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MOLECULAR MECHANISMS OF PHOTOCARCINOGENESIS

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1. ABSTRACT

Photocarcinogenesis represents the accumulation of genetic changes as well as immune system modulation, which ultimately lead to the development of skin cancers. The recent advances in molecular and cellular biology have clarified the mechanisms of photocarcinogenesis, including the formation of DNA photoproducts, DNA repair, mutation of proto-oncogenes and tumor suppressor genes, and UV-induced immunosuppression. The understanding and further investigation of photocarcinogenesis is critical to the development of effective prevention and intervention strategies for human skin cancer.

2. INTRODUCTION

The incidence of skin cancer has been increasing at an astonishing rate over the past several decades, and it is estimated that over one million new cases of non-melanoma skin cancer (NMSC) occur each year in the United States (1). The relevance of sunlight exposure to the NMSC epidemic is well known (2). The skin responds to sun exposure by tanning and skin thickening, which provides some protection from further damage by ultraviolet (UV) irradiation. The degree of pigmentation in the skin and the ability to tan are important risk factors in skin cancer development, and the risk of NMSC is highest in people who sunburn easily and suntan poorly (1).

Recent developments in molecular biology and research using laboratory animals have clarified the central role of UV radiation in NMNC carcinogenesis. UV radiation induces skin cancers by damaging the ability of keratinocytes to control cell proliferation; the cell has mechanisms to counteract this damage before cancer can develop, including DNA repair, apoptosis, and immune

surveillance. UV radiation can damage keratinocytes by forming cyclobutane-type dimers in DNA between adjacent pyrimidine residues, potentially leading to UV 'signature' mutations that can accumulate over time (3). The cell can respond to the damage by repairing DNA to avoid harmful mutations, or if the damage is too serious, by inducing apoptosis to remove potential cancer cells from the population (3). Failure of these pathways can result in the loss of control of cell proliferation and lead to tumor development through the inactivation of tumor suppressor genes or the activation of oncogenes. Immune surveillance in the skin also plays an important role in protecting against skin cancer development. UV exposure depresses the function of the immune system in the skin, creating a more favorable environment for the development and growth of tumors (4). The purpose of this article is to provide an overview of recent advances in molecular mechanism(s) of photocarcinogenesis.

3. THE EFFECT OF UV RADIATION ON SKIN

Sunlight is composed of a continuous spectrum of electromagnetic radiation that is divided into three main regions of wavelengths; ultraviolet (UV), visible, and infrared. UV radiation comprises the wavelengths from 200-400 nm, the span of wavelengths just shorter than those of visible light (400-700 nm). UV radiation is further divided into three sections, each of which have distinct biological effects; UVA (320-400 nm), UVB (280-320 nm), and UVC (200-280 nm). UVC is effectively blocked from reaching the earth's surface by the stratospheric ozone layer, although accidental exposure could occur from man-made sources, such as germicidal lamps. UVA and UVB radiation both reach the earth's surface in amounts sufficient to have important biological consequences to the skin and eyes. Wavelenghts in the UVB region of the solar spectrum are absorbed into the skin, producing erythema, burns, and eventually skin cancer. Although UVA is the predominant component of solar UV radiation to which we are exposed, it is supposed to be weakly carcinogenic. Some laboratory studies, however, have demonstrated that wavelengths in the UVA region not only cause aging and wrinkling of the skin, but they have also been shown to cause skin cancer in animals when given in high doses over a long period of time (5,6).

UV radiation is absorbed by DNA maximally from 245-290 nm (7). UV is able to induce mutagenic photoproducts or lesions in DNA between adjacent pyrimidines in the form of dimers. These dimers are of two main types: cyclobutane dimers (CPD) between adjacent thymine (T) or cytosine (C) residues, and pyrimidine (6-4) photoproducts between adjacent pyrimidine residues. CPD are formed between the C-4 and C-5 carbon atoms of any two adjacent pyrimidines; the double bonds become saturated to produce a four-membered ring (8). Similarly, (6-4) photoproducts are formed between the 5-prime 6 position and the 3-prime 4 position of two adjacent pyrimidines, most often between TC and CC residues (7). CPD are produced overall three times as often as (6-4) photoproducts (7). Both lesions occur most frequently in runs of tandem pyrimidine residues, which are known as 'hot spots' of UV-induced mutations (8). Although both lesions are potentially mutagenic, the CPD is supposed to be the major contributor to mutations in mammals (7); the (6-4) photoproducts are repaired much more quickly than CPD in mammalian cells (9).

If not repaired, UV-induced DNA lesions can lead to mutations in the DNA sequences. These mutations are in the form of C to T and CC to TT transitions, known as UV 'signature' mutations. The 'A rule' has been proposed to explain how UV signature mutations arise from the DNA lesions (10). According to the A rule, the DNA polymerase inserts adenine (A) residues by default opposite the lesion that it cannot interpret. A mutation is then created upon DNA replication of the strands containing base pair changes. The TT cyclobutane dimers do not result in mutations, because A normally is paired with T and no mutation would result from insertion of A residues by default opposite the dimer. However, with CC cyclobutane dimer, a CC to TT transition occurs, because two A residues are placed opposite the dimer by default in the place of two guanine (G) residues. In (6-4) photoproducts between a pyrimidine and a C residue, the 5-prime residue base pairs correctly, but the 3-prime C residue resembles a non-instructional site (8). A C to T mutation occurs because an A residue is placed opposite the C residue by default.

Mutations in genomic DNA can lead to carcinogenesis usually acting as an initiating event. These may remain dormant for a number of years until exposure to a promoting agent. Promoters may or may not be carcinogenic themselves, but can act with the initiating events to cause progression into tumor development. Genes can cooperate to effect carcinogenesis, in which multiple mutations at different loci are required. It has been estimated that between three and seven mutational events are required to transform normal cells into cancer cells, depending on the life span of the cell (8). The transforming mutations are usually in tumor suppressor genes or oncogenes, or other genes that are involved in the regulation of cell proliferation.

