Skin Cancer Prevention

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13.1 Epidemiology of Skin Cancer

Skin cancer is the most common malignancy in North America. This includes primarily non-melanoma skin cancer (NMSC), including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), and melanoma. Of the NMSCs, BCC accounts

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for 80% of all skin cancers. Australia has the highest rates of NMSC in the world with 1000 per 100,000. In Australia, NMSC accounts for 75% of all cancers and is 30 times more prevalent than lung cancer among men and ten times more prevalent than breast cancer among women. Incidence data for NMSC are sparse because traditional cancer registries do not track NMSC; however, it has been estimated that the incidence of NMSC is 18–20 times greater than that of melanoma (Jemal 2011; Lazovich 2012). Incidence rates of NMSC increase proportionally with the proximity to the equator, with high cumulative ultraviolet radiation (UVR) light exposure and with age (Diepgen and Mahler 2002). The incidence of NMSC has until most recently affected the older population—especially men who have worked outdoors; however, the age of onset has steadily decreased. While the incidence rates for NMSC continue to rise, the mortality rate has decreased in recent years; however, there continues to be a substantial impact on morbidity, health, and health-care costs. An upward trend in incidence is seen in Australia, the United States (US), Europe, and Canada with an average of 3–8% increase per year (Narayanan et al. 2010). It is predicted that the incidence of NMSC will double in the next 30 years. As in the US, in Australia NMSC is the most costly cancer with annual expenditures at about $511 million (Fransen et al. 2012). Early diagnosis and appropriate therapy result in a 95% cure rate. Melanoma also shows greater outcomes the earlier it is diagnosed. Clearly, prevention and early diagnosis are the key management tools for skin cancer.

The most aggressive and lethal of the skin cancers is melanoma. It arises from melanocyte cells, which provide pigment to the skin, and can occur at any place that these cells reside. Survival is strongly associated with the thickness of the lesion. Melanoma most commonly arises on the skin surface and is usually diagnosed by visual examination. The ABCDE rule is used to outline the clinical presentation of melanoma: A stands for asymmetry, where half the mole looks different than the other half; B stands for border, which is irregular in melanoma; C stands for color variation rather than a uniform color in normal moles; D represents the diameter where greater than 6 mm is a possible indication of melanoma; and E is the evolution, evaluation, and or enlargement of the lesion. Malignant melanomas will have some but not necessarily all these characteristics (Cummins et al. 2006).

Melanoma accounts for nearly half of all skin cancer deaths but only represents 4% of all skin cancers (Skin Cancer Foundation 2015; Guy Jr. et al. 2015). The number of melanoma cases is increasing faster than any other cancer in the US (Linos et al. 2009). In the US, the lifetime risk of developing melanoma was 1 in 1500 individuals in the year 1935; however, currently it is 1 in 59 overall, 1 in 39 for white men and 1 in 58 in white women (Rigel 2010). Currently, women have a higher incidence rate than men before the age of 50 but rates in men are double than women by age 64 and then triple by age 80 (Cancer Facts & Figures 2018). The suggestion for this is due to recreational and occupational differences in UV exposure. Melanoma in whites is five times higher than in Hispanics and 20 times higher than in African Americans (Narayanan et al. 2010). Mortality rates demonstrate a 1% decline in those older than 50 and a 2.6% decline per year in those younger than 50 (Cancer Facts & Figures 2018).

NMSCs are derived from keratinocytes in the epidermis and are divided into two types, BCC and SCC. BCC is the most common skin cancer that originates

from the basal cells of the epidermis. BCC generally appears without a precursor lesion and the small papule enlarges slowly over months and years. BCC is often difficult to distinguish from benign growths. Metastasis is rare but local growth can be highly destructive (Maden et al. 2010). SCCs comprise about 16% of skin cancer cases. The SCC malignant tumor invades the dermis where local destruction of the tissue can be extensive and metastasizes via the lymphatic system in advanced stages. Metastatic rates are estimated to be 3–10% (Porter 2011). Early clinical presentation can include papules, plaques, nodules, smooth, hyperkeratotic or erosive lesions. Eventually the lesion ulcerates and invades the tissue below. The cost to treat NMSC is estimated to be $4.8 billion annually in the US (Guy Jr. et al. 2015). Of additional concern, individuals who develop NMSC are at increased risk for the development of new skin cancers within the next few years following diagnosis (Diepgen and Mahler 2002). A follow-up study found that 52% of individuals diagnosed with SCC developed subsequent NMSC within 5 years of initial therapy (Frankel et al. 1992). Prevention of NMSC is a sensible strategy to lowering these costs.

13.2 Risk Factors

13.2.1 Ultraviolet Radiation Exposure

Skin cancer is perhaps one of the most preventable cancers because 50–90% of melanoma, 50–90% of BCC, and 50–70% of SCC worldwide are caused by ultraviolet radiation (UVR) (Lucas et al. 2008). Therefore, effective protection from UV irradiation would prevent the majority of cases of skin cancer. Aside from genetics, the major risk factors for all skin cancers are exposure to UVR and skin color or the inability to tan. All skin cancers have been associated with exposure to sunlight; however, the pattern of sun exposure may vary between skin cancer types. Sun exposure factors such as sunburn history, sun exposure habits, sun exposure time of day, and ability to tan play a role in the development of skin cancers.

UVR comprises wavelengths from 200 to 400 nm. The ozone of the earth's atmosphere absorbs most light wavelengths below 290 nm. Therefore, UVB (290–320 nm) and UVA (320–400 nm) are the only portions reaching the earth's surface. UVR light reaching the earth's surface comprises 90–99% UVA and 1–10% UVB. UVR causes many biological reactions in the skin, including inflammatory response in a sunburn, hindrance of immune activity, premature aging, and damage to DNA resulting in potential development of skin cancer (Dissanayake et al. 1993).

Direct damage to DNA in the form of photoproducts, 6–4 photoproducts and cyclobutane pyrimidine dimers, which if gone unrepaired by the nucleotide excision repair pathways result in permanent mutations. UVR exposure causes an increase in reactive oxygen species (ROS) which can overwhelm the natural antioxidant defense mechanisms in the skin. This oxidative stress can result in ROS that interact with proteins, lipids, and DNA (Li et al. 1996; Berton et al. 1997). UVR can result in mutations in genes that regulate cell proliferation and repair. UVR-induced

linkage between two adjacent pyrimidines (cytosine or thymine) on the same DNA strand is usually repaired by nucleotide excision repair enzymes before replication. However, if the repair fails or is delayed, fixed DNA mutations can occur when DNA polymerase inserts adenine dinucleotide (AA) opposite the unrepaired dimer. In the erroneous pairing of AA with CC- or CT-linked photoproducts, mutations are observed as CC → TT and C → T, respectively. These are characteristically induced by only UVR and as such are designated UVR signature mutations (Wikonkal and Brash 1999). Figure 13.1 depicts the adverse biological effects that occur with sun exposure and in turn can result in skin cancer.

Originally, UVB was thought to be more important than UVA in the generation of sun damage and skin cancer. However, UVA has become increasingly suspect in the development of skin cancer (Runger 1999; Skin Cancer Foundation 2015). The photocarcinogenesis of UVA differs from UVB in that UVA is not readily absorbed by DNA, but is absorbed by other molecules within the cell, giving rise to reactive oxygen species, which in turn damage DNA, membranes, and other cellular constituents (de Gruijl 2000). UVA induces oxidative stress in melanocytes and UVA perpetuates accumulation of oxidative stress through mechanisms such as Sestrin2, a negative regulator of Nrf2. UVA's ability to suppress 8-oxoguanine DNA glycosylase 1 (OGG1) causes an impairment in the repair of oxidative lesions and oxidative stress (Sample and He 2017). Melanoma cells have shown a reduction in the ability to repair DNA damage (Budden et al. 2016).

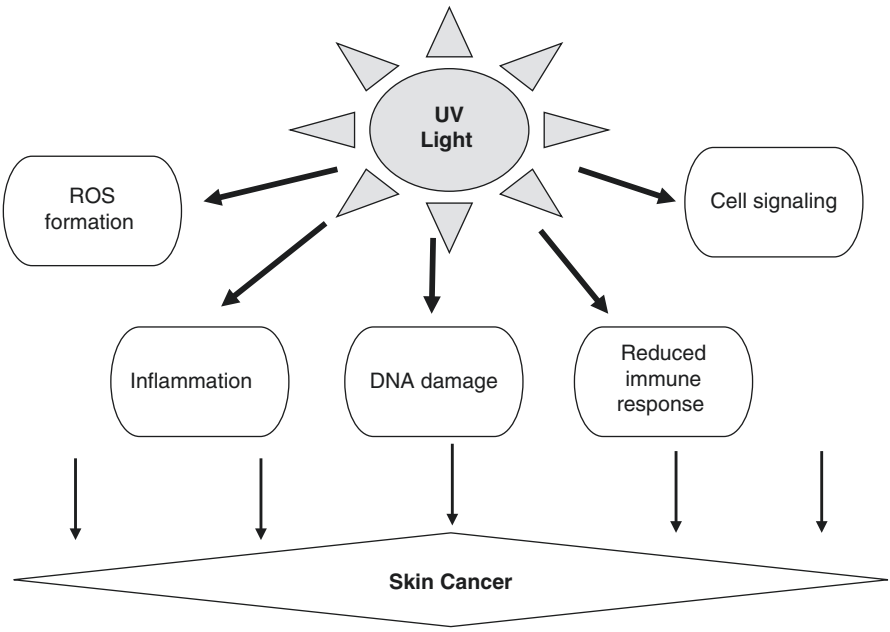

Fig. 13.1 Adverse biological effects of UV light leading to skin cancer

Oxidative damage caused by UVA is recognized by 8-oxoguanine DNA glycosylase 1 (OGG1) and is repaired by base excision repair (Dahle et al. 2008). UVA has been shown to induce mutations in DNA including p53, which is discussed later in this chapter as an important genetic marker for NMSC (Burren et al. 1998). UVA has been found to be an important factor in the development of melanoma. Several investigators have argued that UVA is more relevant in melanoma causation than the UVB range (Setlow et al. 1993). Much of the evidence of UVA exposure as a risk factor for melanoma has come from epidemiological studies of users of sunbeds or tanning equipment with spectral output that is in the UVA range (Swerdlow and Weinstock 1998; Wang et al. 2001). The UV intensity may be 10–15 times higher than that of a midday sun (Gerber et al. 2002). Indoor tanning devices are classified as "carcinogenic to humans" by the International Agency for Research on Cancer (Cancer Facts & Figures 2018). Studies of indoor tanning at early ages have shown an increased incidence of melanoma (CDC 2012). In the US, it is estimated that 40–50% of teenagers utilize tanning beds, and 70% of tanning salon customers are Caucasian females under 30 (Choi et al. 2010; Watson et al. 2017). Melanoma is the second most common cancer among females age 15–29 years. There is a 75% increase in melanoma associated with indoor tanning before the age of 30 (CWG 2006). A study of women from Norway and Sweden found that women who visited a tanning parlor at least once a month were 55% more likely to later develop melanoma than women who did not artificially suntan. Those who used sunlamps during their 20s had the greatest risk, approximately 150% higher than similarly aged women who did not use tanning beds (Veierod et al. 2003). Meta-analysis of 19 studies of indoor tanning and melanoma risk suggests that the use of indoor tanning was associated with a relative risk of 1.15 for developing melanoma, and if the use occurred before the age of 35 years, the relative risk increased to 1.75 (International Agency for Research on Cancer 2007). Additional publications continue to demonstrate the association of indoor tanning and melanoma risk, including an epidemic of melanoma in Iceland possibly due to increased use of sunbeds (Hery et al. 2010; Lazovich et al. 2010). Further contributing to the controversy, a preclinical study showed that UVB, and not UVA, exposure promoted melanoma growth in a mouse model (De Fabo et al. 2004).

Chronic exposure to UVR is the predominant cause of NMSC. Over 80% of these cancers develop on parts of the body exposed to the sun, including the face, neck, and arms (Diepgen and Mahler 2002). Incidence rates of NMSC correspond well with increased UVR exposure as demonstrated by the increased incidence among individuals with occupational or recreational outdoor exposure or who reside at latitudes closer to the equator (Diepgen and Mahler 2002). Many studies have shown an inverse relationship between latitude and NMSC incidence (Almahroos and Kurban 2004). A report from southeastern Arizona suggests that the incidence rates of NMSC in Arizona are three to six times higher than those in subjects with similar skin type and living in regions of higher latitude (Harris et al. 2001a). A compilation of these and several other studies demonstrates a more than 50-fold difference in rates of NMSC incidence between Australia and Arizona (low latitude) and Finland (high latitude), with the higher incidence occurring in the lower latitudes

(Almahroos and Kurban 2004). Several Australian studies have demonstrated that people in countries with high ambient solar radiation have a higher incidence of NMSC than migrants with the same genetic background from countries with lower ambient solar radiation (Almahroos and Kurban 2004). People who move during childhood to the countries of high ambient solar radiation from countries with low ambient solar radiation have equal incidence of NMSC as natives (English et al. 1998). However, individuals who make this same move later in life have a lower incidence. These data support the idea that NMSC develops from a chronic exposure of UVR. The risk factor of skin color for the development of NMSC is demonstrated by the lower risk of ethnically darker skinned migrants.

Melanoma incidence is also associated with exposure to UVR (Khan et al. 2018). Sixty to seventy percent of malignant melanomas may be caused by UVR (Koh et al. 1996). Childhood sunburns and intense intermittent sun exposure are major risk factors for melanoma (Gilchrest et al. 1999). Anatomic locations of melanoma development support the basis for intermittent UVR exposure as a risk factor. Melanoma is most commonly found on the trunk of men and the trunk and lower extremities of women. These sites are not normally acclimated to the sun by chronic exposure, but rather tend to be exposed during outdoor recreational activities. The effects of the sun on the development of melanoma are modulated by skin type. Light pigmentation increases the risk of the development of melanoma.

In a study conducted at the University of Arizona, risk factors for SCC were evaluated among 918 Arizona residents with sun-damaged skin (at least ten clinically assessable actinic keratosis lesions) who had been randomized to the placebo arm of a skin cancer chemoprevention trial (Foote et al. 2001). Risk factors for BCC included older age, male gender, red hair color, and at least 10 years' residence in the state of Arizona, which is located in a lower-latitude region of the US with documented rates of SCC and BCC that are among the highest in the world (Harris et al. 2001a).

13.2.2 Other Risk Factors

The presence of precancerous lesions increases the risk of developing skin cancer. For SCC, the precancerous lesion is actinic keratosis (AK), and for melanoma, the precancerous lesion is thought to be dysplastic nevus (DN). BCC does not appear to have a precancerous lesion; however, the presence of AK can often be an indicator of risk. Additional risk for skin cancer includes genetics, immune suppressive disease, past history of skin cancer, and occupational exposure to coal tar, pitch, creosote, arsenic compounds, or radium. Age, male gender, and DNA repair disorders such as xeroderma pigmentosum are also risk factors for skin cancer. For melanoma, additional risk factors include one or more family members who had melanoma and a large number of moles (risk increases with number of moles) or the presence of DN. Approximately 5–12% of patients with melanoma have a family history of melanoma in one or more first-degree relatives (Goldstein and Tucker 2001). Patients with multiple BCC present with mutations in the tumor suppressor, PTCH1 gene (Craythorne and Al-Niami 2017). Mutations in two melanoma

susceptibility genes, CDKN2A (p16) located on chromosome 9 (9p21) and CDK4 located on chromosome 12 (12q13), have been identified. Mutations in p16 have been identified in 20% of tested melanoma families (Bishop et al. 2002).

13.2.3 Genetic Alterations in NMSC

Genetic studies of AK and SCC have found alterations on possible tumor suppressor genes of chromosomes 9p, 13q, 17p, 17q, and 3p (Quinn et al. 1994; Hunter 1997). The targets for most of these mutations have not been identified except for p53, which lies on chromosome 17p. p53 is a tumor suppressor gene that plays a role in protecting cells from DNA damage. Recognized genetic targets in NMSC include p53 mutations demonstrated in a progression of normal skin to sun-damaged skin to AK to SCC (Einspahr et al. 1999). p53 mutations have also been identified in BCC (Matsumura et al. 1996). Many of these mutations are CC → TT or C → T changes at dipyrimidine sites suggestive of UVR damage (Tsao 2001). Studies indicate that UVA may also contribute to "signature" dipyrimidine mutations instead of only UVB (Runger and Kappes 2008), suggesting that UVA may be a complete carcinogen. Runger et al. (2008) postulate that UVA DNA damage may be more mutagenic than UVB, due to a lack of stimulation of the robust DNA repair response noted with UVB exposure.

Results from an analysis of genetic changes in 36 AKs and 23 invasive SCCs (Rehman et al. 1996) suggest that the relationship between the accumulation of genetic change and behavior for NMSC is complex. However, the overall pattern of autosome loss in AKs was similar to that seen for SCCs. Loss of chromosome 17p was the most frequent target of loss of heterozygosity (LOH), which is consistent with data showing a high rate of UVR-induced mutations in p53 (Brash et al. 1991), detection of p53 mutations in irradiated skin and cultured keratinocytes (Nakazawa et al. 1994), and evidence showing p53 mutations in preinvasive lesions (Einspahr et al. 1999). However, the number of SCCs with chromosome 17p loss far exceeded the number in which mutations were detected in p53 exons 5–8, consistent with the presence of other targets of inactivation on chromosome 17 (Wales et al. 1995). Increased $p21^{WAF1/CIP1}$ immunostaining and p53 immunostaining were observed in 97 and 83% of AKs, respectively, and were observed in lesions without any detectable LOH or p53 mutation, suggesting that changes in proliferation, $p21^{WAF1/CIP1}$ expression, and p53 expression may precede allelic loss or p53 mutation. Pacifico et al. (2008) noted that NMSC also harbors high rates (up to 82%) of deletion of exon 1 or 2 of the CDKN2A locus, which encodes proteins p16INK4a and p14ARF. These tumor suppressors are also linked to the p53 and retinoblastoma pathways. A large number of AKs showing multiple areas of LOH and p53 mutation may not have acquired the relevant genetic change to allow invasion of the underlying dermis (Wales et al. 1995).

