p53 is a common occurrence and may in fact be the initiating step in the development of UV-induced SCC making this gene a likely target for development of chemopreventive strategies. Strategies to replace mutated p53 with a functional p53 gene in cells are currently under development [194].

3.3. Human chemoprevention studies in NMSC

Limiting sun exposure through sunscreen use or by wearing protective clothing can reduce skin cancer risk, but for many reasons these primary preventive measures have had limited success [195]. Sunscreens appear to be beneficial in reducing the number of AK [196,197]. Evidence that sunscreen use can reduce SCC or BCC is mixed, with several prospective trials showing no effect on NMSC incidence [196,198]. A recent study of sunscreen usage demonstrated a reduction in the total number of SCCs with no affect on SCC incidence and no affect on BCC numbers or incidence [199]. Some studies suggest that sunscreen use is associated with an increase in melanoma incidence due to increased exposure times, although this remains controversial [200,201]. Given the limited success of primary prevention, chemoprevention strategies are important in reducing the rate of skin cancer. Ultimately, chemoprevention and primary preventive strategies could be used in combination.

AKs are commonly treated with topical fluorouracil or liquid nitrogen [202]. Both of these methods are

associated with significant adverse side effects including inflammation, erythema and superficial ulceration. Individuals can have numerous AKs on sun exposed body sites making these side effects prohibitive [20]. Less toxic treatments for essentially healthy individuals with severely sun-damaged skin or precancerous AKs are needed, making these individuals excellent subjects for trials of cancer chemoprevention strategies.

Chemoprevention trials focusing on subjects with precancerous lesions, such as AK, can be considered treatment of early disease.

Several large randomized placebo-controlled phase III trials have been performed in subjects at high risk of developing NMSCs (Table 2). These trials were 2–5 years in duration and used oral dosing. The only positive trial involved retinol administration in 2297 subjects with moderate to severe AK. Retinol (25 000 IU/day) resulted in a reduction in SCC, but not BCC [21]. A similar but smaller study of retinol, isotretinoin, or placebo in 719 subjects with BCC or SCC found no difference in development of new NMSCs or in tumor multiplicity [203]. A third study of *cis*-retinoic acid in subjects with two or more BCCs found no affect on new BCC formation [204]. These studies provide evidence that retinoids are more effective in subjects at an earlier stage in the UV-induced skin carcinogenesis pathway (i.e. between AK and SCC) as opposed to subjects with more progressed disease (i.e. new cancers in subjects with resected cancer). Other large phase III skin cancer chemoprevention trials used either β-carotene or selenized brewers yeast. Neither agent affected the incidence of NMSC [205–207]. Although these trials were negative for the skin cancer endpoint, they have been informative in other areas. For example, in a secondary study analysis, selenium supplementation significantly reduced total mortality due to all cancers and significantly reduced the incidence of lung, prostate, and colon cancers providing rationale for chemoprevention trials in these tumor types [132,205].

A number of small trials of short duration (1 month–2 years) have been performed in populations at high-risk for skin cancer (Table 3). These smaller studies found retinoids to be effective in reducing the numbers of new AK or NMSC [208–212]. Additionally trials of high-dose retinoids have been effective in inhibiting NMSC in subjects at very high risk of skin cancer, such as those with genetic defects in DNA repair (xeroderma pigmentosum or XP) or patients who are immunosupressed due to organ transplants [213]. Although these are valuable studies, they likely represent a more homogeneous population, especially those studies using very high-risk subjects such as XP and renal transplant patients, compared with the more heterogeneous populations studied in the larger chemoprevention trials. Moreover, the positive trials primarily focused on AK as an endpoint while the phase III trials focused almost exclusively on a new NMSC in populations with previous NMSC. The one positive phase III trial of retinol studied a population that primarily had AK at baseline with an endpoint of a new NMSC. These studies suggest that chemoprevention or treatment at the precancerous stage may be effective for both reduction of AK as well as inhibition of progression to an SCC. The doses of retinoids used in trials was somewhat similar although the small positive trials used a number of different forms of retinoids compared with β-carotene, retinol, and 13-*cis* retinoic acid used in the phase III trials.

Other chemoprevention strategies include consumption of a low fat diet ($\leq 20\%$ of calories from fat), which reduced the number of AKs in subjects with a history of NMSC [214]. This trial was based on studies in animal models of UV-induced skin cancer where

Table 2
Phase III trials in subjects at high risk for developing a new NMSC

Agent	Route	Dose	Number of subjects	Population	Endpoint	Treatment length	Outcome	Reference
β-Carotene	Oral	50 mg per day	1805	Recent NMSC	New NMSC	5 year	No effect	[207]
β-Carotene[a]	Oral	30 mg per day	1805	Australian Community[b]	New NMSC	5 year	No effect	[199]
Selenium	Oral	200 μg per day	1383	Recent NMSC	New NMSC	4.5 year	No effect	[205]
Retinol	Oral	25 000U per day	2297	>10 AK, <2 SCC/BCC	New NMSC[c]	5 year rx		[21]
					BCC[b]		No effect (BCC)	
					SCC[b]		Positive (SCC)	
13-CIS-RA	Oral	2 mg/kg per day	981	≥2 BCC	New BCC	2 year	No effect	[204]
Retinol/13-CIS-RA	Oral	25 000U per day, 5–10 mg per day	525	≥4 NMSC	New NMSC	3 year	Both no effect	[203]

[a] Sunscreen had a significant effect on total SCC number, but not on incidence. There was no effect on BCC.
[b] Community based study where history of NMSC was not a requirement. Approximately 27% of subjects reported previous NMSC.
[c] There was a significant effect on the incidence of SCC, but not on BCC.