4. DNA REPAIR AND SKIN CARCINOGENESIS

All mammalian cells are equipped with several DNA repair systems, which are able to protect the cell from the effects of DNA damaging compounds by removing DNA lesions (11). Depending upon the primary DNA lesion, one or more repair pathways become active such as direct repair, base excision repair, mismatch repair, double-stranded break repair, and nucleotide excision repair (NER). CPD and (6-4) photoproducts generated by UV irradiation are primarily repaired by NER, which removes bulky DNA damage in two distinct subpathways (12); damages existing in actively transcribed genes are removed by a quick mechanism called transcription-coupled repair (TCR), and the damages prevailing in other parts of the genome are removed in a slower fashion called global genome repair (GGR). The two subpathways are different only in the first step of NER, that is DNA damage recognition (Figure 1). In GGR, the protein complex, XPC/HHR23B is involved in damage recognition, while in

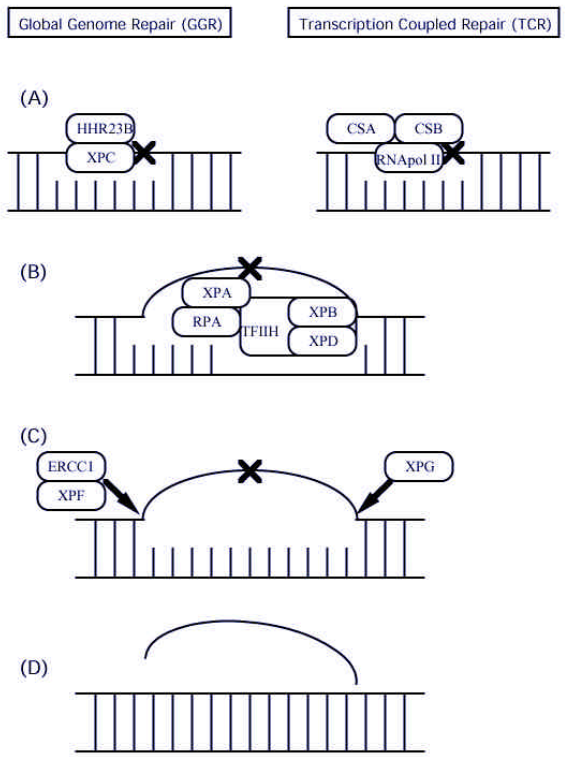

Figure 1. Proposed model for mammalian nucleotide excision repair (NER). It consists of two subpathways: global genome repair (GGR) and transcription coupled repair (TCR). (A) The two supathways differ only in the first step, DNA damage recognition. In GGR, the protein complex XPC/HHR23B binds to the damaged DNA site, recruiting the entire repair protein apparatus to the injury. In TCR, on the other hand, a stalled RNA polymerase II fulfils this function at the site of the DNA lesion, which is facilitated by the Cockayne syndrome proteins CSA and CSB. (B) In the second step, the DNA unwinding, the lesions are opened by the concerted action of XPA, replication protein A (RPA) and the bi-directional XPB/XPD helicase subunits of TFIIH complex. (C) In the next step, the incision of the damaged DNA, ERCC1/XPF complex cuts at the single-strand to double-strand transition 5' of the damage, and XPG cuts at the 3' side of the open complex. (D) In the final stage of NER, the DNA excision and *de novo* synthesis, the repair synthesis occurs by the mammalian DNA replication factors such as RPA, RF-C, PCNA, and DNA polemerase delta and epsilon. The reaction is completed by ligation of the newly synthesized DNA. Adapted from (13) and (14).

TCR a stalled RNA polymerase II itself is the damage recognition signal and CSA and CSB proteins are supposed to facilitate this process. The following stages are the same in GGR and TCR; (B) unwinding of the DNA helix surrounding the DNA lesion, (C) dual incision of the damaged DNA strand, and (D) excision of the damaged stretch and *de novo* DNA synthesis.

Defects in both subpathways of NER can lead to three distinct human diseases; xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD). These diseases provide important model systems for investigating aspects of DNA repair, since they are all deficient in NER but in different subpathways. Among these three different types of diseases, XP patients (divided into XPA – XPF subgroups according to the causative genes) only exhibit skin cancer predisposition as well as photosensitivity (15). The mean age of onset of cutaneous symptoms such as parchment and freckles is 2 years, and patients are associated with more than 1000 fold increased risk of developing skin cancers. BCCs and SCCs appear almost exclusively to sun exposed areas, and less frequently melanomas. Moreover, XP patients have a 10- to 20-fold increased risk of developing several types of internal cancers under the age of 20 years (16). Considering the involvement of NER in repair of chemically induced DNA lesions, as well as in the repair of lesions induced by cellular metabolites, either category of lesions may play a role in the internal neoplasms. CS patients are characterized by prominent cutaneous photosensitivity, however, they are not predisposed to develop skin cancer (17). CS patients display various kinds of skeletal abnormalities as well as physical and mental retardation, which are difficult to comprehend as a consequence of defective NER. That is also true in TTD patients, whose clinical hallmarks are sulfur-deficient brittle hair and ichthyosis (scaling of the skin) (18). Photosensitivity is reported in most, but not all cases of TTD, and the patients have been identified with mutations in *XPB*, *XPD*, and the yet uncloned gene *TTDA* which is supposed to be a TFIIH subunit (19). Although the photosensitivity in TTD is due to a defect in NER, no cutaneous malignancies have been reported (20).

5. TUMOR SUPPRESSOR GENES AND ONCOGENES

Carcinogenesis by UV radiation often involves the inactivation of one or more tumor suppressor genes or the overactivation of growth-stimulatory proto-oncogenes. Tumor suppressor genes are negative growth regulators and usually are recessive in that they require both copies of the gene to be inactivated before loss of control of cell growth occurs. Accumulation of proteins that bind to and sequester tumor suppressor proteins can also make the cell more susceptible to further mutations. Activation of oncogenes is dominant in that a change in only one copy of the gene is required to have an effect. Proto-oncogenes, the normal versions of oncogenes, act to control cell proliferation and differentiation, and are divided into three groups; growth factors and growth factor receptors, signal transduction proteins, and nuclear factors (8). Carcinogenesis can result either from expression of a mutant or altered gene product. Several genes have been extensively studied that have important roles in skin carcinogenesis, including *p53*, *patched*, *p19*, and *ras* genes.