Genetic alterations in NMSC also include mutations in the ras gene. The frequency of ras mutations in SCC ranges up to almost 50% and up to 30% in BCC (Pierceall et al. 1991). Mutations in ras have also been identified in AKs (Spencer

et al. 1995). Different rates reported for SCC and BCC ras mutations may reflect different techniques, different study populations, and/or the differing molecular epidemiology of low and high sun exposure. Additional studies have also implicated viral infection with NMSC progression. Zaravinos et al. (2010) noted that human papillomavirus (HPV) and cytomegalovirus (CMV) infection was detectable in ~30% of AK, SCC, and BCC specimens. Although normal skin did not harbor CMV, this virus was detected in ~20% of skin lesions. These samples were collected from immunocompetent subjects and did not seem to be dependent upon H-ras mutation.

Although more commonly reported in melanoma, studies suggest that alterations in p16 can be found in up to 24% of SCC and 3.5% of BCC (Soufir et al. 1999b). Several of the detected mutations were UVR signature mutations. These mutations may account for alterations observed on chromosome 9p21 in SCC (Tsao 2001).

Genetic alterations in BCC are found in both hereditary and sporadic cases. BCCs arise from activation of the sonic hedgehog pathway resulting from a mutation in the PTCH1 gene (Lear et al. 2007). The PTCH gene is found in patients with nevoid BCC syndrome, characterized by the rapid development of numerous BCCs early in life (Tsao 2001). Studies have demonstrated that 15–39% of these patients harbor mutations in the PTCH gene (Aszterbaum and Rothman 1998). Genes involved in chemical detoxification and abnormal inflammatory responses are also linked to increased risk of BCC (Madan et al. 2006).

13.3 Genetic Alterations in Melanoma

The most prevalent signaling pathway identified in the development of melanoma is the mitogen-activated protein kinase (MAPK) pathway. Genotyping of melanoma tumors demonstrates a range of activating mutations in the BRAF kinase of up to 70%, with a specific point mutation, V600E, in up to 90% (Agarwala et al. 2010; Chin et al. 2006; Dutton-Regester and Hayward 2012). BRAF mutations alone are not sufficient to transform melanocytes into melanoma but UV-induced acquired mutations can synergize with mutant BRAF to drive the transformation (Viros et al. 2014). UV exposure to animal models with BRAF mutations in melanocytes accelerates the development of melanoma. Forty percent of these tumors develop UV-signature p53 mutations which accelerate melanoma development; p53 mutations developed from UV exposure are seen in 20% of human BRAF mutant melanomas. BRAF mutations can also synergize with Arf deletion in vivo to accelerate UV-induced melanoma (Luo et al. 2013). Developments from the Cancer Genome Project revealed that 66% of melanomas tested had a mutation in BRAF with the same single substitution occurring in 80% of melanomas (Davies et al. 2008). This mutation was observed in 68% of melanoma metastases and 80% of primary melanoma. Investigators observed this same BRAF mutation in 63 of 77 (82%) histologically diverse nevi including 4 of 5 (80%) dysplastic nevi (Pollock et al. 2003). This

suggests an early role for BRAF in the development of melanoma. BRAF mutations do not appear to be inherited; instead the mutations are common (59%) in melanomas arising in skin which has received intermittent sun exposure, such as the trunks and arms (Chin et al. 2006). However, the transversion that occurs with this mutation is not classically associated with UV-induced damage. BRAF is known to play a role in cell growth and division. By introducing an activated mutation of BRAF into cultured melanocytes, investigators showed that BRAF can act as an oncogene in early stages of melanoma. This activation resulted in a constitutive activation of MEK and ERK and ultimately tumorigenicity in nude mice (Wellbrock et al. 2004). Dr. Arbiser of Emory University School of Medicine identified the activation of MAPK as an early event in melanoma progression (Cohen et al. 2002). One hundred and thirty-one melanocytic lesions, ranging from atypical nevi to metastatic melanoma, were studied for the expression of phosphorylated (active) MAPK and two target genes known to be induced by MAPK signaling, tissue factor, and vascular endothelial growth factor. While MAPK activation was positive in only 21.5% of benign nevi (with mild atypia), MAPK activation was seen in both radial and vertical growth phase melanomas. These findings suggest MAPK signaling as a potential target of chemoprevention in early melanoma. These findings demonstrate that the mutation of BRAF and activation of the RAS-RAF-MEK-ERK-MAP kinase pathway, which mediates cellular responses to growth signals, are crucial and early steps in the progression of melanoma.

Also part of the MAPK pathway, NRAS is found mutated in 10–20% of melanomas. In one study, 5–35% of various graded melanomas or nevi had some type of RAS mutation (Yasuda et al. 1989). Within the ras family, N-ras has the most significant association with melanoma progression. Herlyn and colleagues found a role for ras in approximately 15–20% of melanomas with a positive association with sun exposure (Herlyn and Satyamoorthy 1996). Mutations in NRAS can also activate the PI3-kinase pathway that can lead to increased cell proliferation, apoptosis, and tumor cell chemoresistance. Mutations in melanoma occur at low frequencies in other components of this pathway including AKT and PIK3CA (Omholt et al. 2006; Davies et al. 2008).

Accumulated mutations in melanocytic nevi as they transform to melanoma include mutations in CDKN2A, TP53, NFI, RAC1, and PTEN (Viros et al. 2014; Melamed et al. 2017).The phosphatases PTEN/MMAC1, located at 10q23,3, have been found deleted in more than 40% of melanoma cell lines (Ortonne 2002). In a study of melanoma progression from normal skin, acquired melanocytic nevi and cutaneous melanoma, nuclear PTEN expression was lost in both benign and malignant melanocytic lesions. However, the benign tumor retained cytoplasmic expression while the cutaneous melanoma demonstrated a complete lack in PTEN expression (Tsao et al. 2003). Bcl-2 has been demonstrated to be overexpressed in melanoma cells (Jansen et al. 2000). In addition, approximately a 20 or 40% increase of Bcl-X_L mRNA levels was detected in primary or metastasized melanoma tissue, respectively. The metastasized melanomas expressed higher Bcl-X_L than their matched primary tumors. These studies also showed that the expression of

Bcl-X_L resulted in UVB resistance in both primary and metastatic melanoma cells (Zhang and Rosdahl 2006). The transcription factor AP-2 and three of its downstream targets, c-kit, E-cadherin, and p21, were found to be involved in later phases of melanoma progression (Baldi et al. 2001).

Another frequently altered chromosome region, 9p21, contains a group of genes involved in cell cycle regulation. Among several potential tumor suppressor genes located on 9p21, p16 (CDKN2A/$p16^{ink4a}$) is the most important melanoma susceptibility gene identified to date with germ line mutations present in 9p-linked melanoma families (Hussussian et al. 1994; Kamb et al. 1994) and in 30–50% of members of melanoma kindreds (Halachmi and Gilchrest 2001). p16 inhibits the ability of cyclin-dependent kinases, CDK-4 and CDK-6, to activate substrates needed for progression past G1 of the cell cycle and therefore acts as a cell cycle check point protein (Liggett Jr. and Sidransky 1998). Germ line mutations in the gene encoding CDK-4 have also been described in a small number of melanoma-prone cases (Zuo et al. 1996; Soufir et al. 1999a). In sporadic tumors, loss of p16 protein expression has been shown to occur only in invasive and metastatic stages of melanoma and to be infrequent in primary thick nodular melanoma (Reed et al. 1995; Straume and Akslen 1997). The loss of p16 also seems to be associated with recurrent disease and has been the most useful marker for progressive disease. Alterations in p16 include CpG island methylation and translation repression mutations in the five prime untranslated regions (Haluska and Hodi 1998). Transcriptional upregulation of p16 has been shown in melanoma cells following UVB irradiation (Piepkorn 2000). UV-induced mutations of p16 have been reported in epithelial skin tumors from sporadic patients and from xeroderma pigmentosum patients, who suffer from hypersensitivity to UVR (Soufir et al. 2000).

Linkage studies of families with multiple cases of melanoma have been important in pursuing genetic analysis; however, the genetic relationship between melanoma and the dysplastic nevus syndrome is complex. Karyotype studies of both familial and sporadic melanomas frequently showed large deletions of band region 1p36, del(1) (p36.1–p36.3) (Dracopoli et al. 1994), suggesting that multiple tumor suppressor genes in this region were deleted. The PITSLRE protein kinase gene locus maps to band region 1p36. Several of its products may affect apoptotic signaling (Lahti et al. 1995). Studies have demonstrated alterations in the PITSLRE protein kinase gene complex in melanomas (Nelson et al. 1999).

Mutational studies of late stage melanomas show that BRAF, NRAS, and c-Kit are optimal targets for therapeutic management. A single institution study, between 1991 and 2015, studied 63 patients with mutated melanoma (Ponti et al. 2017). B-RAF mutations were found in 70% of the melanomas while N-RAS and C-KIT were seen in 19 and 11%, respectively. BRAF were found at sites of intermittent sun exposure, NRAS mutations were found in chronic sun-damaged areas, and C-KIT were located at acral and mucosal sites. While chemotherapy and immunotherapy using these targets do indeed show some activity, the key to determining the early stage targets is what is needed for prevention.

13.4 Screening and Early Detection

Early screening for SCC is often done by the diagnosis of AK. Self-exams are strongly recommended. Warning signs include a skin growth that increases in size or changes color or thickness or a sore that continues to crust, bleed, or itch. Identification of these changes warrants a more extensive checkup from a dermatologist. A precursor lesion to SCC, AK often requires treatment. AKs can be difficult to treat and require frequent visits to a dermatologist, since patients usually have multiple AKs that present at different times. The treatment for AK is usually cryosurgery with liquid nitrogen, excision, including Mohs surgery, or topical 5-FU cream (International Medical News Group 2002). Cryosurgery is the most common treatment but is associated with blistering, scabbing, hypopigmentation, inflammation, and occasionally pain. Treatment with 5-FU often results in severe blistering. Other options for AK treatment include dermabrasion or chemical peeling (Dinehart 2000). Topical diclofenac (Del Rosso 2003), imiquimod (Berman et al. 2004), and aminolevulinic acid (in photodynamic therapy) have also been approved by the US Food and Drug Administration (FDA) for the treatment of AK. While these drugs can be effective, they also cause painful and irritating local skin toxicities. Appropriate chemoprevention strategies of AK or pre-AK treatment would not only reduce incidence of SCC but also eradicate the need for these disagreeable treatments mentioned above.

Survival from melanoma is very dependent on identifying the melanoma at early stages. Screening programs have been analyzed to determine if there is a benefit in reduction of melanoma. Results demonstrate that screening results in an increase in the detection of nonmelanoma, melanoma in situ, and thin melanoma and a decrease in thick melanoma incidence and mortality (Brunssen et al. 2016). The screening is primarily whole body skin examinations by trained physicians. Screening for early melanoma by self-examination is strongly recommended as well. This assessment includes a review of one's moles carefully looking for what the Skin Cancer Foundation calls the ABCDs of melanoma: asymmetry, borders, color, and diameter. Most early melanomas are asymmetrical where the common mole is round and symmetrical. Early melanomas often have irregular borders with scalloped or notched edges. Normal moles have smooth borders. The color of early melanoma tends to have several shades of brown, tan, or black, and as the melanoma progresses, the colors red, white, and blue may appear. Normal moles tend to be a single shade of color. Early melanomas tend to grow larger than normal moles with diameters of at least 6 mm. The discovery of any of these characteristics should be promptly reported to a physician, preferably one that specializes in skin cancer and is trained to identify early signs of melanoma.

It is apparent that with the increase of skin cancer incidence, incomplete resolution by early detection, and the current treatments, there is an urgent need to develop well-tolerated and effective prevention strategies for NMSC and melanoma.

13.5 Prevention of Skin Cancer

13.5.1 Primary Prevention

Since exposure to UVR is a major risk factor in the development of skin cancer, the focus of primary prevention has been to limit exposure to UVR. The 2014 Surgeon General Call to Action to Prevent Skin Cancer (Report 2014) calls for the need to limit UV exposure. The recommendations from the American Academy of Dermatology, the American College of Preventive Medicine, and the American Cancer Society are (1) to reduce sun exposure during peak hours of intense ultraviolet exposure (usually 10 a.m. to 4 p.m.); (2) to wear protective clothing to cover as much of the skin as possible, including long sleeved shirts and hats with wide brims; and (3) to seek shade (Manson et al. 2000). Public health campaigns have been underway since the 1980s for the prevention of skin cancers; however, public campaigns have been met with limited success. These campaigns recommend limited exposure to sun, the use of sunscreen, and early detection through screening. In Australia, where skin cancer rates are the highest in the world, major campaigns have taken place for over 30 years such as the "Slip! Slop! Slap!" ("slip" on a shirt, "slop" on sunscreen, "slap" on a hat), "Sunsmart," and "Me No Fry." While 90% of Australians now recognize the dangers of skin cancer and the associated risks (Borland et al. 1992), the relationship between education and incidence reduction is still unclear. Data regarding these Australian programs do demonstrate a shift in knowledge, attitudes, and beliefs about sun exposure behavior, including suntans, which are losing some popularity, especially in young adults (Marks 1999; Volkov et al. 2013; Youl et al. 2013). Current campaigns focus on reaching young adults via social media and texting. One such program focused on mothers engaging in conversations with their daughters about the dangers of indoor tanning demonstrated reduction in the daughters' desire to get indoor tans (Pagoto et al. 2016).

As mentioned previously, the use of indoor tanning equipment has been associated with increased melanoma and nonmelanoma development. In May 2014, the US FDA (Le Clair and Cockburn 2016) issued regulations to strengthen the warnings for indoor tanning devices, including (1) strong recommendation against the use of tanning beds by minors under 18; (2) the reclassification of tanning beds and sunlamps from Class I to Class II medical devices (meaning moderate to high risk); and (3) mandate oversight, requiring manufacturers to provide safety assurances (warnings of risk for users regarding skin cancer risk and not recommended for minors under the age of 18). The American Academy of Dermatology Association (AADA) (www.aada.org) supports national efforts to place restrictions on indoor tanning for minors. Several states have passed laws to prohibit minors under the age of 18 from indoor tanning. In the 2010 passing of the Affordable Care Act, there was a nationwide 10% tax on indoor tanning implemented in an attempt to curb tanning bed use. A 25% drop in patronage has accompanied this tax (Jain et al. 2012).

The Cancer Progress Report from the US Department of Health and Human Services and related departments reports limited success in the US population's

attitudes toward sun exposure (2001). This report contains data gathered by the Centers for Disease Control and Prevention/National Center for Health Statistics. In the year 2000, 60% of adults said they were likely to seek some sort of sun protection, 31% were likely to use sunscreen, 26% were likely to use sunscreen with a sun protection factor (SPF) of 15 or higher, 32% were very likely to wear protective clothing, and 28% were very likely to seek shade. These data show an increase from 1998 when there was an actual decline in the sun protection from previous years. In the June 3, 2002, issue of the *Philadelphia Inquirer*, the author remarks: "Most adolescents avoid sunscreen like a summer reading list" (Uhlman 2002). The desire for a golden tan and the messy inconvenience of sunscreen outweigh the distant threat of skin cancer in the minds of these adolescents. In a study of sun protection practices in adolescents, only one-third of the respondents reported routine sunscreen use during the past summer (Geller et al. 2002). Eighty-three percent reported sun burning at least once and 36% reported three or more burns during the previous summer. Nearly 10% of all respondents used tanning beds during the previous year, with 24.6% of girls aged 15–18 reporting tanning bed use. Many girls who used tanning beds reported a belief that it was worth getting burned. These findings are very alarming considering that tanning in the teen years is a key factor for lifetime cumulative sun exposure and increased risk for skin cancer, particularly melanoma, which is clearly related to early age sunburns.

13.5.2 Secondary Prevention

The current primary methods for skin cancer prevention, including behavioral modification and the use of sunscreens, have demonstrated a better awareness of the risks for skin cancer. However, the incidence of cancers exemplifies that these campaigns are not sufficient to protect against rise in skin cancer incidence. Therefore, other strategies of prevention need to be coupled with primary prevention. The most promising of these strategies is the development of chemopreventive agents, which target early-stage or precancerous lesions. Sporn and Suh (2000) described chemoprevention as a "pharmacological approach to intervention in order to arrest or reverse the process of carcinogenesis." They emphasize the importance of an increased cancer research effort to control carcinogenesis "rather than attempting to cure end-stage disease." Control of carcinogenesis should be targeted at early stages because "it is easier to fix anything when the smallest numbers of its components are broken." The control of carcinogenesis through chemoprevention gained credibility with the FDA approval of tamoxifen for reducing breast cancer (Fisher and Voorhees 1998; Lippman and Brown 1999) and from FDA approvals of agents to treat intraepithelial neoplasias (IENs) such as diclofenac for AK (O'Shaughnessy et al. 2002) and celecoxib for familial adenomatous polyposis (Steinbach et al. 2000). Agents for chemoprevention are ultimately applied to the general healthy population at high risk for particular cancers. Safety and efficacy must be established in large-scale prospective randomized clinical trials. Furthermore, agents need to be nontoxic, inexpensive, and

available in oral or topical form (for skin). Clinical trials in patients with premalignant lesions are initially performed to investigate the modulation of biomarkers as surrogate endpoints. Lippman and Hong equate the current cancer chemoprevention studies to a delay in cancer development where the measures include a reduction in the rate of tumor development and overall decrease in the incidence of number of tumors (Lippman and Hong 2002). Meyskens described chemoprevention as an interaction between sciences of carcinogenesis, cellular biology, and cancer screening/early detection and cancer prevention/treatment (Meyskens Jr. 1988). Clearly, all of these scientific disciplines are required to develop highly efficacious chemopreventive strategies for skin cancer. Several reviews have been written which describe the current development of chemoprevention of skin cancer (Stratton et al. 2000; Bowden 2004; Wright et al. 2006).

