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MECHANISMS OF INDUCTION OF SKIN CANCER BY UV RADIATION

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1. ABSTRACT

Ultraviolet (UV) radiation is the carcinogenic factor in sunlight; damage to skin cells from repeated exposure can lead to the development of cancer. UV radiation has been mainly implicated as the cause of non-melanoma skin cancer, although some role for UV in malignant melanoma has been suggested. The induction of skin cancer is mainly caused by the accumulation of mutations caused by UV damage. Cellular mechanisms exist to repair the DNA damage, or to induce apoptosis to remove severely damaged cells; however, the additive effects of mutations in genes involved in these mechanisms, or in control of the cell cycle, can lead to abnormal cell proliferation and tumor development. The molecular events in the induction of skin cancer are being actively investigated, and recent research has added to the understanding of the roles of tumor suppressor and oncogenes in skin cancer. UV radiation has been shown to induce the expression of the *p53* tumor suppressor gene, and is known to produce "signature" mutations in *p53* in human and mouse skin cancers and in the tumor suppressor gene *patched* in human basal cell carcinoma. The role of UV radiation in suppression of immune surveillance in the skin, which is an important protection against skin tumor development, is also being investigated. The knowledge gained will help to better understand the ways in which skin cancer arises from UV exposure, which will in turn allow development of better methods of treatment and prevention.

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2. INTRODUCTION

The incidence of skin cancer has been increasing at an alarming rate over the past several decades; it is estimated that over one million new cases of non-melanoma skin cancer (NMSC) occur each year in the United States (1). Mortality from NMSC is low, but there is a considerable morbidity with regard to disfigurement and medical costs; the estimated cost per year for treatment of NMSC is over $500 million (2). The incidence of skin cancer is expected to rise further, emphasizing the importance of increased prevention and treatment efforts.

The relevance of sunlight exposure to the skin cancer epidemic is well known. Increased recreational exposure to the sun has been a major contributory factor in the rising skin cancer incidence (1, 2). The incidence of NMSC increases in proportion to cumulative sunlight exposure, such as in those who work outdoors, and in the elderly (1). The skin responds to sun exposure by tanning and skin thickening, which provides some protection from further damage by the ultraviolet radiation. The degree of

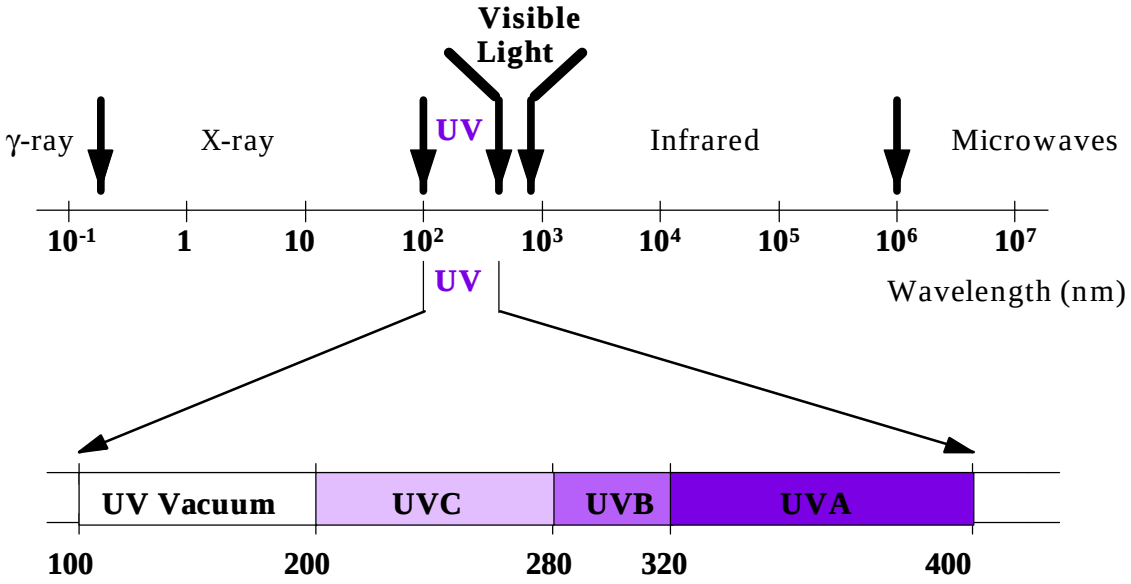

Figure 1. The ultraviolet (UV) component of the electromagnetic spectrum.

pigmentation in the skin and the ability to tan are important factors in the risk of development of skin cancer; the risk of NMSC is highest in people who sunburn easily and suntan poorly, with the least degree of protective skin pigmentation (1). Rising concerns over the depletion of the ozone layer due to human influences, particularly the use of chlorofluorocarbons, has increased the awareness of the dangers of sun exposure and has spurred much research into the ways in which the UV radiation in sunlight causes skin cancer to develop.