5.1. *p53* tumor suppressor gene

The *p53* gene is the most frequent target of genetic alteration identified so far in human cancers (21). Loss or mutation of *p53* has been demonstrated in approximately 50% of all human cancers examined, although the frequency of mutations varies greatly

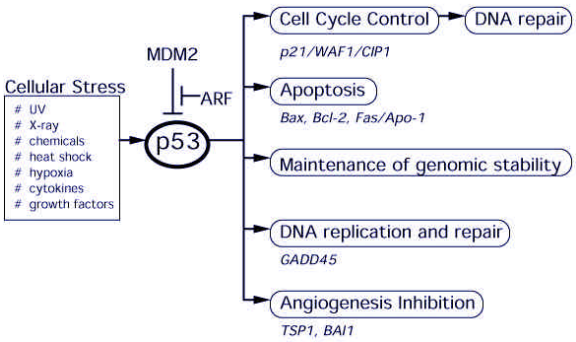

Figure 2. The cellular response against extracellular stress through *p53* gene regulation and its downstream genes.

depending on the type of cancer. The human *p53* gene is localized on chromosome 17 (17p13), contains 11 exons, and encodes 393 amino acids (22). The mouse *p53* gene is localized on chromosome 11 and also contains 11 exons (23). Wild-type p53 binds to specific genomic sites as a tetramer and stimulates expression of downstream genes (24). The p53 protein is a central element in fundamental cellular processes including cell-cycle control, apoptosis, DNA replication and repair, genomic stability and senescence (25). The transcription-activating function of p53 is eliminated by interaction with the MDM2 gene product (26) because MDM2 promotes rapid degradation of a phosphorylated form of p53 (27, 28). On the other hand, p53 activates expression of the MDM2 gene in an autoregulatory feedback loop (26).

5.1.1. Function of *p53* gene

The basic function of p53 protein is to maintain a cell in normal status against various extracellular stress, or to lead the cell into apoptosis when its DNA is severely damaged in order to prevent the carcinogenesis procedure as shown in Figure 2. A variety of cellular stresses including genotoxic agents (UV, X-ray, cytotoxic chemicals and so on), heat shock, hypoxia, and some kinds of cytokines and growth factors activate *p53* by phosphorylating Serine 15 and Serine 20, followed by the dissociation with MDM2 protein (29). The biological functions of *p53* result from transcriptional activation of downstream genes as listed below.

5.1.1.1. Cell-cycle control (p21/WAF1/CIP1)

The accumulation of p53 protein after cellular DNA damage induces a cell cycle arrest at the G1 phase, which allows the repair of DNA damage before its replication in the S phase (30, 31). In this pathway, p21/WAF1/CIP1 was discovered as an inhibitor of cyclin dependent kinase whose induction was associated with expression of wild-type p53 (32, 33). p21/WAF1/CIP1 inactivates the cdk-cyclin complex by forming a Cdk2/Cyclin A or E/Proliferating Cell Nuclear Antigen (PCNA)/p21 complex, thus leading the cell into G1 arrest. Formation of this complex leads to the accumulation of hypophosphorylated pRb, causing the release of E2F, which is necessary for the induction of DNA synthesis (32, 33).

5.1.1.2. Apoptosis (BAX, BCL-2, FAS/APO-1, DR5, IGF-BP3)

Another protection strategy to escape malignant transformation caused by DNA damage is the activation of apoptosis pathways. As a transactivator of transcription, p53 protein can induce apoptosis either by upregulaing the expression of apoptosis-promoting (proapoptotic) genes such as *Bax, Fas/Apo-1, DR5, IGF-BP3,* or by downregulating the expression of apoptosis-suppressing (antiapoptotic) genes such *as Bcl-2, c-IAP2* and *NAIP1* (34). Although p53-induced apoptosis involves several mechanisms that are determined by cell type and apoptotic stimulus, *Bcl-2* gene family plays a crucial role in the p53-induced apoptotic pathway. *Bcl-2* gene family is composed of the proapoptotic gene group (*Bax, Bad, Bak, Bcl-xs, Bik, Bim, Mtd/Bok*) and the antiapoptotic gene group (*Bcl-2, Bcl-w, Bcl-xl, Mcl-1*) (35, 36). Bax and Bcl-2 proteins, which are homologous but have opposing effects on apoptosis, can form heterodimers in cells, and their interaction would be critical to the ability of Bcl-2 to block cell death (24). p53 induces apoptosis by disrupting the balance between them; the upregulation of *Bax* gene and the downregulation of *Bcl-2* gene. Another important p53-induced apoptotic mechanism is through Fas expression. Wild-type p53 can activate *Fas* gene by binding to the transcriptional acitivation site within *Fas* gene as well as its promotor region. (37). At the same time, *p53* promotes the redistribution of cytoplasmic Fas to the cell surface by transportation from the Golgi complex (38).

Several studies have shown that UV-induced sunburn cell formation and apoptosis is medicated by the p53 pathway (39-41). Ziegler *et al* (39) reported that the inactivation of *p53* in mouse skin reduced the appearance of sunburn cells, implying that the elimination system of precancerous keratinocytes does not work well when the cells harbour *p53* mutations caused by UV irradiation. Hill *et al* (40) investigated the function of Fas/Fas-Ligand (FasL) interactions in UV-induced keratinocyte apoptosis and found that accumutation of *p53* mutations in the epidermis of *FasL* deficeint mice was observed at much higher frequency (14 out of 20 mice, 70%) compared with wild-type mice (1 out of 20, 5%) after chronic UV irradiation. They concluded that *FasL*-mediated apoptosis is important for skin homeostasis, and that the dysreguration of Fas-FasL interactions may be central to the development of skin cancer. This notion is supported by the recent study of Ouhtit et al (41). They found the decrease of FasL expression during the first week of UV irradiation on hairless SKH-hr1 mice skin, and the complete loss of its expression after 4 weeks, which is paralleled with the decrease in the numbers of sunburn cells. *p53* mutations were detected in the UV-irradiated epidermis as early as 1 week and continued to accumulate with further UV exposure. These findings suggest that chronic UV exposure would induce a loss of FasL expression and a gain in *p53* mutations, leading to dysregulation of apoptosis, expansion of mutated keratinocytes, and initiation of skin cancer.

5.1.1.3. DNA replication and repair (GADD45)

Several growth-arrest and DNA damage-inducible (or gadd) genes were initially isolated on the basis of induction after DNA damage in Chinese hamster

ovary cells. Among them, *GADD45* gene is rapidly induced by DNA-damaging agents and the normal function of *p53* gene is needed for its induction by ionizing radiation (42). GADD45 interacts with the products of two other *p*53-regulated genes, p21/WAF1/CIP1 and PCNA, and is possibly involved in the NER system (43, 44). Recent studies suggest that GADD45 may also be involved in the G_2/M checkpoint through its interaction with Cdc2 (45, 46).