For skin cancer, the eradication of AK and DN would most likely reduce the incidence of NMSC and melanoma, respectively. The approaches employed in the development of chemopreventive agents include the following: (1) availability of precancerous lesions (AK or DN) to evaluate the potential reduction in risk of progression; (2) identifying target molecules that are often modified and subsequently contribute to skin carcinogenesis; (3) developing animal model systems to test potential chemopreventive agents in skin; (4) delivery of highly potent agents directly into the epidermis, even more specifically through the development of novel formulations; and (5) availability of intermediate molecular or histological markers of the carcinogenic process to be used as endpoints.

13.5.3 Targeting Precursor Lesions for Chemoprevention

Current chemoprevention trials evaluate the efficacy of chemoprevention agents by the eradication or reduction of intraepithelial neoplasias (IENs). In skin, the IENs include AK for SCC and DN for melanoma. Individuals with AK are at increased risk for developing NMSC, and the presence of DN is the single most important risk factor for developing melanoma.

In general, IENs are cancer precursor lesions that have genetic abnormalities, loss of cellular control function, similar phenotypic characteristics of invasive cancer, and are risk markers for cancer. The presence of IENs in an individual is indicative of an increased likelihood of developing invasive cancer as compared to unaffected individuals (O'Shaughnessy et al. 2002). The American Association for Cancer Research (AACR) Task Force on the Treatment and Prevention of Intraepithelial Neoplasia recommends targeting individuals with or at risk for IENs for new agent development because of the potential preventive consequence on developing invasive cancer (O'Shaughnessy et al. 2002). IENs have been described for many types of cancers including colorectal adenomas for colorectal cancer, dysplastic oral leukoplakia for head and neck cancers, Barrett's esophagus for esophageal cancer, cervical intraepithelial neoplasia for cervical cancer, prostatic intraepithelial neoplasia for prostate cancer, transitional cell carcinoma in situ for bladder cancer, and AK for NMSC (Fig. 13.2).

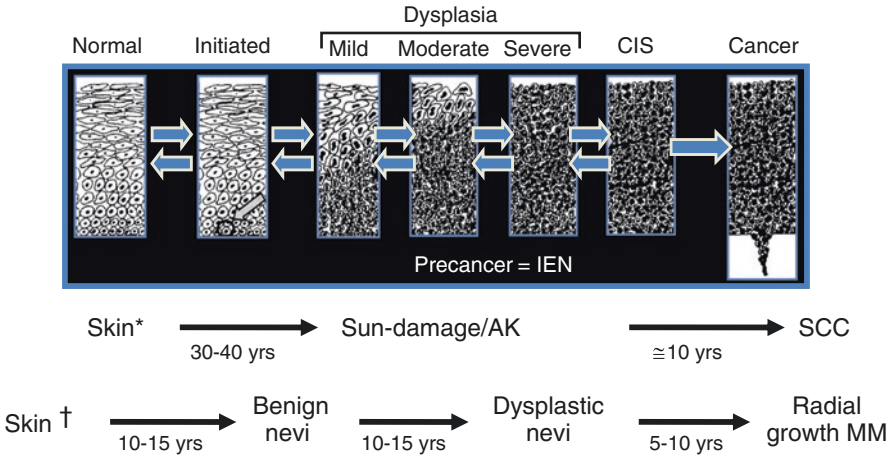

Fig. 13.2 Intraepithelial neoplasia in skin cancer. The strategy for evaluating the efficacy of chemoprevention agents is targeting precursor lesions in the development of skin cancers. These intraepithelial neoplasias in the skin include AK for SCC and dysplastic nevi for melanoma. These lesions are also important risk factors for the development of skin cancer (*Adapted from O'Shaughnessy et al. (2002); †Adapted from Li and Herlyn 2000; Harris et al. 2001a, b; Li et al. 2002)

Targeting precancerous lesions for chemoprevention is a rational strategy for the reduction of SCC incidence. Evidence includes (1) the FDA approval of diclofenac for treating AK as a preventive measure against SCC (O'Shaughnessy et al. 2002) and (2) a report from the Southeastern Arizona Skin Cancer Registry that suggests the leveling of SCC incidence in southeastern Arizona could be due to the removal of the precursor lesion, AK, while BCC incidence appears to continue to rise because there is no known precursor lesion to be removed or treated (Harris et al. 2001a, b). Both surgical and FDA-approved topical drug treatments available for the management of AK are listed in Table 13.1. Of these, only diclofenac is molecularly targeted (i.e., against cyclooxygenase 1 and 2 enzymes).

AKs, also known as solar or senile keratoses, are cutaneous lesions with chromosomal abnormalities that occur primarily on sun-exposed skin surfaces (Callen 2000). AK is a proliferating mass of transformed neoplastic keratinocytes confined to the epidermis. AKs develop on the surface of the skin as thickened, cornified, scaly lesions (O'Shaughnessy et al. 2002). Papules and plaques are often found on a background of sun-damaged skin with telangiectasias, hyper- or blotchy pigmentation, and a yellowish hue. The lesions range in size from 1 to 2 mm papules to large plaques (Callen 2000). AKs are most often diagnosed by histopathologic examination, since diagnosis by appearance can often be unclear as to whether the lesion is an AK or SCC. Typical histological characteristics of AKs include irregular arrangement of cells with atypical, pleomorphic keratinocytes at the basal cell layer demonstrating nuclear pleomorphism, loss of polarity, crowding of nuclei, and disordered maturation (Callen 2000).

Table 13.1 Management of actinic keratosis is done both surgically and by FDA-approved topical drug treatments. Of those listed, the only molecularly targeted treatment is diclofenac (Lober and Fenske 2004; Spencer, Hazan et al. 2005; Martin and Swanson 2013)

Surgical therapy
Cryotherapy
Curettage
Current FDA-approved topical drugs
5-Fluorouracil (e.g., Efudex®, Carac®)
Diclofenac (e.g., Solaraze®)
Aminolevulinic acid HCl (+UV light) (i.e., photodynamic therapy)
Imiquimod (e.g., Aldara®)
Ingenol mebutate gel (e.g., Picato®)

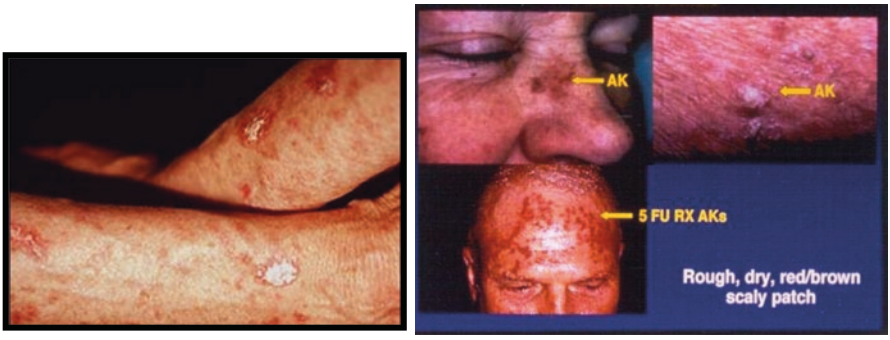

Fig. 13.3 SCC precursor: actinic keratosis (AK). Most AKs spontaneously regress. However, 0.1–16% may convert to SCC. AKs are risk markers for SCC

The lack of significant cytological differences between AK and SCC gives rise to the premise that AKs represent early SCCs (Dinehart et al. 1997). Several investigators consider AKs to be precursors or early forms of SCC (Glogau 2000; Salasche 2000). Inasmuch as AK is well accepted as a precursor to SCC, the US Centers for Medicare and Medicaid Services have added a national coverage policy to include the treatment of AK (2002). As shown in Fig. 13.3, the percent of AKs that progress to SCC is in the range of 0.1–16% (Glogau 2000; Stratton 2001) (Fig. 13.3). Approximately 60% of SCCs have been demonstrated to arise from preexisting AKs or the contiguous skin surface (Sober and Burstein 1995). Therefore, AK can be defined as a potential risk factor for the development of SCC.

Based on histological features, melanoma development has been described by Li and Herlyn as follows: (1) common acquired and congenital nevi with normal melanocytes that have a finite lifespan and no cytogenetic abnormalities; (2) DN that displays both cellular and architectural atypia; (3) radial growth of a melanoma; (4) vertical growth phase of the primary melanoma; and (5) metastatic melanoma (Li and Herlyn 2000).

While there remains controversy over whether DNs progress to melanoma, it is very evident that DNs confer a major risk for melanoma (Elder 2010; Farber

et al. 2012). DNs are found at a much higher frequency in patients with a history of melanoma. Prevalence rates range from 34 to 59% (Farber et al. 2012). One study demonstrated that on average, 34% of patients with melanoma had DN, in comparison with 11% of control subjects. Relative risk ranged from 1.0 to 16.7 for melanoma in the presence of DN. Several studies also reported an increased risk for melanoma with an increase in the number of DN. Cohort studies of patients with familial DN have also provided evidence for the presence of DN as a risk factor for the development of new melanomas (Greene 1997). In a retrospective study drawn from 820 patients diagnosed with a first primary cutaneous melanoma, 82% of 50 examined patients with multiple melanomas were clinically diagnosed with DN (Stam-Posthuma et al. 2001). Histological confirmation was demonstrated in 78% of these patients, and 16 of 37 patients had more than 30 clinically diagnosed DNs, 8 patients had 11–20 DNs, 4 patients had 21–30 DNs, and 9 patients had 1 DN. Finally, prospective studies have concluded that patients with DN and no family history also have an increased risk of melanoma (Greene 1997).

Other studies have investigated the idea that melanoma actually arises from DN (Marras et al. 1999). One such report performed cytogenetic analyses of DN in a young patient with a family history of melanoma (Marras et al. 1999). A t(6;15)(q13;q21) translocation found in one of the DN was similar to a translocation, with a breakpoint at 6q13 reported in a benign, nondysplastic nevi (Richmond et al. 1986) and in a cutaneous metastatic melanoma (Thompson et al. 1995). The repeated occurrence of this rearrangement provides initial support for the hypothesis that melanoma progresses from normal melanocytes to benign nevus, to DN, to early melanoma, to late melanoma, and then to metastatic melanoma.

In a study by investigators at the National Cancer Institute (NCI) and the University of Pennsylvania, almost all members of a family cohort with melanoma also had DN. New melanomas were only diagnosed in family members with DN (Greene et al. 1985a, b). These data suggest that not only are DNs risk factors for melanoma, but they may also be the precursor lesions from which new melanomas evolve.

The use of DN as a precancerous lesion and an indication of chemoprevention efficacy has been used in previous research and is proposed in upcoming trials. To date, four chemoprevention trials with topical tretinoin have been performed on individuals with DN (Stam-Posthuma et al. 1998). In these trials, DNs were targeted as surrogate markers for chemoprevention of melanoma.

13.5.4 Molecular Targets for Chemoprevention Identified in UVR Signaling Pathways

Skin carcinogenesis caused by UVR is a multistep process of initiation, promotion, and progression (Fig. 13.4). The best phases to intervene are the tumor promotion and progression phases, which are slow, rate-limiting stages. The initiation phase occurs rapidly. The targeting of AK and DN is at the promotion phase where several

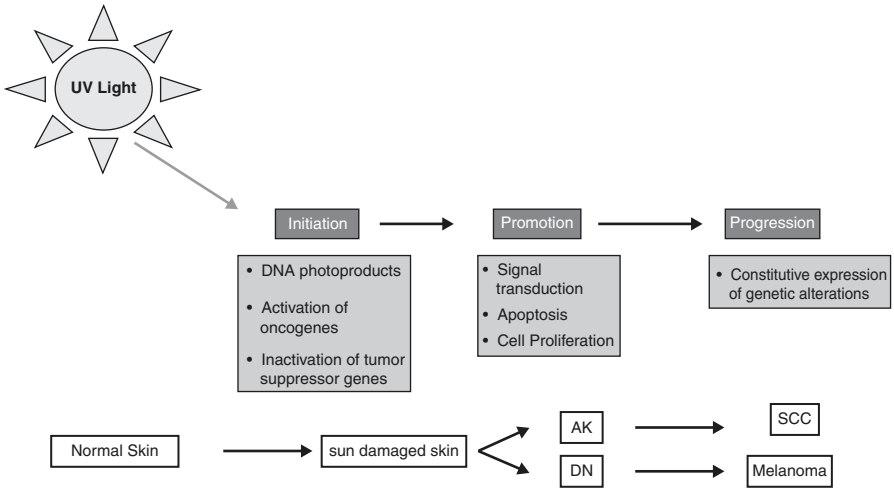

Fig. 13.4 Multistep UV-induced carcinogenesis. This multistep process involves sun exposure of normal skin or benign nevi to develop into AK or DN and progress to SCC or melanoma, respectively. The process involves initiation, promotion, and progression via the formation of photoproducts, activation of oncogenes, or inactivation of tumor suppressor genes to signal transduction, cell proliferation, or apoptosis and finally to constitutive activation of genetic alterations

specific genetic alterations can occur. In order to produce specific chemopreventive agents, it is necessary to first identify important molecular targets, which are modified in the carcinogenesis process. Bode and colleagues (2004) describe three major criteria for "valid" targets for cancer prevention: (1) The target molecule is deregulated in tumor development. The target molecule is affected by a tumor promoter resulting in a cascade of activation or inhibition of signal transduction pathways implicated in carcinogenesis. (2) The outcome of the deregulation of the target molecule results in malignant transformation, cell proliferation, and cell cycle arrest and/or apoptosis. (3) The deregulation of the target molecule significantly impacts carcinogenesis in vivo. For both NMSC and melanoma, many of these targets can be identified by understanding the UVR signaling pathways and identifying the points where alterations occur due to UVR signaling. This chapter briefly discusses select targets which are discussed in more detail in two extensive reviews of chemoprevention of photocarcinogenesis by targeting UVR signaling (Afaq et al. 2005; Bowden 2004). Bachelor and Bowden have reviewed UVA-mediated signaling which may be involved in skin tumor promotion and progression and eventually provide additional targets for chemoprevention (Bachelor and Bowden 2004a). The identification of these targets has been revealed by in vitro and in vivo model systems in the laboratory. The initiating events in skin cancer appear to involve gene mutations in proto-oncogenes or tumor suppressor genes. In the case of UVR-induced skin carcinogenesis, these initiating mutations have been identified in the TP53 tumor suppressor gene as UVR signature mutations (Ziegler et al. 1993). These mutations

have been identified in AK and SCC (Nelson et al. 1994). The initiated cell undergoes a clonal expansion during the promotion phase at which point it is most likely that AK and DN in human skin arise. UVR tumor promotion is carried out by signaling molecules that give rise to altered gene expression. For SCC development, the UVR-induced clonal expansion signaling has been demonstrated to lead to the activation of activator protein-1 (AP-1) transcription factor or to cyclooxygenase-2 (COX-2) expression (Bowden 2004). Three signaling molecules identified in the UVR signaling cascade (Fig. 13.5) during the promotion stage of SCC include MAPKs (Chen et al. 2001a), phosphatidylinositol 3-kinase (PI3K) (Kabuyama et al. 1998), and epidermal growth factor (EGF) receptors (Wan et al. 2001a, b). These serve as excellent targets for the development of chemoprevention.

AP-1 is upregulated in response to UVR-induced MAPK signaling in human keratinocytes in vitro (Chen and Bowden 2000). The transcription factor, AP-1, mediates the transcription of genes containing a 12-O-tetradecanoylphorbol-13-acetate (TPA) response element (TRE) (Lee et al. 1987). AP-1 is made up of homo- and heterodimers of proteins from the Jun and Fos families (Curran and Franza Jr. 1988). These genes are considered early response genes because of their rapid response to environmental changes, such as growth factors, stress, or DNA damage

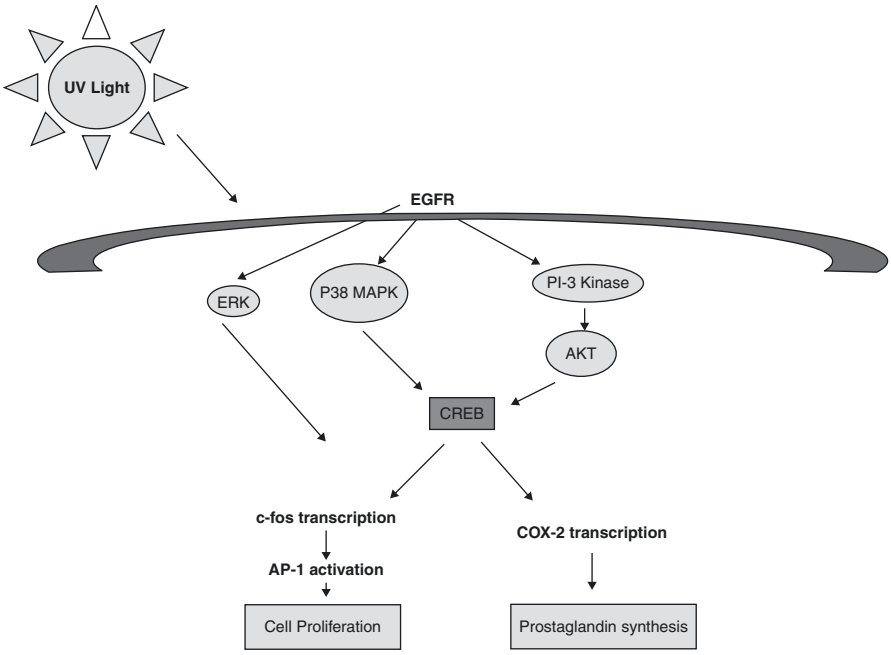

Fig. 13.5 UV signaling pathway via the epidermal growth factor receptor (EGFR) involves ERK, MAPK, and PI-3 kinase activation that leads to the activation of AP-1 and/or COX-2. These markers have been identified as potential targets for chemoprevention in NMSC. Current ongoing investigations are exploring a UV signaling cascade including PI-3 kinase, AKT, MAPK, and Raf as potential chemoprevention targets for melanoma

(Angel et al. 1988; Ryseck et al. 1988). Induction of Jun and Fos results from post-translational modification (Stein et al. 1992). In contrast, UVR activation of AP-1-dependent genes, such as the metalloproteinase genes and c-Fos, appears to require new protein synthesis of Jun and Fos (Konig et al. 1992).

c-Fos is constitutively expressed in both rodent and human epidermis (Basset-Seguin et al. 1990; Fisher et al. 1991) as demonstrated by immunohistochemical localization, suggesting that c-Fos has a role in growth and cell proliferation (Basset-Seguin et al. 1990; Fisher et al. 1991). Additionally, UVB (Chen et al. 1998) and UVA irradiation (Silvers and Bowden 2002) has been shown to induce c-Fos expression in human keratinocytes. Similarly, in rat epidermis, single doses of UVB produced a rapid and sustained increase in c-Fos and c-Jun mRNA and protein throughout the epidermis at early time points, but were restricted to the basal layer at later time points. This suggests their possible role in the induction of both apoptosis and cell proliferation (Gillardon et al. 1994).