UV radiation has mainly been implicated in the development of non-melanoma types of skin cancer. Although the relationship between sun exposure and the incidence of melanoma is not clear, some epidemiological evidence suggests that UV exposure does influence the development of malignant melanoma (1). UV radiation induces cancer in the skin through damaging the ability of skin cells 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 skin cells by forming dimers in DNA between adjacent pyrimidine residues, potentially leading to UV "signature" mutations that can accumulate over time (3, 4). The cell can respond to the damage by repairing the DNA to avoid harmful mutations, or if the damage is too great, by inducing apoptosis to remove potential cancer cells from the population (4). Failure of these pathways can result in the loss of control of cell proliferation and allow a skin tumor to develop, through the inactivation of tumor suppressor genes or the activation of oncogenes. The importance of DNA repair mechanisms in the prevention of skin cancer is evident from the high incidence of skin cancer in patients with diseases that affect such processes, such as xeroderma pigmentosum (XP) (2). Immune surveillance in the skin also plays an important role in protection against the development of skin cancer. UV exposure acts to depress the function of the immune system in the skin, creating a more favorable environment for the development and growth of tumors (reviewed in ref. 5). There has been much research interest in the study of the processes by which skin cancer is induced by UV radiation; the purpose of this article is to provide an overview of recent advances in this area.

3. INTERACTION OF UV RADIATION WITH THE SKIN

3.1 The nature of the UV spectrum.

Sunlight is composed of a continuous spectrum of electromagnetic radiation that is divided into three main regions of wavelengths: ultraviolet, visible, and infrared. This spectrum is diagrammed in figure 1. 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 through being absorbed by the ozone layer of the atmosphere, although some accidental exposure occurs 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 from exposure of the skin and eyes. Wavelengths 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 weakly cracinogenic. Recent studies 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 (6, 7). Interestingly, UVA radiation has been shown to be involved in the development of melanoma in fish (8, 9).

3.2. UV radiation causes DNA damage and mutations.

UV radiation is absorbed by DNA maximally from 245-290 nm (10); UV is able to create mutagenic photoproducts or lesions in DNA between adjacent pyrimidines in the form of dimers. These dimers are of two main types: cyclobutane dimers between adjacent thymine or cytosine residues, and pyrimidine (6-4) pyrimidone photoproducts between adjacent pyrimidine residues. Cyclobutane dimers 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 (3). 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 T-C and C-C residues (10). Cyclobutane dimers are produced overall three times as often as (6-4) photoproducts, although the ratio depends on the DNA sequence and the chromatin environment (10). Both lesions occur most frequently in runs of tandem pyrimidine residues, which are known as "hot spots" of UV-induced mutations (3). Although both lesions are potentially mutagenic, the cyclobutane dimer is believed to be the major contributor to mutations in mammals (10); the (6-4) photoproducts are repaired much more quickly in mammalian cells (11).

In addition to their mutagenic properties, UV-induced pyrimidine dimers may interfere with other important processes of cell cycle regulation involving DNA. Recently, it has been shown that T-T cyclobutane dimers in promoter sequences can strongly inhibit transcription factor binding (12). In addition, pyrimidine dimers can block transcription elongation when present on the transcribed strand (13), and transcribed sequences, especially of active genes, are repaired more rapidly, while promoter sequences are repaired much more slowly (14, 15). UV-induced photodamage to DNA may therefore be an important source of inhibition of transcription factor binding, which could contribute to its carcinogenic effects. Other photoproducts in addition to cyclobutane dimers and (6-4) photoproducts are produced by UV radiation, but these are produced at much lower frequencies and represent less than one percent of all UV-induced photodamage (3). These lesions include pyrimidine monoadducts, purine dimers and an adjacent A-T photoproduct (10). Little is known about the mutagenicity of these lesions (10).