5.1.1.4. Angiogenesis inhibition (TSP1, BAI1)

As normal cells progress toward malignancy, they must switch to an angiogenic phenotype to attract the nourishing vasculature that they depend on for their growth. Overexpression of *p*53 has been found to inhibit angiogenesis possibly through upregulation of Thrombospondin 1 (*TSP1*) (47) and Brain-specific angiogenesis inhibitor 1 (*BAI1*) (48). Early passage Li-Fraumeni (*p53*-deficient) cells secret large amounts of an angiogenesis inhibitor, TSP1, which no longer occurs with late passage angiogenically-prone fibroblasts, and the *TSP1* promoter would be positively regulated by wild-type *p53* (47). *BAI-1* was initially isolated as a transcript which can be upregulated by p53 in glioblastoma cells (48). *BAI1* contains five TSP1/TSP2 repeats in its extracellular region, and is a potent inhibitor of neovascularization in the rat cornea (48).

5.1.2. *p53* mutations in human skin cancers

A number of investigators have detected *p53* gene mutations in a large proportion of human SCCs, BCCs, and actinic keratoses (39, 49-56). Ziegler et al reported that *p53* gene mutations in non-melanoma skin cancers were detected at a higher frequency (about 50-90%) than those of internal malignancy (39). The predominant alterations are C to T and CC to TT transitions at dipyrimidine sites. These types of mutations are preferentially induced by UV radiation, and thus are referred to as "UV signature" mutations. *p53* mutations have also been found at high frequencies in skin cancers from patients with xeroderma pigmentosum (57-59). In such case, the majority of mutations are CC to TT tandem base substitutions and most occur on the nontranscribed strand of DNA, implying preferential repair of UV-induced lesions on the transcribed strand.

The mutations in *p53* gene appear to be an early genetic change in the development of UV-induced skin cancers (39, 55, 59). *p53* gene mutations have been detected in normal sun-exposed human skin (55, 60) as well as UV-irradiated mouse skin (61, 62). In addition, *p53* gene mutations were detected at a high frequency in human actinic keratoses which are considered to be pre-SCCs (39, 54). On the other hand, more recent studies revealed that non-cancerous skin adjacent to skin cancers harbor *p53* gene mutations that are distinct from those present in the skin cancers (63, 64). One report (65) compared multiple NMSCs with and without *p53* mutations in the same XP patient and found that the former tend to exhibit more rapidly-growing and/or histologically immature clinical features, suggesting that *p53* gene mutations would bring more malignant characteristics to NMSCs.

5.1.3. *p53* mutations in mouse skin cancers

In the experiments of photocarcinogenesis using animal models, mutations in the *p53* gene are clearly linked to UVB radiation and the *p53* alterations seem to be essential early in tumor development (66). Most of the *p53* mutations detected in mouse skin cancers were C to T and CC to TT transitions at dipyrimidine sites like those found in human skin cancers, and most were located on the non-transcribed strand (67, 68). However, the frequency and the exons of *p53* mutations differ among mouse strains for reasons that are not yet clear. For example, *p53* mutations were detected in 100% of skin cancers induced in C3H/HeN mice by sunlamp irradiation (67), while 20% of SCC from SKH-hr1 hairless mice and 50% of SCC from BALB/c mice exhibited *p53* mutations in another study (68).

Recently, Ananthaswamy *et al* (69) have shown that UV-induced SCCs from SKH-hr1 mice contained *p53* mutations at a high frequency (87.5%). They have also found that the *p53* mutation spectra seen in solar simulator-induced SKH-hr1 skin tumors are similar to those present in unfiltered and Kodacel-filtered FS40 sunlamp-induced C3H skin tumors (69). Interestingly, the frequency and spectrum of *p53* mutations of UV-induced skin cancers in XPC knockout mice is similar to that of NER-proficient C3H and SKH-hr1 mice (70). The difference between them was that the *p53* mutations persisted after 3-4 weeks of chronic UV irradation only in XPC knockout mice as well as decreased apoptosis and increased proliferation of keratinocytes (70).

The protective role of *p53* against UV irradiation was directly shown using *p53*-/-, *p53*+/-, and wild-type C57BL/6 mice (71). In the report, *p53*-/- mice were extremely sensitive to tumor induction by UV irraditation, wild-type mice were resistant, and *p53*+/- mice showed an intermediate response between the two groups. A recent report (72) has shown the extreme sensitivity of *p53*+/- mice to the development of lymphoid malignancies following exposure to irradiation and indicated a causal relationship between UV irradiation and the development of lymphoid malignancies.

5.1.4. p53 expression in human and mouse skin cancers

Immunohistochemical detection of p53 protein using anti-p53 antibodies has shown that many *p*53 mutations increase the half-life of the protein, allowing cytosolic accumulation of mutant protein (73). Stabilization and elevation of p53 levels may signify an early event in tumorigeneisis. Increased p53 staining is seen in atypical keratinocyte proliferations, actinic keratoses (74) and keratoacanthomas (75), but it is not seen in benign skin lesions (76). Overexpression of mutant p53 was observed in high frequency in human BCCs (77). Increased p53 staining during the progression of keratoacanthomas to SCC (78), and primary SCC to regionally metastatic SCC (79) has also been reported.