Investigators have demonstrated the important role of AP-1 in UVR carcinogenesis in human and mouse keratinocytes as well as transgenic mice. UVB (Chen et al. 1998) and UVA (Silvers and Bowden 2002) were shown to activate AP-1 in HaCaT cells (human keratinocyte cell line) with a correlative increase in c-Fos expression. The blocking of AP-1 transactivation in malignant mouse SCC cell lines inhibits the formation of tumors in athymic nude mice (Domann et al. 1994). c-Fos and junD were identified as the main components of the AP-1 complex induced by UVB. The upregulation of AP-1 by UVB has been demonstrated in mouse skin (Barthelman et al. 1998b) and human skin (Fisher et al. 1998). In studies with mouse epidermal JB6 cells, it has been demonstrated that blocking tumor promoter-induced AP-1 activity inhibited neoplastic transformation by an AP-1-inhibiting dominant-negative Jun (Dong et al. 1994). UVB irradiation studies have demonstrated an induction of AP-1 through the MAPK signaling cascade in human keratinocytes (Chen and Bowden 1999). A critical role for MAPK signaling (p38 and JNK) in AP-1 transactivation has also been demonstrated by UVA irradiation (Silvers et al. 2003). A mouse model that was used for testing the hypothesis that AP-1 activation has a functional role in the promotion of UVB-induced skin tumors is a TAM67 mouse crossed with a mouse expressing an AP-1 luciferase reporter gene. The TAM67 transgenic mouse contains a dominant-negative c-Jun mutant transgene (TAM67) under the control of the human keratin 14 promoter expressed in the epidermis of SKH-1 hairless mice. These mice show a decrease in UVB light-induced AP-1 activation with a signal UVB exposure. The expression of the TAM67 delayed the appearance of tumors, reduced the number of tumors per mouse, and reduced the size of the tumors subsequent to chronic UVB exposure. The data demonstrated that the expression of the TAM67 inhibited UVB-induced AP-1 activation in the epidermis and inhibited UVB-induced skin tumor development. Information gathered from these studies has enabled the formulation of a UVB signaling pathway that leads to AP-1 activation and provides a good molecular target for the development of new chemoprevention strategies to prevent UVR-induced skin cancers (Bowden 2004).

Einspahr et al. (2012) demonstrated protein pathway activations in the progression of normal skin (upper inner arm), AK, and SCC using reverse-phase protein microarray analysis. Two sets of samples looking at 51 signaling proteins in the first set and 102 key signaling proteins in the second set demonstrated that the MEK-ERK pathway was activated in SCC compared with the AK and normal skin. The epidermal growth factor receptor (EGFR) and mTOR pathways were aberrantly activated in SCC. In AK, an increase in Bax and Bak expression was demonstrated compared to normal skin. Several of these connected pathways provide targets for potential chemoprevention agents.

The MAPKs are part of signaling cascades that involve the regulation of cell proliferation and differentiation in human epidermis (Geilen et al. 1996). Mitogen-activated protein (MAP) kinases are a family of serine/threonine protein kinases. These kinases have been found to be important in cellular response to growth stimuli (Peyssonnaux and Eychene 2001). MAP kinases are activated by translocation to the nucleus, where kinases phosphorylate their target substrates such as transcription factors (Coso et al. 1995). The MAP kinase family includes c-Jun-NH_2 terminal kinases (JNKs/SAPKs), extracellular signal-regulated protein kinases (ERKs), and p38 MAP kinases. JNKs/SAPKs and p38 kinases are activated by stress, including UVR irradiation (Kallunki et al. 1994). Investigators have demonstrated that UVA and UVB irradiation causes activation of ERKs, JNKs, and p38 kinases in cell culture (Huang et al. 1997a, Huang et al. 1997b, Huang et al. 1997c; Dong et al. 1998).

p38 MAP kinase plays an important role in UVB-induced c-Fos expression in human keratinocytes (Chen and Bowden 1999). Both p38 and ERK were significantly activated by UVB irradiation in human keratinocytes. Treatment of these cells with a p38 inhibitor, SB202190, inhibited UVB-induced p38 activation but did not induce ERK activation. In addition, the treatment of the cells with MEK1 inhibitor, PD98059, inhibited UVB-induced ERK activation but not UVB-induced p38 activation (Chen and Bowden 1999). The blocking of p38 almost completely abrogates UVB-induced c-Fos gene transcription and c-Fos protein synthesis. Inhibition of ERK partially abrogates UVB-induced c-Fos transcriptional and protein levels. Inhibiting both p38 and ERK completely blocked UVB-induced c-Fos expression but also decreased c-Fos basal gene expression. The p38 inhibitor, SB202190, strongly inhibited UVB-induced AP-1 transactivation as well as AP-1 DNA binding (Chen and Bowden 2000). Transgenic mice expressing dominant-negative p38 in the epidermis showed that genetic inhibition of this signaling pathway significantly blocked UVB-induced skin carcinogenesis in SKH-1 mice (Dickinson et al. 2011). Studies with UVA demonstrated that UVA-induced p38 MAP kinase activity also plays an important role in the survival of keratinocytes (Bachelor and Bowden 2004b). The UVA-induced activation of p38 and JNK plays a role in AP-1 activation, COX-2, and Bcl-X_L (Zhang and Bowden 2012). The inhibition of p38 MAP kinase by SB202190 decreases expression of Bcl-X_L and results in increased apoptosis. Consequently, it was shown that UVA induces p38 MAPK activity and the subsequent increase in Bcl-X_L resulted in a resistance to UVA-induced apoptosis. These data together suggest that the upstream molecules of c-Fos and AP-1 signaling, p38, and ERK are potential targets for chemoprevention in NMSC.

Another target gene in UVB signaling is COX-2. COX-2 is a key enzyme involved in the synthesis of prostaglandins. Prostaglandins have been linked to several important events of the carcinogenesis process. Studies of malignant melanoma progression have demonstrated that no COX-2 expression was observed in dysplastic nevi, primary skin melanoma cells, and vertical and radial growth phase cases, but COX-2 was strongly detected in the metastatic cancer cells (Goulet et al. 2003). In addition, five out of seven melanoma cell lines overexpressed COX-2 compared to normal melanocytes. An increase in COX-2 expression occurs after UVB exposure in both human skin (Buckman et al. 1998) and cultured human keratinocytes (An et al. 2002). There is also an increased expression of COX-2 protein in human squamous cell carcinoma biopsies and when compared to normal non-sun-exposed control skin. Selective inhibition of COX-2 in hairless mice has resulted in a significant reduction of UVR-induced skin tumors in hairless mice (Pentland et al. 1999). Of particular interest is another study which demonstrated that p38 is required for UVB-induced COX-2 gene expression in human keratinocytes (Chen et al. 2001a, b). Inhibition of p38 with SB202190 markedly inhibited UVB-induced COX-2 mRNA. There was no effect when the Mek inhibitor PD98059 was used. UVA has also been shown to induce COX-2 in keratinocytes (Bachelor et al. 2002). The expression of a dominant-negative p38α in the epidermis of SKH-1 hairless mice led to a significant decrease in UVB-induced tumor growth and number compared to wild-type littermates in a UVB skin carcinogenesis model (Dickinson et al. 2011). The expression of this transgene inhibited UVB-induced apoptosis of keratinocytes. The reduction of skin carcinogenesis in this model appears to be due to the inhibition of COX-2 expression and proliferation of UVB-irradiated cells. Since p38 MAPK appears to be an important step in two UV-induced signaling pathways (ending in the transcription factors AP-1 and COX-2), it is an excellent candidate as a target for chemoprevention.

JNK phosphorylates c-Jun (Derijard et al. 1994; Kallunki et al. 1994), a component of the AP-1 transcription factor. There are three JNK genes (JNK-1, JNK-2, and JNK-3) that have been identified in humans. It has been demonstrated that JNK2 knockout ($JNK2^{-/-}$) mice, in a two-stage tumor promotion skin carcinogenesis model with DMBA and TPA, exhibited significant reduction in papilloma burden compared with wild-type controls (Chen et al. 2001a, b). Further studies to look at the UVR signaling pathway for skin carcinogenesis may point toward JNK as another potential target for chemoprevention of skin cancer.

The phosphatidylinositol-3 kinase (PI-3 kinase) pathway regulates cellular proliferation, growth, apoptosis, and cytoskeletal rearrangement. PI-3 kinases are heterodimeric lipid kinases composed of regulatory and catalytic domains (Vivanco and Sawyers 2002). PI-3 kinase is an important enzyme associated with a variety of receptors or protein-tyrosine kinases and acts as a direct biochemical link between a novel phosphatidylinositol pathway and a number of receptor proteins, including the receptors for insulin or platelet-derived growth factor (Downes and Carter 1991). This enzyme is a heterodimer of a 110-kDa unit and an 85-kDa unit (Auger et al. 1989). It can phosphorylate phosphatidylinositol (Ptdins), Ptdins (4) phosphate [Ptdins (4) P], or Ptdins(4,5) bisphosphate [Ptdins(4,5)P2] to produce Ptdins(3)

P, Ptdins(3,4)P2, or Ptdins(3,4,5) trisphosphate [Ptdins(3,4,5)P3], respectively (Whitman et al. 1988; Nomura et al. 2001a; Cohen et al. 2002). Insulin or growth factor stimulation of the associated tyrosine kinase results in phosphorylation of the p85 subunit of PI-3 kinase. This phosphorylation is important for activation of PI-3 kinase (Huang et al. 1997a, b, c). Akt works downstream in the PI-3 kinase pathway to regulate proliferation, apoptosis, and growth (Vivanco and Sawyers 2002). Akt, a serine/threonine kinase, is activated by recruitment to the plasma membrane. Clinical evidence of PI-3 kinase activation has been reported in various cancers, and the identification of downstream kinases provides a potential target for mediating tumorigenesis (Vivanco and Sawyers 2002). Investigators have shown that UVB irradiation activates Akt in JB6, mouse epidermal cells. This activation was attenuated by inhibitors for MAP kinase/ERK kinase-1 and p38 (Nomura et al. 2001b). It has been reported that PI-3 kinase plays an important role in UVB-induced AP-1 and Akt activation (Huang et al. 1997a, b, c; Nomura et al. 2001a, b). Inhibition of PI-3 kinase was found to block UVB-induced activation of p90 ribosomal protein S6 kinase (P70S6K), known to be associated with AP-1 in tumor promoter-induced cell transformation (Zhang et al. 2001).

The T-LAK cell-originated protein kinase (TOPK) is a serine-threonine kinase that is a member of the MAPK kinase (MAPKK) family. TOPK is involved in many cellular functions, including tumor development, growth, apoptosis, and inflammation (Zykova et al. 2006, 2010; Ayllon and O'Connor 2007; Oh et al. 2007; Zhu et al. 2007; Hu et al. 2010; Kim et al. 2012). As noted above, the MAPKK signaling pathway is a major component of the RAS/RAF/MEK/ERKs signaling axis and is implicated in cutaneous SCC (cSCC). Previous studies showed that TOPK is highly expressed in many cancers such as lymphoma, leukemia, melanoma, colorectal, breast, and lung (Abe et al. 2000; Simons-Evelyn et al. 2001; Zhu et al. 2007; He et al. 2010; Park et al. 2010). During mitosis, TOPK and the Cdk1/cyclin B1 complex promote cytokinesis through phosphorylation of protein regulator of cytokinesis 1 (Matsumoto et al. 2004; Abe et al. 2007; Chen et al. 2009; Park et al. 2010). Zykova et al. (2006) reported that TOPK is involved in preventing apoptosis in melanoma cells (Zykova et al. 2006), while Oh et al. (2007) reported that it is a positive regulator of c-Jun-NH_2-kinase 1 (JNK1) signaling and H-Ras-induced cell transformation (Oh et al. 2007). A positive feedback loop also exists between TOPK and ERK2, which may increase the carcinogenic properties of HCT116 colorectal cancer cells, suggesting that TOPK-regulated signaling could be a therapeutic target in colorectal cancer (Zhu et al. 2007). Notably, p53-related protein kinase (PRPK), which is a downstream phosphorylation target of TOPK, was recently discovered to be overexpressed in skin cancer and is another crucial player in skin cancer development.

TOPK directly binds to PRPK and strongly phosphorylates Ser250, a site known to be important for PRPK activity (Facchin et al. 2007). Mutation of residue Ser250 of PRPK to alanine prevents its phosphorylation in colon cancer cells. Pull-down assay results further confirm that TOPK and PRPK directly interact, thereby demonstrating that PRPK is a novel protein-binding partner with TOPK (Zykova et al. 2010). Additional unpublished evidence from the laboratory of Dr.

Zigang Dong indicates that PRPK is activated by TOPK after UV stimulation, and that both proteins increase in phosphorylation after acute UV exposure in human skin (manuscript in preparation). Importantly, the same lab has developed specific pharmacological inhibitors of TOPK (ADA-07 and HI-TOPK-032) and have shown that they suppress UV-induced skin carcinogenesis and colon cancer growth, respectively, in mouse models. (Kim et al. 2012; Gao et al. 2017).

Wan and colleagues demonstrated that solar UVR irradiation of human skin activated EGFR as well as other downstream signals including MAP kinases ERK, JNK, and p38 (Wan et al. 2001a, b). Their investigations revealed activation of the PI3-kinase/AKT survival pathway via EGFR. They also found that EGF crosstalks with cytokine receptors such as IL-1 receptor leading to the activation of c-Jun kinase in response to UVR irradiation of human keratinocytes. Additional investigators have shown that UVA-induced EGFR signaling is required for activation of p90RSK/p70S6K, PI-3 kinase, and ERK (Zhang et al. 2001).

Another transmembrane receptor that has been gaining attention for its possible role in skin carcinogenesis is Toll-like receptor 4 (TLR4). The Toll-like receptors (TLRs) are a family of proteins expressed throughout the body which are stimulated by pathogen-associated and damage-associated ligands. Activation of TLRs results in stimulation of the innate immune response (inflammatory cytokines, recruitment of immune cells), in order to protect the host from the perceived threat. Thus, TLRs are associated with inflammation and wound healing. It is thought that stimulation of TLR7 by topical application of imiquimod is the impetus for recruitment of inflammatory cells to the site of AKs, thus allowing inflammatory-mediated clearing of the precancerous lesion (Gupta et al. 2004). TLR4, on the other hand, is currently a target for both pharmacological stimulation and inhibition in the skin, depending upon the target of interest. TLR4 expression is typically sequestered to the basal layer of the epidermal keratinocytes and is strongly upregulated in cSCC (Weng et al. 2013; Janda et al. 2016). Janda et al. (2016) showed that TLR4 contributes to AP-1 and NF-κB signaling after UV stimulation of cultured keratinocytes, and that inhibition of TLR4 using the targeted pharmacological agent resatorvid (TAK-242) blocks UV-induced signaling both in cell culture and in epidermis, when topically applied (Janda et al. 2016). Investigators have also shown that long-term application of resatorvid significantly blocks UV-induced skin cancer in SKH-1 mice. Notably, both in cell culture and in tumors, resatorvid treatment potentiated apoptosis and inhibited p38 and Akt phosphorylation. Thus, inhibiting TLR4 activity seems to be a viable model for photochemoprevention of NMSC. This is supported by the fact that an alternative TLR4 inhibitor, eritoran, successfully inhibited colon cancer in mice (Kuo et al. 2016). It should be noted that another group has linked TLR4 with suppression of keratinocyte growth, so the matter of the contribution of TLR4 to NMSC is currently under debate (Iotzova-Weiss et al. 2017). Indeed, other groups are currently using agonists of TLR4 in skin-directed cancer therapies. For example, the LPS-mimetic GLA-SE is currently being utilized in clinical trials as an immune stimulant for patients with melanoma or with Merkel cell carcinoma (Clinicaltrials.gov: NCT02320305 and NCT02035657).