If not repaired, UV-induced DNA lesions can lead to permanent mutations in the DNA sequence. These mutations are in the form of CT and CCTT transitions, known as UV "signature" mutations. Various deletions, insertions, and multiple base changes also can occur. The “A rule” has been proposed to explain how UV signature mutations arise from the DNA lesions (16). According to the A rule, the DNA polymerase places A residues by default where the correct base(s) is not indicated. A mutation is then created upon DNA replication of the strands containing base pair changes. The T-T cyclobutane dimers should not result in mutations; because A normally is paired with T, no mutation results from insertion of A residues by default opposite the dimer. In the C-C cyclobutane dimer, a CCTT transition occurs; two A residues are placed opposite the dimer by default in the place of two 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 (3). A CT mutation occurs because an A residue is placed opposite the C residue by default.

3.3. Mutations can lead to loss of cell cycle control and carcinogenesis

UV radiation is a complete carcinogen; it can act alone as both initiator and promoter in carcinogenesis. UV can also act as a promoter with initiating events inside the cell, such as DNA mutations arising from DNA polymerase incorporation errors, depurination, deamination of 5-methylcytosine, or oxidative damage from free radicals (3). UVA, while a complete although far less efficient carcinogen, can act as an initiator with UVB as promoter in skin carcinogenesis; this was first suggested in studies in which mice were irradiated with combinations of UVA and UVB (17, 18). UVA alone was relatively ineffective as a carcinogen, but it increased the carcinogenic effects of UVB when mice were irradiated with both. UVA also increased the carcinogenic effect of UVB when mice were exposed to it several months after the initial UVB exposure (19, 20); these studies were meant to be analogous to the popular tanning habit in which sunbathing was followed by UVA solaria treatments. Recently, UVA was found to have less of an impact on the cell cycle than UVB radiation in epidermal cells of exposed mice (21), further suggesting differences in carcinogenic effectiveness between UVA and UVB.

Mutations in genomic DNA can lead to carcinogenesis through changes in the function of genes that influence cell growth. The complex series of events in carcinogenesis involves three stages: initiation, promotion, and progression (3). Mutations in the DNA may act as initiating events. These may remain dormant for a number of years, until exposure to a promoting agent occurs. 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 that 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 (3). The transforming mutations are usually in tumor suppressor genes or oncogenes, or other genes that are involved in the regulation of cell proliferation.

3.4. DNA repair and apoptosis are defenses against carcinogenesis

The cells of the skin contain protective mechanisms to prevent DNA damage from UV and other sources from resulting in tumor formation. One of these mechanisms is growth arrest followed by DNA repair, and the other is cell death by apoptosis. Both of these mechanisms prevent the transmission of mutations to daughter cells that can lead to transformation and carcinogenesis. Failure of these pathways can result in abnormal cell proliferation. DNA repair processes are very important in the prevention of skin carcinogenesis. This importance is evidenced by the extreme susceptibility of patients with genetic diseases impairing DNA repair processes to skin cancer development. In XP, an autosomal recessive disorder caused by defects in DNA repair and synthesis, the thymine dimers occurring in DNA from UV

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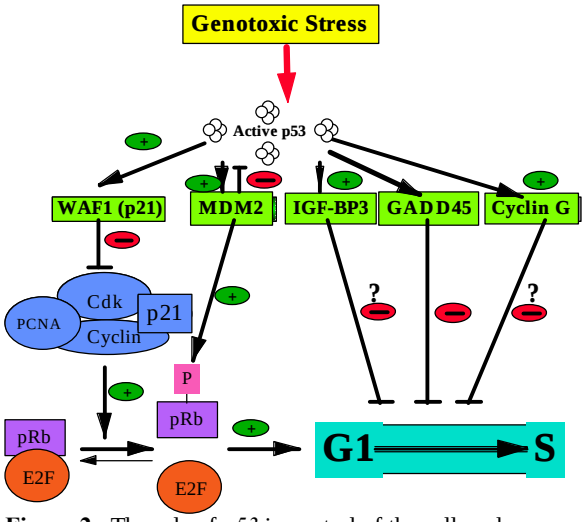

Figure 2. The role of *p53* in control of the cell cycle

exposure of the skin fail to be repaired (29). These defects cause an extreme sensitivity to UV radiation and a high risk of development of non-melanoma skin cancers (3). Similar UV sensitivity and skin cancer risk is seen in Cockayne Syndrome, in which nucleotide excision repair is impaired (30).