Although immunohistochemistry has the obvious advantages of being faster and less expensive as a screen for *p53* mutations than DNA analysis, there does not seem

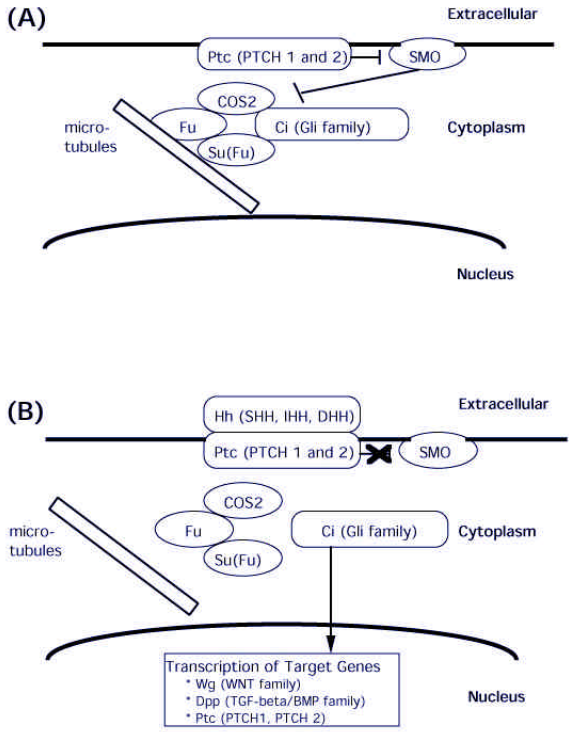

Figure 3. A proposed model for hedgehog signaling pathway. (A) In the absence of hedgehog, the patched protein (Ptc) inhibits the activity of smoothened (SMO). Ci forms a complex with COS2, Fu, and Su(Fu) (suppressor of Fu), by which it associates with microtubules. (B) When hh (SHH, IHH, DHH) binds to Ptc, the inhibitory effect upon SMO is suppressed, followed by the activation of SMO signal pathway. The COS2-Fu-Su(Fu) complex dissociates from microtubules, and an active form of Ci translocates to the nucleus where it switches on transcription of the target genes such as *Wg*, *Dpp* and *Ptc*.

to be a strong correlation between p53 immunostaining and the presence or absence of *p*53 mutations (80). Some tumors harboring missense mutations have been negative for p53 expression by immunostaining, and conversely, tumors staining positively for p53 do not always contain mutations (81). The lack of correlation between *p*53 mutation and immunocytochemical expression could be the result of several factors. Certain *p53* mutations may make the protein less stable and more susceptible to degradation. Alternatively, some antibodies directed against the mutant p53 protein may also cross-react with wild-type protein.

5.2. Ptc and Hh signaling pathway

The *Ptc* gene was first cloned in Drosophila by two different groups (82, 83). Ptc is a transmembrane protein with 12 membrane-spanning domains and two extracellular loops (84), and serves as a receptor for the secreted protein Hh (85, 86). In Drosphila, *Ptc* is a segment polarity gene which acts to control the number and identity of the body segments in a developing Drosophila embryo (87). In vertebrates, Ptc and Hh signaling pathway is important in development of the pharyngeal arches, limb buds, neural tube, and somites (84), and at many sites as hair, whisker, and tooth formation (88). In 1996, the *Ptc* gene was linked to the basal cell nevus syndrome (Gorlin's syndrome) (89, 90). The syndrome shows autosomal dominant inheritance and is characterized by a number of abnormalities including palmar and plantar pits, cleft lip and palate, hyperterolism, bifid ribs, calcification of the falx cerebri and macrocephaly (91). Several tumors feature in the syndrome, including medulloblastoma, ovarian fibroma, jaw cysts, and meningioma, but the most frequent tumor is BCC, occurring in 80-90% of cases (92). The development of tumors in the syndrome is in keeping with Knudson's 'two-hit hypothesis' (93). Germinal mutations in *Ptc* gene have been found in families with Gorlin's syndrome (90, 94-96). Germline *Ptc* gene mutations in *de novo* cases of Gorlin's syndrome, but not their relatives, have also been found and are especially indicative of a causal link between the *Ptc* gene and the syndrome (90).

5.2.1. Function of hedgehog pathway

The Hh signaling pathway has been clarified through the study of *Drosophila*, where a number of proteins are recognized to be important for pathway function. The *Ptc* gene controls the activities of genes that drive cell growth and differentiation by repressing their activities in cells where *Ptc* is expressed (97) (Figure 3). This is accomplished by opposing the function of the *hedgehog* (*Hh*) gene, which encodes a secreted signaling protein that induces cell growth and differentiation (98). *Ptc* inhibits the Hh pathway by repressing the activity of *smoothened* (*Smo*), which is another transmembrane membrane protein (99, 100). The *Ptc*-mediated inhibition is removed when Hh binds to *Smo*. *Cubitus interruptis* (*Ci*) is a transcription factor which is activated at the downstream of *Smo* (101). Ci protein is thought to form a cytoplasmic complex with the proteins fused (Fu) and costal2 (COS2) (102-104). Fused is a serine-threonine kinase (105) and costal 2 is a kinesin-related protein that binds the Ci-containing complex to microtubules (103). *Smo* activity favors translocation of an active form of Ci from the cytoplasm to the nucleus, where it promotes transcription of downstream target genes (106), including *Ptc* (107), decapentaplegic (*Dpp*) (103), and wingless (*Wg*) (108, 109). In summary, the transcription of downstream target genes is switched on when Hh is bound to Ptc, and off when it is not.

The vertebrate homologues of *Hh* include *Sonic*, *Indian*, and *Desert* (110, 111), and the *Ptc* homologues are *Ptc1* and *Ptc2* (112). *Smo* and *Ci* also have homologues in vertebrates (113, 114). The latter is related to vertebrate *Gli* transcription factors, which are overexpressed in human gliomas (115, 116). This suggests that the entire pathway is conserved through evolution. The downstream target genes identified in *Drosophila* also have vertebrates homologues. *Dpp* shows homology with the bone morphogenic proteins (BMPs), and is a member of the TGF-beta superfamily (111, 117). *Wg* shows homology with *Wnt-1*, a signaling protein (118).

5.2.2. Disorder of hedgehog pathway in mouse models

In vivo models of Hh pathway deregulation have been used to assess the functional consequences of Hh

pathway deregulation in skin. In these studies, the *Hh* gene was overexpressed in epidermal cells of transgenic mice (119), and in transgenic reconstituted human skin (120). In the latter case, the skin was grafted onto the back of immune-deficient mice. The transgenic models resulted in activation of the Hh signaling pathway as shown by increased expression of downstream target genes. Both of these studies describe the formation of BCC-like tumors showing morphological and gene expression profile resembling that of human BCC. The tumors in transgenic mice occurred within the first few days of skin development but the mice died perinatally. In transgenic human skin, the tumors arose within 4 weeks occurring throughout the epidermis. The tumors ranged from small buds to more deeply penetrating epithelial islands. The rapid induction of BCC coupled with the absence of deliberate mutagenesis in these model systems strongly suggests that activation of the Hh signaling pathway alone initiates BCC formation. In addition, transgenic mice in which there is excess epidermal expression of normal Gli1 or Gli2, or of mutant Smo all develop cutaneous tumors with a histology well resembling human BCCs (121-123). These mice provide compelling evidence for the importance of the hedgehog signaling pathway in BCC genesis and suggest that hedgehog target gene activation may not only be necessary for BCC genesis but also may be sufficient for the development of these tumors.