Table 3
Smaller trials in subjects at high risk for developing AK or a new NMSC

Agent	Route	Dose	Number of subjects	Population	Endpoint	Treatment length	Outcome	Reference
Retinoids								
Etretinate	Oral	75 mg per day	50	AK	AK	2 months	Positive	[212]
All-trans RA versus Arotinoid	Topical	0.05% cream	25	≥3 AK	#AK	4 months	Both positive	[211]
Methyl Sulfone Arotinoid	Oral	1 μg/kg per day	16	AK, NMSC	#AK, SCC	28 days	Positive	[209]
13-CIS-RA	Oral	2 mg/kg per day	5	XP patients	New NMSC	2 years	Positive	[210]
Acitretin	Oral	30 mg per day	44	Renal transplant patients	NMSC	6 months	Positive	[208]
Other agents								
Low fat diet	Oral	20% calories, fat	76	NMSC	#AK	24 months	Positive	[214]
DFMO	Topical	10% ointment	47	≥10 AK	#AK	6 months	Positive	[217]

high fat diets increased incidence of skin cancers, while low fat diets reduced incidence [215].

Another chemopreventive strategy employed in clinical trials is the inhibition of polyamines. DFMO, an irreversible inhibitor of ornithine decarboxylase (the first step in polyamine synthesis) prevented tumorigenesis in UV-induced skin carcinogenesis models [121,216]. We recently completed a trial of topical DFMO ointment (10% w/w bid) applied for 6 months in 42 subjects with at least 10 AK on their dorsal forearms. A significant reduction was seen in the numbers of AK (23.5% reduction) and in the skin level of spermidine (21% reduction), a polyamine known to be involved in cell proliferation. Measures of skin polyamine levels were included in the study as an index of drug effect since DFMO is a polyamine synthesis inhibitor. p53 protein expression also was measured because p53 mutation and p53 overexpression are early events in AK and SCC. p53 expression was significantly reduced (26% reduction) by DFMO, while cell proliferation was not affected (data unpublished) [217]. A second larger trial in the same population is underway. Taken together, these studies demonstrate that dietary/nutrient or chemical interventions can be effective strategies for skin cancer chemoprevention.

It is of interest that all the studies that focused on early (premalignant) endpoints in the carcinogenesis pathway (e.g. AK) were positive, although the majority of these studies were small and must be tested in larger populations. NMSC-naive patients with AK may represent a high-risk population that is more likely to respond to chemoprevention strategies than groups with a previous history of NMSC.

4. Conclusion

NMSC is a significant and increasing health problem in the United States. Epidemiologic evidence has determined that chronic exposure to sunlight is the etiologic agent responsible for skin cancer. Subsequent studies in animal models and in vitro have identified UV radiation, particularly UVB, as a complete carcinogen capable of damaging DNA at adjacent pyrimidines resulting in characteristic double cytosine (CC) to double thymine (TT) transition mutations [30]. UV-induced human skin carcinogenesis is a multistep process having discreet steps that include chronically sun-damaged skin, AK, and SCC. This multistep process provides an excellent model for testing chemopreventive strategies to reduce the incidence of SCC and its precursor lesion, AK. Dysplastic nevi, a likely precursor of melanoma, is also a potential target for chemopreventive interventions. Primary prevention strategies have had limited effectiveness. A sensible approach would be to combine primary prevention and secondary chemoprevention to maximize their effectiveness [196,198].

A number of the molecular and cellular mechanisms in multistep skin carcinogenesis giving rise to SCC are well understood. Consequently, these events in the natural history of the disease are useful targets for the development of new chemoprevention strategies and the development of new drugs and natural products as chemoprevention agents. An example is the UVB signal transduction pathway leading to the activation of the transcription factor complex AP-1 which has been functionally implicated in skin tumor promotion [69,70,72,218–220]. Natural products, like those

derived from green tea [98,99] and perillyl alcohol or limonene derived from citrus peel [132], block UVB induced AP-1 activation in both cultured keratinocytes and in the epidermis. Chemoprevention agents may act through multiple mechanisms. This is the case for retinoids, which can inhibit AP-1 and activate gene expression via nuclear retinoid receptors [158]. These studies show that the UVB signal transduction pathway is a viable target pathway for the development of chemoprevention strategies. In recent years many new molecular targets and chemoprevention agents have been developed and this will be an area of intense interest for future skin cancer prevention studies. Future studies should also target multiple molecular alterations and/or pathways with combination chemoprevention.

One goal of skin cancer chemoprevention studies is to reduce the size, cost, and length of studies with SEBs. Obvious potential SEBs include known genetic alterations (i.e. mutation of the tumor suppressor gene p53 as well as components of the UVB signal transduction pathway) and epigenetic alterations (i.e. cell proliferation or apoptosis). It is important to use a combination of SEBs that will adequately model the particular cancer type as well as verify the activity of intervention agents.

Leads for chemoprevention agents have come from in vitro and in vivo experimental models of UV-induced skin carcinogenesis and observation/epidemiologic studies. Recently, agents have been developed with activity towards specific cellular targets [83]. The most promising agents are studied in small short term trials in high risk subjects, followed by larger chemoprevention trials of longer duration. If successful, effective strategies for reducing the risk of skin cancer will result from these trials. Hopefully, these efforts will lead to a reduction in the incidence, morbidity, and mortality of skin cancer.

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Biography

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