Upon DNA damage by UV or another agent, the cell cycle may be arrested at at least two checkpoints: at the G1/S phase before DNA replication, or at the G2/M phase before chromosome segregation (22); UV radiation is known to influence the activities of genes active in cell cycle control and growth arrest (23-27). Upon arrest, DNA repair pathways are activated which repair the damage and return the cell to the normal state. If the damage is too severe and cannot be repaired, apoptotic pathways are activated which kill the cell and prevent the formation of a tumor cell population by loss of cell cycle control. DNA damage induced by UVB has been shown to induce apoptosis in human keratinocytes; recently, it has been shown that high doses of UVB irradiation causes the cells to undergo apoptosis, where low doses result in DNA repair (28). Intermediate doses caused a mixture of cells undergoing apoptosis or repair. These observations correlated with differences in the perinuclear versus nuclear localization of the p53 protein, suggesting that p53 plays a role in both repair of UV-damaged DNA and induction of apoptosis.

4. TUMOR SUPPRESSOR GENES AND ONCOGENES IN SKIN CANCER

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

Table 1. Genes involved in ultraviolet radiation-induced skin cancer

Gene	Function	Location
p53	Tumor suppressor; induction of DNA repair and apoptosis	Perinuclear/nuclear
patched	Tumor suppressor; regulation of cell proliferation/differentiation signaling by hedgehog proteins	Transmembrane
ras, (H-,K-,N-)	Protooncogenes; signal transduction by GTP binding	Cell membrane

more susceptible to further mutations (31). Activation of oncogenes is dominant in that a change in only one copy of the gene is required to have an effect. Protooncogenes, 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 (3). Carcinogenesis can result either from overexpression of the normal gene product or from expression of a mutant or altered gene product. Several genes have been extensively studied that have important roles in skin carcinogenesis. These include the tumor suppressor gene *p53* and the *ras* oncogenes. Other candidates are currently being investigated; one such gene is *patched*, which may act as a tumor suppressor. Information about these genes is summarized in table 1 (see also figure 5).

4.1. The *p53* tumor suppressor gene

The *p53* tumor suppressor gene codes for a DNA-binding protein and mutations or loss of p53 plays a key role in the process of carcinogenesis. It is the most frequently altered gene in human cancers (>50%). The human *p53* gene is localized on the chromosome 17 (17p13) and contains 11 exons spanning 20 kilobases. The mouse *p53* gene is localized on chromosome 11 and also contains 11 exons (32). Recent work indicates that the *p53* protein is a central element in fundamental cellular processes, including gene transcription, repair of DNA damage, control of the cell cycle, genomic stability, chromosomal segregation, senescence and apoptosis (33).

4.1.1. p53 and the cell cycle

The cell cycle is under a positive and direct control of the cyclin-dependent kinase family (Cdk) and their regulatory subunits. Cdks control the cell cycle in part by hyperphosphorylation and inactivation of negative regulators of the cell cycle, such as the Rb protein responsible for susceptibility to retinoblastoma, and associated proteins p107 and p130. It has been proposed that after genotoxic stress, the accumulation of p53 protein induces a cell cycle arrest at the G1 phase; this arrest allows the repair of DNA damage before its replication in the S phase (34, 35). However, it has been found that DNA damage might induce an irreversible arrest of normal human fibroblast mitosis (36). The function of p53 in control of the cell cycle is shown diagrammed in figure 2. Accumulation of active p53 induces the expression of different proteins that regulate the cell cycle. p21 (encoded by the*Waf1* gene, called also *Cip1*, *Sdi1* or *Pic1*) inactivates the Cdk-Cyclin complex by forming a Cdk2/A or E Cyclin/Proliferating Cell Nuclear Antigen/Waf1 complex.

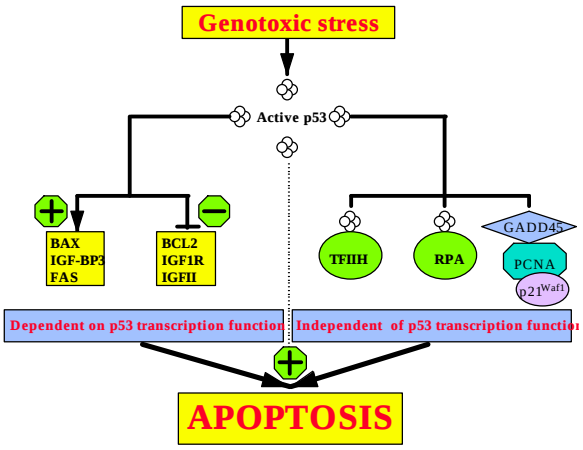

Figure 3. The role of p53 in apoptosis

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 (37, 38). p21 can also be induced by a p53-independent pathway (39). Although it is known that Gadd45, IGF-BP3 and G or D1 Cyclin G, and likely other proteins, can induce cell cycle arrest, the molecular mechanisms are not yet fully understood.