BCCs are unusual among malignancies because they almost never metastasize. The failure of cells to separate from the primary tumor and grow in another tissue environment may reflect a need for interaction with a conditioned stroma. Transplantation of human BCCs to nude mice provides some evidence that surrounding stroma is necessary for the maintenance of the malignant state (124, 125). Paracrine activity of hedgehog target genes in neoplasia has not been explored extensively. However, members of the TGF-beta family have been shown to play a role in enhancing growth conditions for BCCs by altering surrounding tissue (126).

5.2.3. Disorder of hedgehog pathway in human skin cancers

Statistical analysis of the distribution of BCCs in BCNS patients suggested that tumors in the syndrome arise through a two-hit mechanism and that the underlying defect might be mutations in a tumor suppressor gene. This hypothesis was strongly supported by the mapping of the causative gene (*patched*) to chromosome 9q22-31 and the demonstration that the exact same region was deleted in a high percentage of BCC and other tumors related to the disorder (127). In addition to germline mutations in BCNS patients, *patched* mutations and allelic loss containing *patched* locus in sporadic BCCs (127-131) and in BCCs associated with xeroderma pigmentosum (132, 133) have been reported. In sporadic BCCs that appeared on normal (DNA repair proficient) individuals, *patched* gene mutations were detected in 12-40% (89, 90, 127-131), and allelic loss was found in 42-69% of the examined specimens (127, 129). Asterbaum *et al* reported that three among 26 sporadic BCCs in which *patched* mutations were detected were also accompanied with allelic loss of the other *patched* locus, suggesting that *patched* acts as a classic tumor suppressor gene requiring 'two hits' for tumorigenesis in at least some BCCs (129). Like germline mutations in BCNS, a high proportion of mutations in sporadic BCCs are predicted to lead to premature protein termination (127). The minute BCCs are as likely as large tumors to have mutations, and all histological subtypes have almost the same frequency of *patched* disorder. The mutational spectrum of *patched* in sporadic BCCs is rather diverse and only 41% (11 in 27 examined samples) exhibited the typical UVB signature (89, 90, 128-131). This result is different from the previous analyses of *p53* gene mutations in sporadic BCCs, in which most of the mutations were presumably related to UVB irradiation (50). The lower incidence of UVB signature mutations in *patched* suggests that mutagenic events other than UVB irradiation may also cause *patched* inactivation and trigger tumorigenesis. Furthermore, this relatively low fraction of BCC cells that have UVB induced mutations is consistent with epidemiologic studies that have found poor correlation between UVB dose and incidence of BCC, unlike the better correlation between UVB dose and the incidence of SCC (134, 135).

In contrast to sporadic BCCs, those in patients with xeroderma pigmentosum have a high rate of *patched* gene mutations (73-88%), as well as higher frequency of UV-specific mutations (75-79%), indicating that the inablility of XP patients to repair UV-induced *patched* mutations would significantly contribute to the BCC tumorigenesis observed in those patients (132, 133).

The activating mutations in the *Smo* gene have also been detected in a proportion of sporadic BCCs (123). One common mutation (Trp535Leu) in the seventh transmembrane domain of Smo has been detected in almost all BCCs lacking *PTCH* mutations. Since the activating mutation of *Sonic hedgehog* itself has been detected very rarely in BCCs (119), inactivation of *patched* or oncogenic activation of *Smo* would play a crucial role in human BCC tumorigenesis through dysregulation of the Hh signaling pathway.

5.3. $p16^{INK4a}$ and $p19^{ARF}$ genes

It has been postulated that the causative gene(s) of non-melanoma skin cancers would be located in chromosome 9p, since loss of heterozygosity occurs frequently at the locus in SCCs and actinic keratoses (136) as well as, less frequently, in a subset of BCCs (137). Soon after, *CDKN2A* (cyclin-dependent kinase inhibitor 2A) was identified on chromosome 9p21 as a causative gene of familial melanoma (138, 139), and was suspected as the candidate involved in the carcinogenesis of SCCs and BCCs. Interestingly, the locus encoding CDKN2A gives rise to two distinct transcripts from different promoters (140); $p16^{INK4a}$ and $p19^{ARF}$ ($p14^{ARF}$ in human). $p16^{INK4a}$, which is composed of exons 1alpha, 2 and 3, is a cyclin-dependent kinase inhibitor that specifically inhibits progression through G1 phase of the cell cycle in cells that express the retinoblastoma protein (pRb). *p16* maintains pRb in its activating state by blocking cyclin-dependent kinase 4 (CDK4) from phosphorylating pRb (141). $p19^{ARF}$,

which is composed of exon 1beta, 2 and 3, stabilizes p53 by inhibiting MDM2-dependent p53 degradation, thus specifically activating the p53 pathway (142).

p16^{INK4a} is considered to be a key tumor suppressor gene, and its inactivation by deletions, mutations, or methylation can occur in a wide range of human cancers (143). p16 ^{INK4a} accumulates in HeLa cells after nonlethal UV irradiation and causes cell cycle arrest, suggesting that alteration in *p16* ^{INK4a} would constitute an important step in UV-induced carcinogenesis (144). *p19* ^{ARF} is also assumed to be involved in skin carcinogenesis, since the targeted disruption of *p19* ^{ARF} renders mice susceptible to SCCs (145).