Signaling cascades due to UVR stimulation that leads to skin carcinogenesis of melanoma are not defined as extensively as for NMSC. Investigators have outlined UVR signaling pathways for melanogenesis (Tada et al. 2002). However, there are thus far only a few potential molecular targets (Raf and MAPK) for chemoprevention of melanoma. The Raf kinases were the first Ras effectors identified and have been the most extensively studied (Hunter 1997). Ras associates with and activates Raf-1, which in turn phosphorylates and activates MEK kinase, which in turn phosphorylates the MAP kinases ERK1 and ERK2 (Liaw et al. 1993; Samuels et al. 1993; Warne et al. 1993; Ghosh et al. 1994). Activated MAP kinases translocate to the nucleus where they can modulate gene expression (Hill and Treisman 1995; Marshall 1995). Raf-1 has also been shown to interact with PKC, a key regulatory protein associated with a second signal transduction pathway (Kolch et al. 1993). Two well-established biological events that are associated with activation of the Raf/MEK/ERK pathway are cell proliferation and cell cycle progression. Halaban and colleagues have observed that several of the mitogenic factors for melanocytes, bFGF, MCGF, and HGF/SF, stimulate ERK1/ERK2 phosphorylation (Funasaka et al. 1992; Halaban et al. 1992). Others have demonstrated that Raf plays an important role in progression of melanoma (Pollock et al. 2003). The data from these studies identified a particular mutation that was found in 68% of metastatic melanoma, 80% of primary melanoma, and 82% of a diverse set of nevi. These findings implicate Raf as a potential target for chemoprevention of melanoma, since Raf mutations are evident at the early stage of primary melanoma and nevi. The identification of MAPK as an early event in melanoma progression (Cohen et al. 2002) provides another potential target for the chemoprevention of melanoma.

A review by Zhang (Zhang and Rosdahl 2006) describes the targeting of Bcl-X_L for both the prevention and therapy of skin cancer. Bcl-X_L is localized on the mitochondrial outer membrane and plays a critical role in the homeostasis of both the intrinsic and extrinsic apoptotic pathways. Several studies have shown an antiapoptotic role of Bcl-X_L in skin. In immortalized keratinocytes, Bcl-X_L has been shown to be protective against UVA-induced apoptosis (Bachelor and Bowden 2004b). Zhang and Bowden describe the plausibility of targeting Bcl-X_L for NMSC and melanoma (Zhang and Rosdahl 2006). Melanoma cell lines have shown higher expression of Bcl-X_L than melanocytes (Bush and Li 2003), and both primary and metastatic melanomas have demonstrated increased expression (Zhang and Rosdahl 2006). Bcl-X_L has been shown to render primary and metastatic melanoma cells resistant to UVB irradiation. In a chemically induced skin carcinogenesis mouse model, expression of Bcl-X_L in a transgenic mouse resulted in a twofold increase in the number of papillomas formed compared to the wild-type mouse (Pena et al. 1998). In addition, more than half the transgenic mice developed SCC within 7 months of treatment, while none of the wild-type mice had SCC in the same time. The critical role in several stages of skin carcinogenesis, including initiation and promotion, makes Bcl-X_L a very plausible target for prevention of skin cancer through the development of chemopreventive agents.

13.5.5 Animal Models for Studying Chemoprevention Agents

In order to understand the mechanism of carcinogenesis and investigate efficacy of chemoprevention agents prior to clinical application, animal models that closely resemble human disease must be developed. The SKH-1 hairless mouse is a model for the studies of skin cancer pathogenesis and the evaluation of chemoprevention of UVB-induced skin cancer (Bowden 2004). The most obvious advantage of these mice is that they are hairless and therefore do not require any removal of hair that may actually protect the skin from UVR light. With increasing UV dose level, three times a week for 25 weeks, nearly 100% of the mice develop at least one skin tumor with an average of seven to nine tumors per mouse. Most of these tumors are SCC, which arise from benign papillomas. UVB irradiation is used as a complete carcinogen in these mice. Another protocol used with these mice is UVB exposure twice a week for 20 weeks. This results in epidermal hyperplasia; no immediate tumors occur but there is a high risk of developing skin tumors during the next several months in the absence of any further UVR. This latter model system resembles humans who are heavily exposed to UVR early in life with reduced exposure later in life. Chemoprevention agents can be tested in these models.

A mouse strain with abnormalities in the hedgehog signaling pathway develops neoplasms that closely resemble human BCC. These mice contain a heterozygous allele in the PTCH gene (Ptc+/−). Chemoprevention studies with green and black tea have been performed in this mouse model (Herbert et al. 2001).

Multiple animal models of melanoma have been reported; however, difficulties with these models for studies of chemoprevention are that tumors develop at a low incidence rate and the latency period is often very long. There are two prominent models for melanoma that are useful for the study of chemoprevention agents. Broome Powell et al. (1999) report the development of a transgenic mouse for which when chemically induced develops melanoma. The mouse line expresses a mutated human Ha-ras (TPras) gene driven by a mouse tyrosinase promoter. This transgene is therefore expressed in pigment-producing cells of the mice. The protocol for inducing melanoma in these mice is topical application of 50 μg 7,12-dimethylbenz-[a]anthracene (DMBA) once a week for 5 weeks. Development of melanoma occurs around 15 weeks. Tumors only occur in the mice expressing the transgene, and no tumors develop in the negative littermates. Tumors develop in more than 80% of the treated mice. No spontaneous cutaneous melanoma or other skin cancers develop in these mice. Metastatic lesions have been observed in the skin, lungs, and lymph nodes of the DMBA-treated transgenic mice (Broome Powell et al. 1999). Melanomas isolated from TPras transgenic mice display alterations and/or losses of p16 (Gause et al. 1997) much like human melanoma. Early experiments with these TPras mice did not result in UVR-induced melanoma, perhaps because of the highly pigmented skin of the adult TPras mouse. Further investigations using this model have found ways to produce UVR-induced melanoma in this model

system. The first was a single neonatal exposure (2–3-day-old mice) of UVR light which resulted in a penetrance of 57% by 12 months (Hacker et al. 2005). Another development of this model has been to cross it with an activated Cdk4 mouse. This resulted in spontaneous melanomas with an increase of penetrance of 83% when treated with UVR (Hacker et al. 2006). Another model is a transgenic model, which utilizes a metallothionein-gene promoter driving a hepatocyte growth factor/scatter factor (c-Met receptor tyrosine kinase ligand) gene based on the albino FVB background (Noonan et al. 2001). Development of melanoma occurs in this model system after a single acute exposure of an erythemal dose of UVR irradiation. Development of invasive melanoma occurs in 80% of the animals. These melanomas closely resemble human melanoma in terms of the development between the dermis and epidermis. Another mouse model, the HGF/SF-Tg model, produces melanoma with the exposure to UVB but not UVA (De Fabo et al. 2004)

One concern with UVR studies is the ability of the experimental UVR exposure to imitate the true solar spectral output. Many studies with UVR use light sources which produce primarily UVB output with minimal UVA output. One such debate has been brought forward in the study by Ibuki and colleagues (Ibuki et al. 2007), which produced data suggesting that UVA produces a protective role against UVB by inhibiting UVB-induced apoptosis. However, a published commentary (Runger 2007) to this publication noted that since UVA radiation inhibits UVB-induced apoptosis, this may only increase the mutation burden that would normally be eliminated by UVB-induced apoptosis and therefore increase skin cancer formation. With these questions still left to be answered, it is best to choose animal models that use UVR sources that combine both UVA and UVB spectral output, which best mimics the solar output for any studies of chemoprevention agents to be proposed for future human, clinical trials.

13.5.6 Endpoints for Evaluating Efficacy of Chemoprevention Agents

Because the process of carcinogenesis can take many years, assessment of clinical chemoprevention trials using cancer incidence as an endpoint requires a long follow-up period and large sample sizes. In addition to evaluating the modulation of targets for a specific chemopreventive agent, such as those involved in the UV signaling pathway discussed above (p38 MAPK, PI-3 kinase, etc.), biomarkers are useful for evaluating the efficacy of a chemopreventive agent. The rationale for the use of intermediate biomarkers is to circumvent these issues in chemoprevention trials (Einspahr et al. 1997), since biomarkers occur at steps preceding the occurrence of malignancy. As discussed by Lippman and colleagues (Lippman and Brown 1999), biomarkers of intermediate endpoints can be defined as measurable markers of cellular or molecular events associated with specific stages of the multistep progression of carcinogenesis. Thus, the risk of carcinogenic transformation, whether in

the skin or other sites, can be correlated with the quantitative degree and pattern of biomarker expression. Criteria for identifying and evaluating the potential efficacy of biomarkers are as follows:

- Variability of expression between phases of the carcinogenesis process (i.e., normal, premalignant, malignant)
- Ability for early detection in the carcinogenesis pathway
- Association with risk of developing cancer or recurrence of the precancer
- Potential for modification by a chemopreventive agent
- Presence in tissues that are easily accessible for multiple biopsies
- Capability to develop adequate assay quality control procedures

Markers of cellular proliferation can be used as an intermediate biomarker to evaluate the efficacy of chemoprevention agents in clinical trials and animal model systems. Enhanced cellular proliferation has been closely associated with the process of tumorigenesis in numerous tissues including the skin (Einspahr et al. 1996). Proliferating cellular nuclear antigen (PCNA) functions as an auxiliary protein to DNA polymerase d and e in DNA replication and repair (Hall et al. 1990). Expression of PCNA increases late in G1, is maximally expressed in S, and decreases in the G2/M phases of the cell cycle. Therefore, PCNA can be used to evaluate cell proliferation and possibly chemoprevention efficacy. Studies have indeed found a significant difference in PCNA expression in AK compared to sun-damaged skin (Einspahr et al. 1996, 2006) but not sun-damaged forearms compared to forearms from subjects with AK (Einspahr et al. 2006). PCNA was not useful in detecting an effect of the chemoprevention agent difluoromethylornithine (DFMO) (Einspahr et al. 2002). These investigators suggest that PCNA may be useful in combination with the number of apoptotic cells as an endpoint for clinical trials with chemoprevention agents. Another extensively used marker for proliferation is Ki67 which is present in all active phases of the cell cycle (G1, S, G2, and mitosis) but is absent from resting cells. MIB-1 is a commonly used monoclonal antibody that detects the Ki-67 antigen. Bordbar and colleagues (2007) evaluated the MIB-1 antibody in its usefulness in differentiating benign, premalignant, and malignant skin lesions.

Apoptosis also serves as biomarker for the efficacy of chemoprevention agents in clinical trials and animal model systems. Apoptosis is a unique mode of cell death, characterized by ultrastructural changes distinct from necrosis (Kerr et al. 1972). In the developing animal, programmed cell death removes cells during remodeling of a number of organs (Haake and Polakowska 1993). Apoptosis is also involved in tissue regression following hormone stimulation or deprivation in hormone-sensitive tissues, such as the prostate, and functions in development of the immune system (Haake and Polakowska 1993). In continually renewing tissues such as the epidermis, homeostasis is maintained through a balance between cellular proliferation and cell death. Apoptosis may also play an important role in regression of neoplasms (Haake and Polakowska 1993). Alterations in either cell proliferation or cell death can lead to loss of growth control, thereby playing major roles in the process

of tumorigenesis. Apoptosis is characterized by cell shrinkage, plasma membrane blebbing, nuclear fragmentation, and chromatin condensation. Apoptotic cells are rapidly phagocytosed by neighboring cells in order to prevent the release of cell contents. In contrast to necrosis, apoptosis is an organized and controlled process of cell death (Kerr et al. 1972). Measurements for apoptosis may include morphology, in situ TUNEL, and caspase-3 detection.

Investigators have shown that p53 mutations increase through the progression of normal skin, sun-damaged skin, AK, and SCC. While the frequency of p53 mutations was 14% in normal skin, this percentage rose to 38.5% in sun-damaged skin, 63% in AK, and 54% in SCC. Proliferation was also increased through this same progression. SCC samples demonstrated an increased presence of Bax compared to AK (Einspahr et al. 1999). An additional study confirmed the use of p53 expression as a valid biomarker in the progression of sun-damaged skin to AK to SCC (Einspahr et al. 2006). This latter study demonstrated that p53 expression as well as expression of selected polyamines is effective in differentiating early stages of skin cancer progression and was not affected by sunscreen use. These data support the use of p53 as a biomarker for disease progression when evaluating the efficacy of chemoprevention agents. A presented study demonstrated that vascular endothelial growth factor (VEGF) may serve as a biomarker for detection and chemopreventive modulation for melanoma studies. Investigators found that there was a higher VEGF expression in DN compared to benign nevi (Thomas and Bozzo 2006). Biomarkers that measure micronutrient and biochemical levels in tissue and blood may also be useful biomarkers in studies that evaluate chemopreventive agents' ability to slow or inhibit progression from a benign to premalignant to malignant stage.

Karyometric evaluation of the epidermis has been used as a developmental secondary endpoint in clinical studies (Bozzo et al. 2001). Ranger-Moore and colleagues have published a complete review of karyometric measures in intraepithelial lesions, discussing the usefulness of karyometry as an integrating biomarker for evaluating progression and effectiveness of chemopreventive agents (Bartels et al. 2002; Ranger-Moore et al. 2005). The advantage to this type of biomarker is that it can detect activity of a chemopreventive agent even when the mechanism for a given progression pathway is unknown or when multiple pathways exist. Nuclear chromatin patterns can be used diagnostically to assess changes in the development of cells, particularly the development into a cancerous cell, which could then be correlated with the prognosis of individual patients. Image analysis of nuclear chromatin patterns provides a quantitative approach (Weyn et al. 2000). With image analysis, karyometric features are described by the arrangement of a combination of pixels. These features are then combined by means of multivariate analysis of criteria used for prognosis. Digital microscopic studies of epithelia from the ectocervix (Wied et al. 1980), lung mucosa (MacAulay et al. 1995), colonic mucosa (Bibbo et al. 1990), glandular epithelium of the thyroid (Bibbo et al. 1986), breast (Susnik et al. 1995), bladder (Sherman and Koss 1983), and prostate (Irinopoulou et al. 1993) have detected very subtle, possibly preneoplastic changes in the organization of nuclear chromatin in biopsies from individuals with premalignant and

malignant lesions of these organ sites. When these same tissue sections were examined with standard histopathological techniques, no abnormalities were detected. Thus, digital microscopy can provide highly sensitive detection of early change and may provide novel diagnostic clues. Digital imagery can reliably detect very early subtle changes in the organization of nuclear chromatin in epithelial cells that appear to be entirely normal during histopathologic examination. This technology, which uses high-resolution imagery of cell nuclei to assign values to karyometric features, may enable the quantitative assessment of progressive change from normal-appearing to severely sun-damaged skin to AK to SCC as well as from DN to melanoma. Nuclear karyometric measurements have been performed on both benign and malignant melanocytic lesions (Bjornhagen et al. 1994; Stolz et al. 1994). Using imprint specimens, Stolz et al. (1994) found five features (mean value and standard deviation of nuclear area and the 80th, 90th, and 95th percentiles of the DNA distribution) to be significantly different between benign melanocytic lesions and melanoma. A second report (Stolz et al. 1994) found significant differences between benign melanocytic tumors and malignant melanoma for the following features: mean nuclear area, coefficient of variation (cv) of nuclear area, cv of nuclear shape, nuclear contour index, mean and cv of nuclear area, and DNA distribution rates. Investigators have conducted feasibility studies for the karyometric assessment of skin shave biopsies of AKs and for the assessment of the effects of chemopreventive intervention, using quantitative characterization by digital microscopy (Bozzo et al. 1998). Sections of shave skin biopsies were digitized and a minimum of 100 nuclei from each was recorded per case. After image segmentation, feature extraction software produced 93 karyometric features per nucleus that were stored for analysis. Discriminant functions were derived according to differences between normal nuclei and those with sun damage. Profiles commonly found in malignant cells were seen in the AK lesions. Using these features, a grading score was developed based on a plot of degree of solar damage versus the mean discriminant function. While upper inner arm (minimally sun-exposed) skin biopsies demonstrated as few as 3% of nuclei affected by sun damage, the AK lesions included approximately 50% affected nuclei. Discriminant functions derived from values obtained from samples ranging from normal to sun-damaged to premalignant (AK or DN) to malignant (SCC or melanoma) phenotypes establish a progression curve that can be used to determine the efficacy of applied chemopreventive agents (Bozzo et al. 2001) (Fig. 13.6). They have also applied this novel technology to demonstrate the efficacy of two chemoprevention agents, α-DFMO and vitamin A, in patients with moderately severe sun-damaged skin (Alberts et al. 2004). More evidence that this technology is useful in predicting prognosis and risk of skin cancer patients was published (Glazer et al. 2011). In these studies, the investigators demonstrated that aggressive cutaneous SCCs have a unique karyometric pattern distinct from nonaggressive cutaneous SCC lesions in low-risk patients. The classification accurately categorized 80% of the patients in this study as either aggressive cutaneous SCC or nonaggressive SCC.