4.1.2. *p53* and apoptosis

p53 can induce apoptosis (programmed cell death) by two independent mechanisms, as shown in figure 3. One pathway depends on the function of p53 as a transactivator of transcription by upregulating the expression of Bax, IGF-BP3 and Fas proteins, and by downregulating the expression of Bcl2, IGF-1R and IGFII. The up- and down-regulation of these proteins, respectively, has been correlated with the induction of programmed cell death processes (reviewed in 33). The second pathway is independent of the *p53* transcriptional function but is dependent on *p53* protein-protein interactions: *p53* protein can bind to cellular proteins involved in DNA synthesis such as replicating protein antigen (RPA) (40), and in DNA repair such as TFIIH, including xeroderma pigmentosum group B (XPB) and D (XPD) DNA helicases, p62 and topoisomerase I (41-42).

4. 1. 3. Inactivation of the *p53* gene

Some mutations in the *p53* gene frequently lead to the production of a protein that cannot bind specifically to DNA, and therefore loses its transactivation activity (43). However, it has been found that some mutations such as 175Pro, resulted in the loss of the ability of the p53 protein to induce apoptosis in certain cells, but these cells are still able to induce growth arrest (44). Three types of genetic alterations may affect the function of the *p53* protein: (i) Partial or total deletion of the gene, which is sufficient to abrogate the tumor suppressor function, contributing to the development of tumors, as has been shown in the *p53* knockout mice (45); (ii) A dominant negative effect of some critical mutations which can suppress the function of the wild type p53 protein through inhibition of its DNA binding activity and thus its transactivation function (46). It has been reported that a transgene carrying one mutated allele, which codes for mutant *p53* protein 135Val, results in loss of tumor suppressor function and contributes to increased tumorigenicity by a dominant negative effect, probably by forming inactive hetero-oligomers with wild type protein, generated from the normal allele (47). It has been shown recently that all hotspot mutations in the *p53* gene generate mutant proteins capable of inhibiting the transcriptional activity of the wild type protein (48). (iii) Certain "gain of function" mutants of p53 acquire an oncogenic potential. For example, the 175His *p53* protein was characterized by the loss of tumor suppressor function, a dominant negative effect gain of function (47) and the ability to increase the tumorigenic and metastatic potential of cells missing the wild type p53 protein (49).

Mutational analysis of the *p53* gene provides a unique opportunity to investigate the etiology, epidemiology, and pathogenesis of human cancer (50-52). It is the most commonly mutated gene in human cancers (50). To date, more than 5000 mutations have been identified and classified that are available electronically through the network databases (50). Interestingly, the sites of the point mutations are nonrandom, with more than 90% occurring in highly conserved regions of the middle third of the gene (52).

4.1.4. *p53* mutations in UV-induced skin cancers

Analysis of human skin cancers and UV-induced mouse skin cancers for *p53* mutation have provided new insights into the molecular mechanisms by which UV radiation induces skin cancer. The *p53* gene has been found to be mutated at a high frequency in human (53-55) and mouse UV-induced skin cancers (56-58). Most hotspot mutations detected in human and mouse UV-induced skin cancers inactivate critical p53 functions. Over 90% of human cutaneous SCCs and about 56% of human BCCs contain unique mutations at dipyrimidine sites. A number of studies have found *p53* mutations in precancerous actinic keratosis and sun-damaged skin (59, 61). An interesting aspect of UV-induced mutations in the *p53* gene in human skin cancers is that they are frequently CT or CCTT base substitutions at dipyrimidines sites. These types of *p53* mutations are not commonly present in human internal cancers or in mouse skin cancers induced by chemical carcinogens. In XP tumors, including BCC, SCC and sarcoma, 60% of the *p53* mutations detected were tandem CCTT transitions and occurred at sites previously identified as hotspots (62, 63). Because of the unique nature of CCTT tandem mutations, they are termed UV "signature" mutations (64). Most of the mutations detected in XP skin tumors occurred at the non-transcribed strand implying a preferential repair of UV-lesions on the transcribed strand in human tissues.