5.3.1. *p16*^{INK4a} and *p19*^{ARF} mutations in squamous cell carcinomas

Kubo *et al* (146) reported that three (14%) out of 21 SCCs developed in Japanese showed the hemizygous mutations in CDKN2A gene. All the mutations were found in exon 2, which is common in *p16* ^{INK4a} and *p19* ^{ARF}. Among them, two SCCs were seen on sun-exposed areas. One of them showed a deletion of 21 base pairs, and the other showed CC to T (a combination of deletion and transition) at a dipyrimidine site, both of which had no mutation in *p53*. A third SCC occurred on a burn scar of lower leg, showed C to T transition at dipyrimidine site and had a *p53* gene mutation as well. Sourfir *et al* (147) examined 20 human SCCs and found four (20%) different mutations. All four mutations lead to both *p16* ^{INK4a} and *p19* ^{ARF} gene alterations, and three (75%) were UV-signature mutations. No SCCs had simultaneous alterations of *p53* and *p16* ^{INK4a} */p19* ^{ARF}, confirming a reciprocal relationship between them (148). Soufir and colleagues also examined *p16* ^{INK4a} */p19* ^{ARF} mutation in SCCs developed in XP patients (149). Among 18 SCCs, six (33%) SCCs showed 10 mutations *in p16*^{INK4a}*/p19* ^{ARF} genes and seven (70%) were either C to T transition at dipyrimidine sites or CC to TT tandem mutations. Considering only a few reports have been published so far concerning the mutations of *p16* ^{INK4a} */p19* ^{ARF} genes, further accumulation of mutational analyses as well as methylation of promoter region is necessary to clarify the impact of *p16* ^{INK4a} */p19* ^{ARF} in the carcinogenesis of SCCs.

5.3.2. *p16*^{INK4a} and *p19*^{ARF} mutations in basal cell carcinomas

Compared with the involvement of *p16* ^{INK4a} */p19* ^{ARF} disorders in SCCs, the contribution of these genes in the development of BCCs seems far less. Kubo et al (150) examined 25 sporadic BCCs for the mutation of *p16* ^{INK4a} */p19* ^{ARF} genes, and found no mutations. Soufir *et al* (147) found only one (3.5%) mutation out of 18 BCC samples. The mutation was C to T transition at a dipyrimidine site. However, its pathogenic effect is not clear because the sample also contained *p53* gene mutation. Saridaki *et al* (151) investigated 67 sporadic BCCs and found allelic loss of 9p21-22 locus in 55% (37 of 67) of the specimens, whereas no mutation of *p16* ^{INK4a} */p19* ^{ARF} genes were detected. They concluded that these genes seem not to be implicated by mutational inactivation in the development of BCC, and other (yet unidentified) tumor suppressor gene(s) located in the locus could be involved in BCC carcinogenesis.

5.4. *ras* oncogenes

Proto-oncogenes act as crucial growth regulators in normal cell division, differentiation, and apoptosis. They encode growth factors, receptors, extranuclear proteins involved in signal transduction, kinase and other regulatory factors, and nuclear transcription modulatory facors (152, 153). Proto-oncogenes are activated to cancer-causing oncogenes by point mutations, gene amplification, and gross DNA rearrangements (154). Among various kinds of oncogenes which have been analyzed in rodent and human tumors, *ras* oncogenes have the greatest impact as causative factors of carcinogenesis. The family of ras oncogenes consistis of three members, *H-ras*, *K-ras*, and *N-ras* genes, which encode 21-kDa guanosine triphosphate-binding proteins located on the inner surface of the cell membrane (155). These proteins participate in signal transduction from the cell surface to the nucleus and participate in growth control through intrinsic GTPase activities (155). The majority of *ras* mutations found in various types of human cancers occur in codons 12, 13, and 61 (156), and result in the continuous activation of *ras*-mediated signal transduction. Activated *ras* genes may initiate papillomas (157), and, in cooperation with at least one other genetic alteration, they can induce malignant conversion (158).

5.4.1. *ras* mutations in mouse skin cancers

In analyses of mouse skin cancers initiated by ultraviolet irradiation, *ras* gene mutations were detected in about 20-40% (159, 160). The detected mutations occurred at codon 61 of N-*ras*, codon 13 of H-*ras*, and codon 61 of K-*ras*. Contrary to the case of *p*53 gene mutations in mouse skin cancers, C to T transversions were most frequently observed among them, suggesting other types of DNA damage than pyrimidine dimers and (6-4) photoproducts. One candidate is 8-hydroxydeoxyguanosine (8-OHdG) at the 5' site of 5'-GG-3' sequence in double-stranded DNA produced by UV radiation with riboflavin (161). 8-OHdG is one of the oxidative DNA products induced by reactive oxygen species (ROS), and causes a G:C to T:A transversion by pairing with A in 50% probability. Considering it increases several times higher than the basal level in mouse skin after UV irradiation (162), 8-OHdG could possibly induce *ras* mutations.

Interestingly, *ras* activation may depend on genetic background; mutations of N-*ras*, especially at codon 61, were observed in 20% of skin tumors in the C3H strain of mice but not in similarly induced tumors in SKH mice (159). In view of the clinical features, amplification of a mutant H-*ras* allele, or loss of an allele, is generally seen only in high-grade or spindle-cell tumors, with good correlation among such allelic imbalance, tumor progression, and the degree of invasiveness of the malignant cells in the analysis of mouse skin tumors (163, 164).

In the UV-induced experiments using v-Ha-*ras* transgenic (Tg.AC) mice(165), three exposures at various

UV intensities resulted in a dose-dependent squamous papilloma formation and about 60% of the papilloma-bearing mice went on to develop SCCs or anaplastic spindle tumors. Interestingly, *p53* mutations were not detected in UV-induced tumors, except for one papilloma, suggesting that *p53* would not be a target for UV-induced tumorigenesis in the Tg.AC mouse.

5.4.2. *ras* mutations in human skin cancers

There are discrepancies with respect to the frequency of *ras* mutations in human non-melanoma skin cancers reported by various investigators (166-171). In one study, G to T mutations at the second position of codon 12 in H-*ras* gene have were detected in about 40% of the examined specimens (166). Other studies, however, reported a lower frequency of *ras* gene mutations in human non-melanoma skin cancers (167-171). The variation in frequency of *ras* gene mutation may be a reflection of the carcinogen exposures, ethnic heterogeneity, and/or genetic makeup of the groups studied. In addition to activating point mutations, amplification of ras gene has also been reported in human skin cancers (166, 170). In many cases, amplification is accompanied by point mutations or other rearrangements, but some cases exhibit simple amplification of the unmutated sequences. Since the frequency of *ras* gene mutation or amplification is rather low, they could be either coincidental to skin cancer formation or represent only one of several possible pathways by which cells lose growth regulation (172).