Optical coherence tomography (OCT) is a technique for identifying and characterizing AKs and monitoring their response to chemoprevention agents (Barton

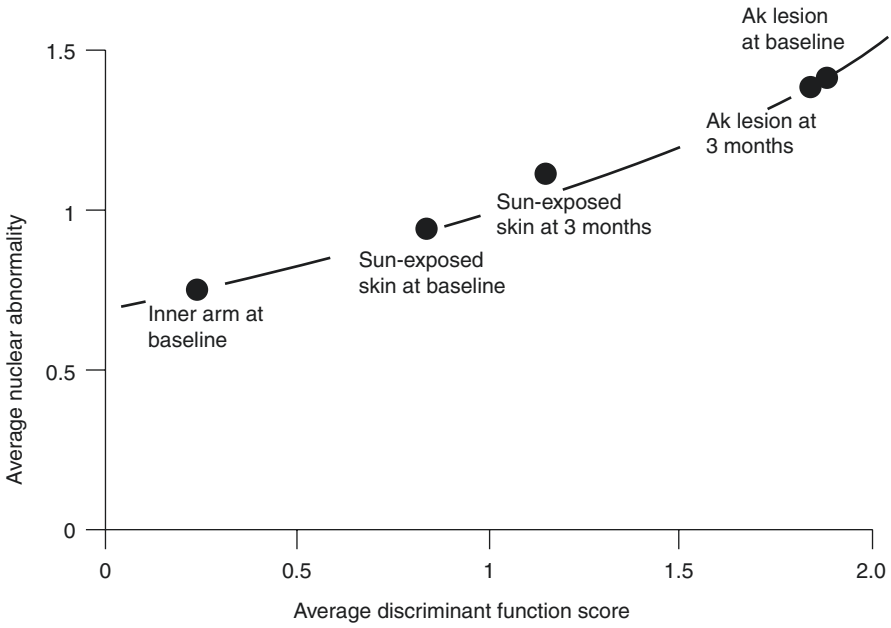

Fig. 13.6 Average nuclear abnormality versus average discriminant function scores for the 10% worst nuclei from the upper inner arm at baseline, sun-exposed skin at baseline and 3 months, and AK lesions at baseline and 3 months (Bartels et al. 2002)

et al. 2003). Based on Michelson interferometry, this technique was first introduced for investigations of the human eye (Fercher et al. 1988). This noninvasive technique uses coherent light operating in the near-infrared region of 1300 nm to produce two-dimensional images of the skin (Welzel 2001). The resulting photons have a typical penetrating depth of 1.0–1.5 mm allowing for multiple layers and structures to be distinguished. The resolution afforded by this technique makes it possible to distinguish features such as stratum cornea, epidermal layer, hair follicles, sebaceous glands, and blood vessels. In addition, it is possible to evaluate the efficacy of topical application of ointments and similar treatments, as these compounds tend to increase the detection/penetrating depth of the coherent light (Welzel 2001). In OCT, images of epithelial skin tumors and cell aggregation from the epidermis are visible. In some cases, lateral borders of the tumor adjacent to healthy skin are detectable and BCC can be distinguished from fibrous stroma. It is also possible to diagnose various inflammatory skin diseases such as psoriasis and eczema. The OCT measurement is an unobtrusive and safe technique with no side effects for the patient.

In a pilot study on 20 subjects to investigate the OCT appearance of upper inner arm, sun-damaged skin, and mild AKs (Barton et al. 2003) and to determine if features or quantitative measures in OCT images could be used to reliably differentiate between these categories, OCT images of upper inner arm showed skin layers and features (stratum corneum, epidermis, dermis, blood vessels) seen in previous

studies; additionally in this subject base, the subcutaneous fat layer was usually seen. Sun-damaged skin was characterized by increased signal in the epidermis and rapid attenuation of light. AKs were diverse in appearance but frequently characterized by high surface reflection, presence of a low-signal band in the stratum corneum, and heterogeneous appearance in the epidermis/dermis. Significant differences were found between skin categories using measures of stratum corneum and epidermal/dermal depths and intensities. The presence of a dark band in the stratum corneum was 79% sensitive and 100% specific for AK. This study suggests that OCT may be a useful noninvasive technique for monitoring AK during the clinical studies to evaluate the efficacy of chemoprevention agents.

13.6 Potential Chemoprevention Agents for Skin Cancer

The previous chapter (Chap. 12) describes a novel way to deliver chemoprevention compounds to the skin by the development of topical prodrug formulations. Therefore, this chapter focuses primarily on agents that have not yet been discussed in the previous chapter nor the technology used for prodrug formulation for skin chemoprevention agents. However, many of these compounds could also be considered for prodrug formulation. Figure 13.7 depicts potential UVR-induced targets and chemoprevention agents which may act on these targets for the prevention of skin cancer progression. The investigators at the Arizona Cancer Center use a decision tree which results in leads for chemoprevention agents that will potentially

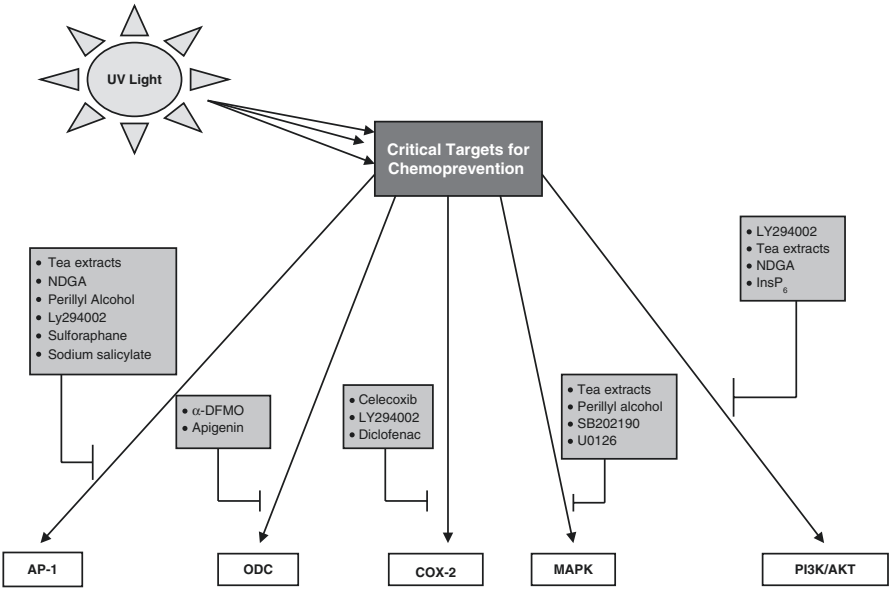

Fig. 13.7 UVR-induced targets can be modulated by specific potential chemoprevention agents

result in a clinical trial (Einspahr et al. 2003). The agents are selected based on epidemiological literature and activity in in vitro and in vivo models of UV skin carcinogenesis. Agents with novel mechanisms of action that are active against identified molecular targets are tested for their ability to modify the target and inhibit tumorigenesis in the animal models. Subsequent to toxicological evaluation and proper formulation, promising agents then progress to human phase I and then to phase II trials in subjects with AKs, DNs, or sun-damaged skin. Intermediate endpoints are evaluated to identify efficacy of the select agent. The following discussion provides an overview of chemoprevention agents for skin cancer which have been or are currently in clinical trials as well as future agents which may result in clinical trials due to their activity toward modulation of molecular targets previously discussed for NMSC and melanoma in in vitro or in vivo model systems.

Several potential chemopreventive agents have been taken through phase III clinical trials in people at high risk for NMSC (Stratton 2001; Bowden 2004). The agents include beta-carotene (Greenberg et al. 1990), selenium (Clark et al. 1996), retinol (Moon et al. 1997), and 13-cis-retinoic acid (Tangrea et al. 1992). Of these trials, the only one with positive results involved oral administration of 25,000 U/day of retinol in 2297 subjects with moderate to severe AK (Moon et al. 1997). This trial resulted in a reduction in SCC but not in BCC. The hazard ratio for first new SCC was 0.74 when comparing subjects from the retinol-supplemented group to placebo. Vitamin A (retinol) has been demonstrated to be necessary for cell growth and differentiation of human epithelial tissues, reproduction, and visual function (Gudas 1994). Retinoids have been shown to be involved in cell growth, cell differentiation, and carcinogenesis, all mediated in part by nuclear retinoic acid receptors and retinoid X receptors (Mangelsdorf et al. 1994; Xu et al. 1994).

There have been several smaller phase II trials in subjects at risk for NMSC that have resulted in positive outcomes. A phase IIa/IIb safety, dose-finding, and efficacy study of orally administered vitamin A in participants with sun-damaged skin resulted in a positive outcome (Alberts et al. 2004). The results were evaluated using karyometric analysis (described previously). One hundred and twenty randomized participants were given daily oral placebo, 25K, 50K, or 75K units of vitamin A (retinyl palmitate) for 12 months. The primary endpoints included quantitative, karyometric image analysis and assessment of retinoid receptors in sun-damaged skin. This analysis suggests that orally administered vitamin A is effective as a skin cancer chemopreventive agent by reducing levels of actinic nuclear damage as measured by average nuclear abnormality levels and discriminant function scores derived from appropriate karyometric features (Fig. 13.8). The dose effects of vitamin A correlated with increases in retinoid receptors, RAR-α, RAR-β, and RXR-α, at the 50,000 IU/day vitamin A dose.

Vitamin A has also shown activity in melanoma. In melanoma cells, vitamin A inhibits growth, invasion, and metastasis as well as impairment of UV-induced tumorigenesis by preventing UV-induced oxidative stress accumulation (Russo et al. 2015; Weinzweig et al. 2003; Wood et al. 1990). Clinical studies, however, have shown that retinol intake reduces melanoma risk, although vitamin A and beta-carotenes did not demonstrate an association in the reduction of melanoma

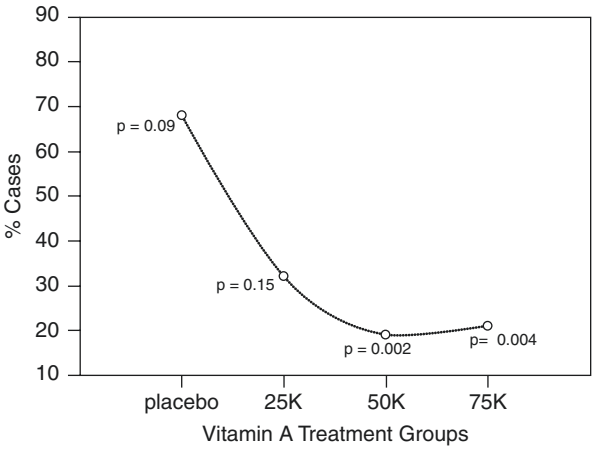

Fig. 13.8 Dose response to vitamin A treatment as demonstrated by percent of cases with increased actinic damage decreases based on karyometric analysis (Adapted from Bartels et al. (2002))

(Asgari et al. 2012; Zhang et al. 2014). Other vitamins, such as vitamin C, D, E, and K, have also shown some activity in melanoma. Vitamin D production in the skin protects against irradiation and therefore suppresses melanomagenesis. Vitamin D has been shown to reduce oxidative and genotoxic DNA damage caused by UV irradiation (Demetriou et al. 2012; Drane et al. 2004; Gordon-Thomson et al. 2012). DNA damage repair is induced by vitamin D, and proliferation and invasion of melanoma cells in vitro and in vivo are inhibited by vitamin D (Russo et al. 2015). An expanded review of vitamins and melanoma is discussed by Russo et al. (2015). Vitamin E, tocotrienols, and tocopherols have also shown suppression of melanoma growth and progression. Alpha-tocotrienol induces apoptosis and suppresses cell proliferation in vitro (Fernandes et al. 2010). Melanomagenesis and progression are inhibited by tocotrienols in vivo (Montagnani Marelli et al. 2016). Finally, although limited studies have been done, several forms of vitamin K, including K3 and K5, increase apoptosis and proliferation of melanoma cells, and a vitamin K analog, menadione, inhibits melanoma xenograft tumors (Ishibashi et al. 2012; Shah et al. 2009).

Another clinical trial performed by Alberts and colleagues demonstrated that topical 2-(difluoromethyl)-dl-ornithine (α-DFMO) can reduce spermidine concentrations and the number of AK lesions in patients at high risk of skin cancer (Alberts et al. 2000). Forty-eight participants with moderately severe AKs on their forearms were assigned randomly to topical α-DFMO treatment. A reduction of 23.5% in the number of AK lesions was seen from baseline to the 6-month follow-up. Spermidine concentration was reduced by 26% in skin biopsies from α-DFMO-treated arms. No systemic toxicities were detected; however, 7 of the 48 (14.6%) participants experienced severe (4.2%) or moderate (10.4%) inflammatory reaction on their α-DFMO-treated arms. In skin biopsies from this study, investigators were able to demonstrate a significant reduction of 22% in p53-positive cells (Einspahr et al. 2002). However, there were no significant changes in PCNA index, apoptotic indices, or p53 mutation frequencies. With karyometric analysis, α-DFMO

treatment markedly decreased the discriminant function score indicating effectiveness in reducing nuclear abnormalities. α-DFMO is an irreversible inhibitor of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine synthesis, and may exert its chemoprevention effects by inhibiting growth and/or inducing apoptosis. α-DFMO inhibits polyamine biosynthesis by covalently binding to ODC, thus inhibiting proliferation and inducing apoptosis. Leads for the use of α-DFMO came from previous studies where α-DFMO had been demonstrated as an antitumor agent in several animal models for carcinogenesis including a report that oral α-DFMO inhibited cutaneous carcinogenesis and immunosuppression in a mouse model (Gensler 1991). In Xpa knockout mice, α-DFMO, given in drinking water, reduced UVR-induced skin tumors in mice (Takigawa and Enormoto 1990). Tumor-suppressive activity was demonstrated for α-DFMO in melanoma (in vitro and in metastatic melanoma in a clinical trial) (Bregman and Meyskens Jr. 1986; Meyskens et al. 1986).

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) often used alone for degenerative arthritis management or with opioids in the treatment of pain associated with cancer. Investigations have shown that diclofenac has activity in the treatment of AK, thereby potentially preventing progression to SCC. An early study of 29 subjects assessed the efficacy and safety of topical diclofenac (Solaraze). Three percent diclofenac in 2.5% hyaluronic acid gel was generally well tolerated with the exception of seven (24%) patients who experienced irritant-type contact dermatitis (Rivers and McLean 1997). Additional clinical trials have further explored the potential therapeutic effect of this gel formulation. One randomized double-blind controlled trial of 130 patients, which did not include a follow-up period, did not find a significant difference between the use of diclofenac/hyaluronan and placebo in the eradication of AKs (McEwan and Smith 1997). However, two other randomized, double-blind, placebo-controlled studies found treatment with 3.0% diclofenac to be effective in the treatment of AK. A study of 195 patients with at least five AKs investigated the duration of treatment for 30 or 60 days with two daily applications of the gel (Rivers et al. 2002). While no significant difference was seen after 30 days, the 60-day treatment group showed a statistically significant difference in the number of patients (33–10%) with complete resolution of all target lesions in treated areas when compared to placebo group. The 60-day treatment group was also significantly different than placebo in the number of patients with resolution of target and new lesions in treated area, visible but no longer palpable lesions, and investigator and patient global improvement indices. Another study of 96 subjects also demonstrated a statistically significant difference in a 60-day treatment regimen when comparing the above criteria (Wolf Jr. et al. 2001). In both these studies, the gel was well tolerated with only a few subjects reporting skin reactions. The mechanism of action of the 3.0% diclofenac, with regard to tumor resolution, is unknown. As an NSAID, diclofenac inhibits cyclooxygenase enzymes (COX-1 and COX-2). A case-control study in women found an inverse association of NSAIDs intake and malignant melanoma (Harris et al. 2001a). One group demonstrated that 26–28 primary melanoma cell lines expressed COX-2 (Denkert et al. 2001).

The COX-2 inhibitor, celecoxib, has been shown to suppress the formation of UV-induced skin cancers when given orally to mice (Fischer et al. 1999; Pentland et al. 1999). Topical application of celecoxib reduced cutaneous inflammation (Wilgus et al. 2000). Reduction of the inflammatory response could prove protective against long-term UV exposure and the development of skin cancer and the conversion of benign AK to cancerous ones. An UVR-induced tumor mouse model demonstrated a lengthening in tumor latency period and reduced tumor multiplicity when mice were treated with a COX-2 inhibitor, celecoxib (Orengo et al. 2002). An increase in arachidonic acid metabolism in keratinocytes due to exposure to UVB (Buckman et al. 1998) may be a target for this type of chemoprevention. A study of a topical combination of celecoxib and 5-FU, a common treatment for AK, found that the combination was 70% more effective in reducing the number of UVB-induced skin tumors than with 5-FU alone (Wilgus et al. 2004). A randomized, double-blind, placebo-controlled trial demonstrated that individuals with extensive actinic damage (number of AK at screening was approximately 24) given twice daily oral administration of 200 mg of celecoxib for 9 months demonstrated fewer nonmelanoma skin cancers, both BCC and SCC, in the celecoxib arm compared to the placebo arm (Elmets et al. 2010). These results were seen 11 months after randomization. These studies indicate a genuine possibility of celecoxib being a chemopreventive agent for skin cancer at least for those with extensive actinic damage who are then at high risk for the development of nonmelanoma skin cancers. Given that increased levels of COX-2 are associated with melanoma progression and is overexpressed in melanomas, this is an ideal target for melanoma chemoprevention. One study showed that COX inhibition resulted in a decrease in the number of new tumors, disease recurrence and metastases were observed in melanoma patients (Ramirez et al. 2005). These results do show that COX-2 inhibitors such as celecoxib, etoricoxib, and rofecoxib could be potential chemopreventive agents in patients which are at risk for melanoma.

An agent developed at the University of Arizona, Melanotan-1 (MT-1), could potentially be used as a chemopreventive agent against melanoma and NMSC. This agent is a superpotent melanotropic peptide that results in darkening of the skin (Fig. 13.9). In clinical studies with MT-1, there was no improvement of tanning in doses greater than 0.16 mg/kg/day for 10 days by subcutaneous (SC) injection. No moderate toxicities occurred at this dose as in the higher doses (Levine et al. 1999); a single exposure of three minimal erythema doses (MED) of UVB did not enhance eumelanin content of the skin either before or after MT-1 administration (Dorr et al. 2000). While treatment showed darkening of the skin on days 14 and 21, there was no significant difference from baseline to 3 weeks after dosing. Investigators found that the most effective delivery was by SC administration, which resulted in an increase in eumelanin and measured tanning by reflectance in the forearm and forehead. For the purpose of prolonging skin darkening by the use of MT-1, investigators formulated a controlled release MT-1 implant formulation based on a PLGA polymer. In studies with an in vitro frog skin bioassay, they found that the implants (1 mg of peptide) showed that the melanophores migrated to the dendritic processes of the pigment cells, which resulted in skin darkening. In in vivo studies

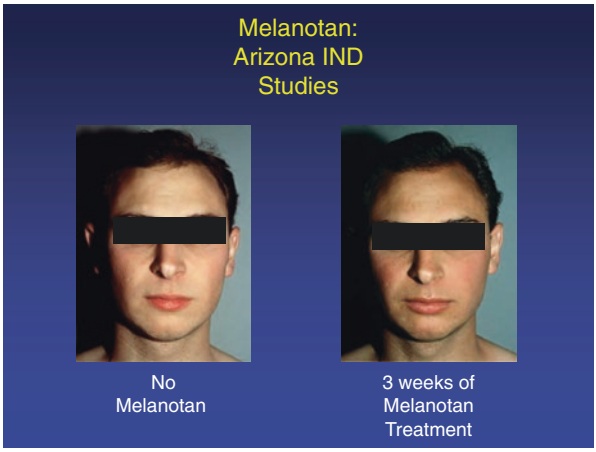

Fig. 13.9 Early clinical trials (Levine 1991) demonstrated skin tanning. *Left*: pretreatment state. *Right*: tanning of the face and neck after start of therapy

using pigmented haired and hairless guinea pigs with the MT-1 implants (4 mg peptide), increase of skin darkening was observed for up to 3 months and eumelanin content demonstrated a 2.5-fold increase in 1 month and persisted for 3 months. This prolonged increase in pigment, specifically eumelanin, can be favorable to the prevention against photodamage induced by UVR radiation.