UV-induced mouse skin cancer provides an ideal model to investigate the molecular mechanisms involved in the multistep process of carcinogenesis. Analogous to human skin cancers, UV-induced mouse skin cancers also display *p53* mutations (56-58), although the frequency of mutations and the exons in which they occur differ among mouse strains, for reasons that are not yet clear. For example, in our study, *p53* mutations were detected at 70-100% frequency in UV-induced SKH-hr1 and C3H mouse

skin tumors, respectively (56, 57). In contrast, 20% of SCC from SKH-1/hr hairless mice and 50% of SCC from BALB/c mice exhibited *p53* mutations in another study (58). Nonetheless, most of the mutations detected in UV-induced mouse skin tumors were CT and CCTT transitions at dipyrimidine sites, like those found in human skin cancers. Another important finding from these two studies is the fact that most of the *p53* mutations were located on the non-transcribed strand, which is in accordance with the principles of preferential DNA repair. In other words, transcribed strands are repaired preferentially over non-transcribed strands (65, 66). Several of the UV-induced C3H mouse skin cancers contained multiple mutant *p53* alleles (56) suggesting that mutant *p53* alleles with single base changes were targets of secondary mutation events, perhaps due to the continued exposure of UV radiation during tumor progression.

4.1.5. *p53* mutations arise early during UV skin carcinogenesis

The progression of cancer from its initial benign stages to malignancy is generally through stages exemplified by the adenoma-carcinoma sequence in colon cancer (67). These morphologically defined stages provide milestones for recording the timing of *p53* mutations. In colon cancer, for example, *p53* mutations are generally a late event marking the progression from the late adenoma to carcinoma stages (67). However, in the case of skin cancers where UV is the etiological agent, mutations in the *p53* gene appear to have an earlier onset (57, 59, 60, 68). Using sensitive PCR- and ligase -chain reaction based methods, hotspot tandem mutations at codons 245 and 247/248 were detected in UV-exposed normal human skin cell cultures and normal human skin biopsies (69, 70). In addition, in a case-control study conducted recently in Australia, Ouhtit *et al*. (71) found a correlation between mutation frequency in normal skin biopsies (tandem CCTT transitions) at codons 247/248 of the p53 gene and the risk of BCC. A study by Ziegler *et. al.* (60) revealed *p53* mutations in 60% of all AK samples examined, and a high proportion (89%) of them were of the UV "signature" type. More importantly, when normal skin flanking the AKs was examined, the frequency of *p53* mutations was exceedingly small (less than 10^{-3}). Whole mount preparations of sun-exposed and sun-shielded normal human skin revealed clonal population of p53-mutated cells in the sun-exposed skin, arising from the dermal-epidermal junction and from hair follicles (59). Recently, a combination of immunohistochemistry and sequencing analyses has revealed the presence of *p53* immunopositive clonal patches that contain predominantly “UV signature” mutations in normal human sun-exposed skin (59, 75). Clones were both more frequent and larger than in sun-shielded skin, indicating that UV radiation acts as an initiator and a promoter by favoring the clonal expansion of *p53*-mutated keratinocytes (76).

Berg *et al*. (72) analyzed UVB-irradiated mouse skin for the presence of cells expressing mutant p53 protein and found several clusters of cells in the epidermis that reacted with an antibody specific for mutant p53 protein after 17 or 30 daily UVB exposures, which would cause skin tumors around 80 or 30 weeks, respectively. Such clusters expressing the mutant p53 protein persisted in the skin for at least 56 days after UVB irradiation. *p53* mutations have also been detected in UV-irradiated mouse skin months before the gross appearance of skin tumors. The *p53* mutations in mouse skin arose as early as the 4th wk of UV-irradiation, and the frequency of *p53* mutations increased progressively and reached 50% at 12 wk of chronic UV exposure (57). In addition, around week 16, when the *p53* mutation frequency reached maximum, the first mouse developed a skin tumor, and 50% of the mice developed skin cancer by wk 25. These results suggest that *p53* mutations arise well before skin cancer development and that they can serve as a surrogate early biological endpoint in skin cancer prevention studies.