6. MALIGNANT MELANOMA AND UV IRRADIATION

Malignant melanoma incidence and mortality rates are increasing in most countries throughout the world where they are being recorded (173). The annual increase in incidence rate varies between populations, but in general it has been in the order of 3-7% for fair-skinned Caucasian populations (174). The likelihood of melanoma occurring in any individual is a combination of inherited or constitutional predisposition and exposure to environmental factors relevant to tumorigenesis. The major constitutional risk factor for melanoma is skin color and skin reaction against sunlight exposure. Fair-skinned people who burn only and never tan after exposure of sunlight have relatively higher incidence of melanoma (175). The only environmental risk factor that has been shown relevant to the development of melanoma is exposure to sunlight. A history of exposure to large doses of sunlight sufficient to cause sunburn in childhood is particularly important in the formation of melanoma which could occur many years later (176-178).

Some studies also suggest that recreational activity leading to sunburn in adulthood is also associated with the risk of melanoma (178, 179).

6.1. *p16/CDKN2A* gene disorder in human melanoma

A great advance has been seen in the analyses of molecular mechanism of melanoma carcinogenesis in the recent decade. Linkage analysis of familial melanoma suggested presence of a melanoma susceptibility gene on chromosome 9p21 (180, 181), and a few years later, *p16* or *CDKN2A* gene at the locus was identified as a melanoma susceptibility gene (138, 139). The germline mutations in *p16/CDKN2A* gene have been identified in familial melanomas (182, 183). To date, it is estimated that approximately 20% of melanoma families world-wide are linked to *p16* mutations (184, 185). Some melanoma families are linked to the 9p21 locus without *p16* mutations which suggests that another tumor suppressor gene may lie close to the *p16* locus (186, 187). Alternatively, *p16* may still be involved with gene methylation, mutations in the promoter region of the gene, which may explain the lack of *p16* mutations in 9p21 linked families (188). Melanoma has also been linked to chromosome1p36 by linkage and in loss of heterozygosity (LOH) studies. This locus has been implicated in other cancers as well, suggesting the presence of a new tumor suppressor gene in that locus (189).

The intragenic mutations of *p16* gene in human sporadic melanomas were detected at frequencies of 0-26% (190-195). UV-signature mutations occupy approximately half of the whole mutations, implying that the causal role of UV in *p16* gene mutation is not as clear cut as in the case of *p53* gene mutations in non-melanoma skin cancers.

6.2. Animal models of UV-induced melanoma

Animal models for studies of UV-induced melanoma have been established in fish (Xiphophorus) (196), guinea pigs (197), mice (198-200), and opossum (Monodelphis domestica) (201-204), as well as transgenic mice genetically engineered to develop melanoma (205-209). Setlow and colleagues (210) have analyzed the UV spectrum causative of melanoma in backcross hybrid of Xiphophorus. In this model, single UV exposures to 7-day-old fish induce melanomas readily observable by 4 months.

By analyzing the tumorigenic effect of different wavelengths of UVB, UVA and visible light, they emphasized the importance of UVA irradiation in melanoma development in their model. Opossum (Monodelphis domestica) model is unique in that non-familial melanoma can be induced directly by exposure to UV radiation with no prerequisite genetic manipulations or exposure to chemical carcinogens, and it mimics the etiology of UV-induced melanoma initiation and progression in humans (204).

Recently, two important findings were observed in mouse melanoma models. One is that a single dose of burning ultraviolet radiation to neonates, but not adults, of *HGF/SF*-transgenic mice is necessary and sufficient to induce melanomas with high penetrance (211). This observation provides experimental support for epidemiological evidence that childhood sunburn poses a significant risk of developing melanomas (176-178). Another comes from two distinct experiments using $p16^{INK4a}$ targeted mice which retain normal function of $p19^{ARF}$ gene (212, 213). The results were; (i) $p16^{-/-}$ mice show higher spontaneous tumor formation (sarcoma, lymphoma, melanoma) (212); (ii) higher rate of tumor formation (lymphoma, sarcoma, melanoma, etc) in $p16^{+/-}$ mice compared with $p16^{+/+}$ mice,

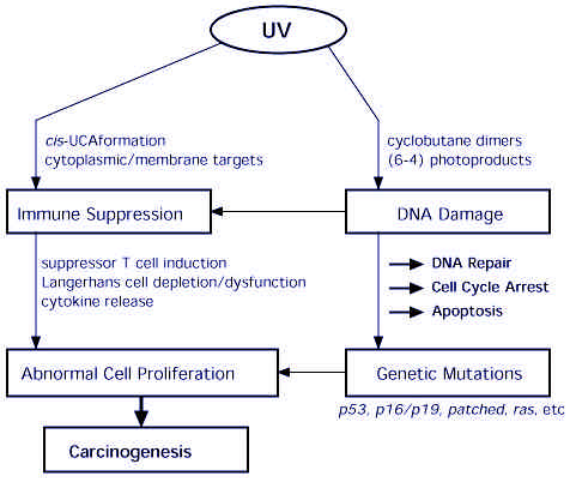

Figure 4. Overview schema of photocarcinogenesis.

and highest rate in *p16*$^{-/-}$ mice, after treatment with DMBA (212, 213); and (iii) frequent metastasis of DMBA-induced melanoma in *p16*$^{-/-}$ mice (213). These data demonstrate that *p16*^{INK4a} acts as a tumor-suppressor gene in mice, and also prove the usefulness of animal models in the analysis of carcinogenesis.

7. SUMMARY

The development of skin tumors is a multistep process involving induction of mutations and escape from immune surveillance (Figure 4). DNA photoproducts induced by UV irradiation may be repaired, lead to cell death, or result in a mutation. Mutations may occur in the repair genes themselves, or oncogenes and tumor suppressor genes. Impairment of immunologic containment of outgrowth of the transformed cell may be initiated *via* DNA damage, photoisomerization of urocanic acid, cell membrane damage or cytokine release. Topical application of sunscreens can block both of the pathways, thus protecting skin cancer development (61, 62, 214). The continuing studies will result in a greater understanding of the genetic and immune suppression pathways mentioned in this review, as well as novel participants in photocarcinogenesis yet to be discovered. These efforts may lead not only to protective measures and adequate treatment of skin cancers, but also to actual repair of pre-existing photodamage, photoimmunosuppression, and thus photocarcinogenesis.

8. ACKNOWLEDGMENT

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