Three phase I clinical trials performed at the Arizona Health Sciences Center in Tucson demonstrated that MT-1 can be safely combined with UVB light or sunlight and appears to act synergistically in the tanning response to light (Dorr et al. 2004). In these studies, enhanced tanning was achieved in the groups receiving MT-1 as well as 47% fewer sunburn cells at the irradiated site. Subcutaneous doses of 0.16 mg/kg/day for 10 days provided an increased darkening and a maintained tanning for at least 3 weeks longer than that observed among participants exposed to sunlight exposure alone. The sun exposure time for equivalent tanning in the sunlight-only controls required 50% more time for an equivalent tanning. Currently, MT-1 is under development in Australia, licensed by Clinuvel Pharmaceuticals, Ltd. (formerly Epitan, Ltd.) under the proprietary name CUV1647. Two objectives for the Australian studies have been (1) to determine the efficacy of MT-1 in at-risk skin-damage-prone populations and (2) the development of an improved formulation/schedule for delivery, namely, a slow-release depot formulation designed to release drug from a single subcutaneous injection over several months (Hadley and Dorr 2006). In phase I/II trials, the 0.16 mg/kg/day injection dose caused increased eumelanin deposition in the skin, similar to results reported in the Arizona studies (Fitzgerald et al. 2006). No dose-limiting side effects were noted. Of additional interest, patients with variant MC1R genotypes were evaluated for their response to MT-1 to evaluate if it is useful for individuals most in need of photoprotection (Fitzgerald et al. 2006). These variants such as Arg151Cys, Arg160Trp, and Asp294His are associated with fair skin color and red hair (Valverde et al. 1996; Box et al. 1997; John and Ramsay 2002). Individuals with one or more of these variants have been shown to be less able to tan naturally with UV light (Healy et al. 2000), and the variants have been associated with an increased risk of skin cancer

(Palmer et al. 2000; Bastiaens et al. 2001; Kennedy et al. 2001). The study on the effect of MT-1 on humans with MC1R variant alleles demonstrated that the agent effectively increased the melanin content in the skin of individuals with variant alleles and therefore those most in need of photoprotection (Fitzgerald et al. 2006). A human depot formulation of MT1 (20 mg) successfully produced pigmentation in a pilot phase I study (Hadley and Dorr 2006). Following pharmacokinetic studies in healthy volunteers, the "controlled release" implants contained 16 mg of MT-1. The increased pigmentation of the skin appeared approximately 4–5 days after implantation and may last for several months (www.clinuvel.com). In 2007, this agent, afamelanotide formulation, CUV1647, began phase III clinical trials for polymorphic light eruption (sun poisoning) and erythropoietic protoporphyria (absolute sun intolerance). In November of 2007, Clinuvel Pharmaceuticals, Ltd. initiated a phase II trial in Australia and Europe for CUV1647 as a preventive for sun damage and AK in fair-skinned, immune-compromised organ transplant recipients (FDA News 2007). The trial evaluates the ability of the agent to reduce the number of AK on the head, back of hand, and forearms during a 24-month test period. A secondary endpoint will determine the effect of this agent on the number of SCC on the head, back of hand, and forearms during the 24-month test period. In 2009, Clinuvel began a study to determine whether afamelanotide (CUV1647) is effective in reducing the number of AKs and SCCs developing in immune-compromised organ transplant recipients, who are at particularly high risk, over a 24-month test period. Most recently, a 3-times-weekly narrowband UVB exposure along with a 4-monthly implant containing a 16 mg afamelanotide demonstrated potential efficacy in the treatment for vitiligo (Grimes et al. 2013). The afamelanotide induced repigmentation within 2 days to 4 weeks of the initial implant.

Statins are known to inhibit the isoprenoid protein modification and therefore may be inhibiting ras farnesylation and cause a downregulation of ras oncogenic potential in melanoma. Dellavalle and colleagues (2003) reviewed the role of statins or fibrates in melanoma chemoprevention. Results from two large clinical trials demonstrated a decrease in melanoma incidence in subjects given lipid-lowering medications for coronary artery disease. In another study, 27 melanomas were newly diagnosed in 3301 placebo-treated patients, whereas only 14 melanomas were diagnosed in 3304 lovastatin-treated patients (Buchwald 1992). The incidence of all other cancers was not statistically different. In another study with gemfibrozil, a hypolipidemic medication, nine melanomas were diagnosed in 1267 patients treated with placebo and only one melanoma was diagnosed in a 5-year period in 1264 gemfibrozil-treated patients (Rubins et al. 1999). Again, all other cancers were not significantly different. Contradictory studies discussed in a meta-analysis found no evidence of reduced melanoma incidence in patients using statins (Li et al. 2014).

The chemopreventive activity of aspirin and sodium salicylate was investigated in a UVB-induced NMSC hairless SKH-1 mouse model (Bair 3rd et al. 2002). While sodium salicylate significantly inhibited UVB-induced tumor formation, aspirin had only a moderate effect. The protection supplied by sodium salicylate appears to be in part due to its sunscreen effect, which was demonstrated by the reduction of

thymine dimers in the epidermis of mice treated with sodium salicylate. Aspirin was unable to prevent dimer formation (Bair 3rd et al. 2002)

In vitro studies revealed that a derivative of nordihydroguaiaretic acid (NDGA), tetra-*O*-methylnordihydroguaiaretic acid, inhibited growth of several tumor cell lines, including a melanoma line where there was morphologic evidence of apoptosis. This compound also inhibited the synthesis of DNA and caused cell cycle arrest in G_0/G_1 and G_2/M phases of the cell cycle. Growth inhibitory effects of this compound were also exhibited in vivo (Lambert et al. 2001). Bowden and colleagues have also identified NDGA as an inhibitor of UVB-induced c-Fos and AP-1 transactivation by inhibiting the PI-3 kinase signal transduction pathway (Gonzales and Bowden 2002).

A potential chemopreventive agent for melanoma, apomine, has been studied in clinical and preclinical settings. Apomine is a bisphosphonate ester that has been reported to activate the farnesoid X receptor, increase the rate of degradation of HMG-CoA reductase, and induce apoptosis (Niesor and Flach 1999). Apomine has been shown to inhibit the growth of many tumor cell lines, including those derived from leukemia, colon, liver, ovary, and breast (Flach et al. 2000). Growth inhibition and apoptotic activity were compared to those of simvastatin, farnesol, and 25-hydroxycholesterol, which all affect HMG-CoA reductase. Apomine was most like farnesol, a non-sterol regulator of cholesterol synthesis. In a phase I trial at the Arizona Cancer Center, it was demonstrated by plasma pharmacokinetics that a daily dose of 125 mg/m^2 of apomine was sufficiently bioavailable to levels used in in vitro studies that demonstrated activity against fresh human solid cancers. In preliminary studies in a TPras transgenic melanoma mouse model (Powell and Alberts 2002), apomine caused a 55% reduction in melanoma development induced by DMBA. In vitro studies with melanoma cell lines derived from the transgenic TPras mouse model and treated with apomine demonstrated a significant reduction in Ras detected in the membrane fraction (activated Ras). Apomine was also able to reduce UVR-induced Akt phosphorylation but had no effect on phosphorylation of ERK1/2 (Powell and Alberts 2002). A study in human melanoma cells found that apomine cytotoxicity to the cells was mediated primarily through a non-apoptotic pathway (Pourpak and Dorr 2005). In a phase I clinical trial at the Arizona Cancer Center, apomine expressed prolonged cancer stabilization in patients with metastatic melanoma and recurrent ovarian cancer with minimal or no toxicity (Powell and Alberts 2002). A topical formulation with apomine is in preparation for clinical trials in skin cancer. Investigators at the University of Arizona Cancer Center have developed the high-performance liquid chromatography method to analyze apomine in topical cream formulations (Kuehl et al. 2006).

Future agents will most likely be identified by their mechanism of action. The selected agents will have specific targets such as those described earlier as important in the UV signaling pathways and carcinogenesis process of skin cancer development (Fig. 13.3). For p38 MAPK, there are inhibitors which are a group of polycyclic pyridinylimidazole compounds. SFK86002, a bicyclic pyridinylimidazole, first reported to inhibit LPS-stimulated cytokine production (Lee et al. 2000). Early reports indicated a role of cytokine inhibition as a potential mechanism

for the potent anti-inflammatory activity of these compounds (Lee et al. 1988). Subsequently, SB203580 and other 2,4,5-triaryl imidazoles were prepared as a tool for finding the molecular target involved in cytokine regulation (Lee et al. 1993). Later discoveries indicated p38/CSBP as the molecular target of these compounds (Gallagher et al. 1997). One such compound, SB202190, inhibits p38 phosphorylation of myelin basic protein (MBP) while not affecting ERK or JNK MAP kinases. The compound also inhibits p38 phosphorylation of activating transcription factor 2 and blocks LPS-induced TNF and interleukin biosynthesis as well as induces LDL receptors in vitro. Investigators have used SB202190 to understand the mechanisms of UVB- and UVA-mediated p38 MAPK (Bachelor and Bowden 2004b; Bachelor et al. 2005). Topical treatment to mouse epidermis with SB202190 resulted in a 84% decrease in UVB-induced AP-1 activation as well as COX-2 expression (Bachelor et al. 2005).

SP600125, an anthrapyrazole, is an inhibitor of JNK catalytic activity (Bennett et al. 2001). This inhibitor was identified in a high-throughput biochemical screen by using purified recombinant JNK2 and c-Jun. SP600125 demonstrated inhibitory activity consistent with the role of JNK in CD4+ cell activation and differentiation, in CD14+ cell gene expression, and in thymocyte death. SP600125 inhibits c-Jun phosphorylation in cells and also COX-2, IL-2, IFN-c, TNF-α, IL-10, and MMP gene expression (Han et al. 2001). In vivo studies demonstrated that SP600125 inhibited LPS-induced TNF-α expression in mice (Bennett et al. 2001). SP600125 also prevented anti-CD3-mediated thymocyte apoptosis in a C57BL/6 mouse model (Bennett et al. 2001). In addition, this inhibitor of JNK blocked cell proliferation but did not kill CD4+ cells, resulting in a cytostatic effect on T cell proliferation. Although several anthrapyrazoles have been identified as chelators of DNA (i.e., doxorubicin), SP600125 did not exhibit characteristics of a strong interchelator of DNA in competitive binding assays. SP600125 also did not induce apoptosis (Bennett et al. 2001). Both compounds, the MAPK inhibitor, SB202190, and the JNK inhibitor, SP600125, were able to inhibit UVA-induced AP-1 and c-Fos transactivation as well as c-Fos expression in the HaCaT cell line transfected with a luciferase reporter (Silvers et al. 2003).

Inositol hexaphosphate ($InsP_6$) is a direct inhibitor of PI-3 kinase in vitro (Huang et al. 1997a, b, c). This agent has also demonstrated inhibition of EGF-induced AP-1 activation and cell transformation of JB6 epidermal cells (Huang et al. 1997a, b, c). $InsP_6$ also inhibits UVB-induced AP-1 and NF-jB transcriptional activity. This compound is similar in structure to a potent PI-3 kinase inhibitor, D-3-deoxy-3-fluoro-PtdIns (Powis et al. 1995).

Three specific inhibitors of EGFR tyrosine kinase, PD153035, AG1478, and ZD1839, may be potentially useful as chemopreventive agents. PD153035 is a 4-anilinoquinazoline compound that acts via competitive binding at the ATP site with the RGF receptor (Fry et al. 1994). AG1478 is a member of a family of tyrosine phosphor kinase inhibitors called tyrophostins (Gazit et al. 1996), which were designed to mimic the tyrosine substrates. Investigators have shown that these inhibitors (Zhang et al. 2001) can block UVA-induced EGFR signaling. ZD1839 (Iressa; AstraZeneca Pharmaceuticals) is another inhibitor of EGFR, which could

be considered for topical formulation development as a chemoprevention agent. ZD1839 has been shown to inhibit activation in a variety of human skin cell types in vivo subsequent to oral therapy (Albanell et al. 2002). In association with the EGFR inhibition, MAPK activation and keratinocyte proliferative rates decreased and an increase in the apoptotic index also occurred during therapy. ZD1839 is a substituted anilinoquinazoline that selectively inhibits EGF-stimulated tumor cell growth and blocks EGF-stimulated autophosphorylation in tumor cells (Wakeling 2002). Clinical trials with oral ZD1839 have shown this compound to provide well-tolerated antitumor activity in patients (Wakeling 2002; Lorusso 2003).

As discussed earlier, BRAF is a potential molecular target for the chemoprevention of melanoma. BAY 43–9006 is a potent inhibitor of Raf kinase (Lyons et al. 2001). Oral administration of this compound has shown significant activity in four different human tumor types including colon (Gianpaolo-Ostravage and Bankston 2001), pancreatic, lung, and ovarian tumors carried out in xenograft models. Clinical testing of this compound in cancer patients began in July 2000 (Strumberg et al. 2001). Preliminary clinical data reported the compound to be well tolerated. At least 37% of patients in the initial study had stable disease lasting longer than 12 weeks. This compound could be a promising agent for chemoprevention specifically for melanoma.

Several phytochemicals have been investigated for their ability to prevent photocarcinogenesis and signaling that occurs with skin carcinogenesis (Chun et al. 2014) (Montes de Oca et al. 2017). One compound that has shown an induction of apoptosis via the activation of the Bcl-2 family in human keratinocytes is sanguinarine (Adhami et al. 2003). This agent is derived from the root of *Sanguinaria canadensis* and is also found in poppy and *Fumaria* species (Shamma and Guinaudeau 1986). The agent has been found to act as an antioxidant (Vavreckova et al. 1994) as well an antimicrobial (Mitscher et al. 1978) and anti-inflammatory compound (Mandel 1988). Sanguinarine has demonstrated potential as a chemopreventive agent in UVB-irradiated human keratinocytes (Reagan-Shaw et al. 2006) and resulted in a decrease in UVB-mediated skin edema, skin hyperplasia, and infiltration of leukocytes in mice treated with topical sanguinarine (Ahsan et al. 2007).

It has been demonstrated that topical application of the phytochemical, resveratrol, demonstrates chemopreventive effects against multiple short-term UVB exposures to the skin of hairless mice by decreasing the UVB-mediated upregulation of MAPKK and the 42 kDa isotope of MAPK (Reagan-Shaw et al. 2004). Resveratrol is a naturally occurring antioxidant phytoalexin produced by some plants subsequent to injury or fungal infection and is found in red wine and grapes (Aziz et al. 2003, 2005). This agent has shown cancer chemopreventive effects in a variety of tumor bioassays (Jang et al. 1997; Kapadia et al. 2002; Aziz et al. 2003) and has been attributed to the "French Paradox" (Kopp 1998; Sun et al. 2002; Aziz et al. 2003). Early studies demonstrated that resveratrol inhibited chemically induced skin tumorigenesis in a mouse model (Jang et al. 1997). Aziz and colleagues demonstrated that topical resveratrol was a chemopreventive agent in long-term UVB exposure of a mouse skin carcinogenesis model (Aziz et al. 2005). The untreated group resulted in SCC, Bowen's disease, invasive carcinomas in situ, and AK. The

resveratrol-treated group (pre- and post-treated) had a significant reduction in tumor incidence and the majority of the lesions were AKs, suggesting that the agent was inhibiting the malignant conversion of AKs. The chemopreventive effects of resveratrol appear to be against modulation of cki-cyclin-cdk network and MAPK pathway (Reagan-Shaw et al. 2004). The activity of the agent appears to be through signaling pathways rather than a sunscreen effect (the treatment was also effective post UV irradiation) (Aziz et al. 2005). These studies suggest that the target for resveratrol is the modulation of the expression and function of survivin. Survivin is a critical regulator of cell survival/death (Altieri and Marchisio 1999; Altieri 2001). Deregulation of survivin has been shown to inhibit melanoma tumor growth (Grossman et al. 2001) and found to prevent papilloma regression and promote conversion to SCC (Altieri 2003). Most recently, resveratrol demonstrated the ability to overcome the resistance against the BRAF inhibitor, vemurafenib, through the dephosphorylation of AKT-serine/threonine kinase indicating the ability for resveratrol to not only prevent melanoma but also prevent recurrence in patients who were previously treated with vemurafenib (Luo et al. 2016).

Other phase II trials have focused on the potential chemoprevention activity of topical green tea extracts (e.g., Polyphenon E) in patients with AK on their arm (Stratton 2001). Animal studies have demonstrated a chemopreventive effect of epigallocatechin gallate (EGCG). Investigators have reported a reduction in tumor incidence with topical application of EGCG in UVB-irradiated mice. Mice were irradiated at a total dose of 2.1×10^6 J/m^2. Skin cancer developed in 96% of control mice and 62% of mice given 10 mg of EGCG and 39% of mice given 50 mg of EGCG. EGCG did not affect immunosuppression and oral administration did not decrease UVR-induced skin tumor incidence. In the investigation of a mechanism of action for EGCG, it was demonstrated that EGCG can inhibit UVB-induced AP-1 activity in a dose range of 5.45 nM to 54.5 μM in human keratinocytes when applied before, after, or both before and after UVB irradiation (Barthelman et al. 1998b). Inhibition of AP-1 by topical EGCG application was also evident in a transgenic mouse model. EGCG inhibited UVB-induced steady-state message and transcriptional activation of the c-Fos gene as well as the accumulation of the c-Fos protein. Upstream of c-Fos, EGCG significantly inhibited activation of p38 MAPK yet did not affect JNK or ERK activation. AP-1 inhibition potentially, through the reduction of c-Fos by EGCG, may be the mechanism by which EGCG inhibits UVB-induced tumor formation in mice (Chen and Bowden 1999). Theaflavins from black tea demonstrated a stronger inhibition of AP-1 than EGCG and inhibition of the activation of ERK and JNK was also significant with theaflavin treatment (Nomura et al. 2000). In addition, Nomura and colleagues demonstrated in mouse epidermal cells that EGCG and theaflavins inhibited the activation of UV-induced PI3K and attenuated UV-induced Akt and p70 S6-K, both downstream effectors of PI3K (Nomura et al. 2001b). Studies with oral administration of green tea or caffeine to mice reported chemopreventive effects on UV-induced carcinogenesis mediated through stimulation of UV-induced increases in the number of p53-positive cells, p21$^{Waf1/cip1}$-positive cells, and apoptotic sunburn cells (Lu et al. 2001).

Investigators have developed a 10% (w/w) EGCG formulation in hydrophilic ointment USP for topical application. An intradermal uptake of 19 and 0.9% of the applied dose was evident in the mouse and human skin, respectively, while transdermal penetration was observed only in the mouse skin (Dvorakova et al. 1999). The 10% EGCG formulation was used in a phase I clinical trial to assess safety and the sun protection factor (SPF). An SPF of 3.6 was recorded for this ointment, applied to buttock skin. No systemic toxicities with topical application to the arms were seen in 19 participants that completed the study. However, 42% of the participants reported moderately severe skin reaction, and histological evaluation corroborated the clinical findings.

Ingenol mebutate (Picato, PEP005) is a natural product derived from the sap of the *Euphorbia peplus* plant (a.k.a. petty spurge or cancer weed), which grows in many parts of the globe. The gel form of this compound has been approved by the FDA as a topical treatment for AK, requiring only a few applications for effectiveness. However, the individual response to ingenol mebutate treatment can be quite variable and unpredictable, with some patients developing painful, excessive inflammatory reactions which are not easily managed. The mechanism of action of this compound is thought to involve the induction of necrosis through disruption of mitochondrial membrane potential (Ogbourne et al. 2004), as well as the activation of several PKC isoforms, particularly PKCδ (Hampson et al. 2008). The literature suggests that there may be a dose-dependent effect of topical administration of ingenol mebutate in which the upper layers of the differentiated epidermis, which are exposed to the highest concentration of the drug, respond through activation of necrosis, while lower layers of the epidermis induce apoptosis, and in the deeper layers of the epidermis, ingenol mebutate is believed to stimulate neutrophils which help to clear the residual tumor cells and prevent recurrence (Kedei et al. 2004; Challacombe et al. 2006; Stahlhut et al. 2012). The literature now contains an ever-increasing cadre of case reports from clinicians using ingenol mebutate for off-label treatments (e.g., genital warts, on the chest, on intraepidermal squamous cell carcinoma) (Larsen et al. 2017; Rallis et al. 2017; Wu et al. 2017). Larger studies have determined that ingenol mebutate may be most effective when used on female patients, younger than 70 years of age, and when applied to the face and scalp (Ortega Del Olmo and Salido-Vallejo 2017). Further studies are exploring wider dosing ranges and new treatment schedules (Hanke et al. 2017), and even whether ingenol mebutate might be efficacious for both improving photodamaged skin aesthetically (Handler et al. 2017) and preventing nonmelanoma skin cancer in some populations (reviewed in Erlendsson 2017).

LY294002 is a morpholino derivative of the broad-spectrum kinase inhibitor quercetin. This compound is also an inhibitor of PI-3 kinase. This agent has been shown to cause inhibition of UVB-induced COX-2 promoter activity and protein expression of COX-2 in human keratinocytes (Tang et al. 2001). Topical LY294002 treatment in SKH-1 mouse epidermis demonstrated a significant induction of UVB-induced AP-1 activation as well as COX-2 expression (Bachelor et al. 2005). Studies using TPras transgenic melanomas in SCID mice demonstrated a reduction in invasion, correlated with a reduction in MMP2, when treated with LY294002

(Bedogni et al. 2004). Using the TPras mouse model for melanoma development, discussed earlier in this chapter, topical application of LY294002 resulted in a delay in melanoma development by 8 weeks (Bedogni et al. 2006). In addition, treatment with LY294002 after tumors had developed in this model resulted in only a 17% progression compared to 93% progression in the control mice. There was a 67% partial regression and a 17% regression in the mice treated with LY294002. Most interesting of these studies is that a combination of LY294002 and U0126, a specific inhibitor of MEK 1/2, increased the effectiveness. In the topical combination treatment, 70–75% of the mice did not develop melanoma while the control group only contained 9% of mice that were melanoma-free at the end of the study. The response to these agents corresponds to increased apoptosis and decreased proliferation both in vitro and in vivo as well as a reduction in tumor angiogenesis. These studies support the role of PI-3 kinase/Akt and Raf/MAPKK pathways as important in the development of melanoma. In addition, the studies point to a potential for using LY294002 or other PI-3 kinase inhibitors as topical chemopreventive agents in both melanoma and nonmelanoma and a combination of LY294002 with U0126 in melanoma. Finally, treatments of these compounds in animal models demonstrated no systemic toxicities or skin irritations.

Another phytochemical agent with potential chemopreventive activity in both melanoma and NMSC is perillyl alcohol (POH). POH is a cyclic monoterpene that reduces the amount of Ras and Ras-related proteins and has been reported to induce apoptosis. POH is found in the essential oils of numerous plants including citrus fruit, cherries, and mint. Limonene (a precursor of POH) has been demonstrated to reduce the incidence of spontaneous lymphomas in $p53^{-/-}$ mice and to inhibit the development of chemically induced rodent mammary, liver, lung, and forestomach tumors (Crowell 1999). Ras oncogene-induced mammary carcinoma development has also been inhibited by limonene (Gould et al. 1994). POH has demonstrated chemopreventive properties in several types of cancers, including liver cancer in rat (Mills et al. 1995), pancreatic cancer in hamsters (Stratton et al. 2000), and mammary tumors in rats (Haag and Gould 1994). POH and limonene as oral agents have also been used in clinical trials (Gould et al. 1994; Crowell 1999). Chemopreventive properties of topical POH have been demonstrated in a nonmelanoma and a melanoma mouse model (Barthelman et al. 1998b; Lluria-Prevatt et al. 2002). In both models, topically applied POH significantly reduced the incidence of tumors. Investigators also reported that POH reduced detectable levels of Ras, inhibited the activation of Akt and MAPK, and reduced UVR-induced reactive oxygen species in melanoma cells (Lluria-Prevatt et al. 2002). POH inhibited UVB-induced AP-1 transactivation in vitro and in vivo (Barthelman et al. 1998a). The mechanisms of action for POH identified thus far include inhibition of cell proliferation, induced tumor cell differentiation (Morse and Stoner 1993), and increased apoptosis (Mills et al. 1995). POH has been shown to inhibit protein isoprenylation in Ras (Hohl and Lewis 1995; Stayrook et al. 1998). Evidence of chemopreventive activity in mouse models and the suspected molecular targets for POH makes it an ideal compound for potential chemoprevention studies in melanoma and NMSC. For clinical studies,

POH has been formulated into a topical cream (Gupta and Myrdal 2004). The formulation was found to be physically and chemically stable over a period of 1 year at 4° and 25° C. A phase IIa randomized, placebo-controlled, double-blind trial of topical POH in sun-damaged skin was initiated at the University of Arizona Cancer Center in Tucson, Arizona, to evaluate its chemopreventive activity in humans (Stratton et al. 2010). Karyometric evaluation demonstrated a slight reduction in the histopathologic score in those treated with a topical 20 mmol/L formulation compared to the placebo group. A statistically, significant reduction in the proportion of nuclei deviating from normal was observed by karyometric analysis in the group treated with 50 mmol/L formulation. However, there was no change observed in p53 expression, cellular proliferation, or apoptosis in either treatment group. The study suggests that an improved delivery into the epidermis may be necessary to deliver the appropriate dose to see an effect other than by karyometric analysis.

A review by Juge and colleagues (Juge et al. 2007) describes the chemopreventive effects of sulforaphane, a natural isothiocyanate found in broccoli seeds, sprouts, and plants. The chemopreventive properties of sulforaphane include inhibition of phase 1 cytochrome P450 enzymes, induction of phase 2 metabolism enzymes, antioxidant functions through increased tissue GSH levels, apoptosis-inducing properties, induction of cell cycle arrest, anti-inflammatory properties, and inhibition of angiogenesis. Many of these effects are downstream of sulforaphane's ability to activate the Nf-E2-related factor-2 (Nrf2) transcription factor, which regulates many cytoprotective and antioxidant genes in the cell. Topical application of sulforaphane demonstrated an inhibition of skin tumorigenesis when applied to a mouse model system that uses DMBA as an initiator and TPA as the promoter of tumorigenesis (Gills et al. 2006; Juge et al. 2007). The topical application of sulforaphane was effective during the promotion phase where it caused a significant decrease in both the percent of mice with tumors and tumor multiplicity. In addition, the agent inhibited TPA-induced ODC activity in the mouse skin which has also been demonstrated in mouse epidermal cells in culture (Lee et al. 1999). Further studies found that the effects of sulforaphane on DMBA-/TPA-induced tumorigenesis are dependent upon the presence of the Nrf2 transcription factor (Xu et al. 2006).

As discussed previously in this chapter, UVB can induce the activation of AP-1, and it is suggested that AP-1 plays a critical role in UVB-induced skin tumor development (Barthelman et al. 1998a; Huang et al. 2000). Investigators have demonstrated that sulforaphane can inhibit UVB-induced AP-1 activation in human keratinocytes by inhibiting AP-1 DNA binding activity (Zhu and Bowden 2004; Dickinson et al. 2009). Other researchers have used a hairless mouse model to demonstrate that topical sulforaphane substantially inhibited skin carcinogenesis induced by UVR (Dinkova-Kostova et al. 2006; Dickinson et al. 2009). One study showed a 50% reduction in tumor burden, incidence, and multiplicity in animals which received topical sulforaphane in the form of broccoli sprout extract, or BSE, after the completion of a 20-week regimen of UV irradiation. Another study showed that purified sulforaphane can also inhibit tumorigenesis in mice

when used concurrently with UVB (Dickinson et al. 2009). A dose escalation safety study in healthy humans revealed no adverse reactions with cumulative doses up to 450 nmol of topical sulforaphane (in BSE) and showed an increase in NAD(P)H:quinine oxidoreductase 1 (NQO1), therefore demonstrating an induction of phase 2 response in humans (Dinkova-Kostova et al. 2006). Sulforaphane is also effective at reducing UV-induced inflammation: treatment with BSE significantly reduced UVB-induced erythema in a clinical study (Talalay et al. 2007), and mice fed 1 mg/day of sulforaphane by oral gavage demonstrated significantly reduced skin thickening and COX-2 activation associated with UVB exposure (Shibata et al. 2010). The evidence presented here provides evidence that topical sulforaphane should be pursued as a potential chemoprevention for skin cancer.

Additional potential chemopreventive agents for skin cancer include curcumin, which induces apoptosis in human melanoma cells through a cell membrane-mediated mechanism independent of the p53 pathway by induction of the Fas receptor and activation of caspase-8 (Bush et al. 2001), and topical apigenin, which has been shown to be effective in preventing UV-induced mouse skin tumorigenesis by inhibition of ornithine decarboxylase (ODC) activity (Birt et al. 1997). Fisetin is a naturally occurring flavonoid that has demonstrated antioxidant, antiproliferative, and anti-inflammatory properties against melanoma and nonmelanoma skin cancer (Pal et al. 2015, 2016). Lycopene is a carotenoid found in tomatoes that when used as a topical application on SKH-1 hairless mouse skin demonstrated a reduction of UVB-mediated apoptosis by inhibiting activation of caspase-3. In vitro this phytochemical resulted in a decrease in cells in the G0/G1 phase followed by delay at the S phase of the cell cycle. Modulating cell cycle events could protect against photodamage (Fazekas et al. 2003; Ascenso et al. 2016). Table 13.2 lists phytochemicals, their source, and references demonstrating the agents' ability to prevent skin carcinogenesis.

Meyskens et al. (2004) present a review of studies that suggest that ROS may be central to the pathogenesis of melanocyte transformation and melanoma progression. They suggest that a critical early pathogenic event is the change of antioxidant to pro-oxidant melanin, the pigment produced by melanocytes. Once the melanin is oxidized by ROS generated by UV, an accumulation of metals occurs, and the antioxidant response is depleted, the buildup of ROS occurs. This, in turn, leads to melanosomal damage, DNA mutations, transcription activation, and enhancement and activation of an antiapoptotic (drug-resistant) phenotype of melanocytes. Chemoprevention of melanoma within the context of this etiological hypothesis may involve the early use of antioxidants.

Other UVR-induced oxidative stress studies are emerging to understand the signaling pathways leading to antioxidant response elements (ARE) for its potential in developing skin cancer chemoprevention strategies. These investigations have found that UVB irradiation can interrupt the signaling of ARE through the JNK pathway in human keratinocytes (Zhu et al. 2006). Additional findings include

Table 13.2 Phytochemicals that have shown activity toward preventing skin carcinogenesis making them candidates for chemoprevention agents

Phytochemicals	Source	Refs.
Sanguinarine	Poppy, *Sanguinaria canadensis*	Chun et al. (2014) and Montes de Oca et al. (2017)
Resveratrol	Grapes, red wine	Reagan-Shaw et al. (2004), Aziz et al. (2005), and Luo et al. (2016)
Epigallocatechin-3-gallate (EGCG)	Green tea	Stratton (2001)
Ingenol mebutate	Euphorbia peplus plant sap	Erlendsson (2017) and Handler et al. (2018)
Quercetin	Onion, apple, tea, red wine	Tang et al. (2001) and Bachelor et al. (2005)
Perillyl alcohol	Citrus, cherries, mint	Mills et al. (1995), Lluria-Prevatt et al. (2002), and Gupta and Myrdal (2004)
Sulforaphane	Broccoli	Gills et al. (2006) and Shibata et al. (2010)
Curcumin	Turmeric	Bush et al. (2001)
Apigenin	Parsley, onions	Birt et al. (1997)
Fisetin	Strawberries, apples, cucumbers	Pal et al. (2015, 2016)
Lycopene	Tomatoes	Fazekas et al. (2003) and Ascenso et al. (2016)
Capsaicin	Red chili peppers, jalapenos	Hwang et al. (2010), Oyagbemi et al. (2010), and Bode and Dong (2011)

UVB-induced glutathione depletion in cultured keratinocytes through the caspase cascade (Zhu and Bowden 2004). Therefore, targeting events in the JNK or caspase pathways may be suitable as a chemopreventive measure to allow the signaling of the ARE during UVR exposure that causes damage in skin cells that can develop into skin cancer.

Development of a group of novel agents for skin photoprotection called quencher of photoexcited states (QPES) (Wondrak et al. 2005) may also be included as strategies of chemoprevention. These compounds directly antagonize the potentially damaging excited state of skin chromophores and molecular oxygen which cause damage to cellular targets leading to skin photoaging or photocarcinogenesis. These compounds suppress skin photooxidative damage upstream of ROS formation. Investigators suggest that these compounds be used in combination with antioxidants and sunscreens for a complete form of photoprotection. With a thorough screening process in place for QPES agents, Wondrak et al. (2005) suggest proline ester derivatives to be optimized for topical application in the skin.

As gut microbiome is being studied for its ability to play a role in cancer chemoprevention, skin microbiome and its role in the immune system may also be a target for future chemoprevention studies (Sherwani et al. 2018).

13.7 Conclusion

Skin cancer is a major health problem in the US as well as in countries such as Australia. With high health-care costs, at 5% of Medicare health-care expenses, increasing incidence, limited treatments, and a significant loss of life specifically for melanoma, prevention of this disease is imminent. Primary prevention strategies focus on an avoidance of sun exposure and the use of sunscreen compounds; however, new individualized prevention strategies will revolutionize these extremely important areas of dermatologic research. Significant advances in molecular biology in combination with pharmaceutical developments have opened the door for research in the field of chemoprevention and personalized medicine. For skin cancer, the formulation of a UV-induced signal transduction pathway (Fig. 13.3) that identifies important molecules involved in the carcinogenesis process has provided molecular targets for the development of target-specific agents. This pathway has been developed by the use of animal and cellular model systems of skin carcinogenesis. These targets include AP-1 and COX-2, as well as upstream targets such as EGFR, PI-3 kinase, MAPK, JNK, RSK-2, and Raf. Ongoing and future clinical trials will evaluate agents that act specifically to block molecules that are altered early in the development of skin cancer. These agents will most likely be delivered in a topical formulation using technology (e.g., prodrug development) that allows for maximum epidermal delivery with minimal systemic toxicity. The combination of several chemoprevention agents working in a synergistic fashion in these topical formulations will provide a promising strategy for the prevention of skin cancer.

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