4.2. The *patched (ptc)* gene is a tumor suppressor in humans

4.2.1. *ptc* gene activity is conserved in Drosophila and vertebrates

The *ptc* gene was recently cloned in Drosophila by two different groups (77, 78). The predicted novel protein has 1286 amino acids and is 143 kilodaltons (kDa) in molecular weight; it is also predicted to be an integral membrane protein. In Drosophila, *ptc* is a segment polarity gene; these genes act to control the number and identity of the body segments in a developing Drosophila embryo. Development of each segment is controlled by a system of carefully regulated intercellular signals for proper growth and differentiation; the expression patterns of *ptc* and other genes act to specify the cell identities that eventually determine the segmental pattern.

The *ptc* gene controls the activities of genes that drive cell growth and differentiation by repressing their activities in cells where *ptc* is expressed. This is accomplished by opposing the function of the *hedgehog* (*hh*) gene, which encodes a secreted signaling protein that induces cell growth and differentiation. Cells receiving the *hh* signal activate transcription of target genes that result in a growth response; the transcription of *ptc* is also induced in these cells. Ptc responds to *hh* signals from adjacent cells by blocking transcription of *hh* target genes; these include *ptc* itself, in a negative autoregulatory function. Transduction of the *ptc* signal pathway involves the *smoothened* (*smo*) gene, another membrane protein that has characteristics of G-protein receptors; it acts downstream of or parallel to *ptc* (79). An additional gene further downstream in the pathway is *cubitus interruptis* (*ci*), which encodes a zinc finger protein that activates expression of *hh* target genes and is part of a negative feedback loop with *ptc* (80). Normally, there exists a balance between expression of the opposing activities of *hh* and *ptc* that determines the level of target gene expression. The *hh* target genes are ectopically activated in *ptc* mutants (81), so that abnormal cell growth may be expected to occur when hh signaling goes unchecked. Current research is underway in order to elucidate the molecular details of this important developmental pathway.

Murine (81) and human (82, 83) homologs of the *ptc* gene have recently been cloned. The mouse and human *ptc* genes are very similar, with 96% amino acid sequence identity, compared to 40% similarity to Drosophila *ptc*. The gene structure spans over 32 kb of genomic DNA and contains at least 23 exons; the open reading frame has 1447 amino acids. The N- and C-termini of the mouse and human genes

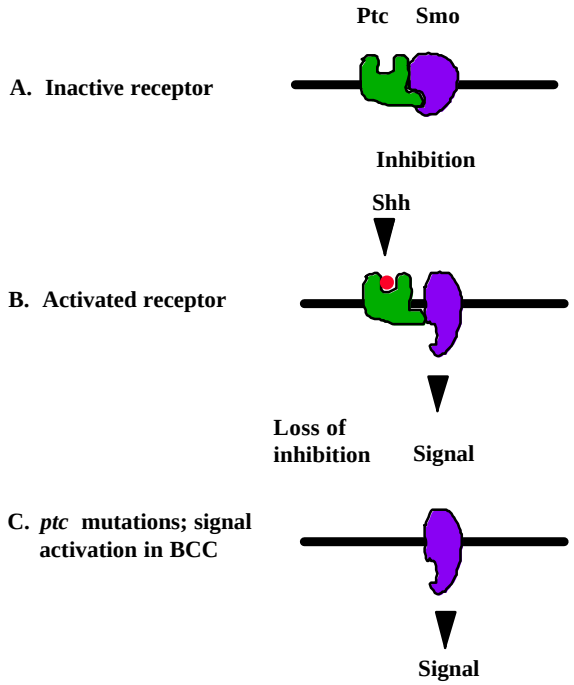

Figure 4. Model for Ptc signaling and a possible role for *ptc* mutations in BCC

correspond. Murine *ptc* was found to be transcribed in many tissues near cells producing either *Sonic* or *Indian hedgehog*, signaling proteins analogous to *hedgehog* in Drosophila. Ectopic *Sonic hedgehog* (*Shh*) expression in the mouse central nervous system also induced *ptc* transcription (81). Homologs of *hh* have been found in other vertebrates, including humans (84). These results suggest that the ptc signaling pathway is conserved in Drosophila and vertebrates (81), which could greatly aid in understanding of the cellular pathways involved in the function of *ptc* as a tumor suppressor in humans. The Ptc protein was recently shown to act as a receptor for Shh, in the mouse (85). Hh was demonstrated to bind to Ptc with high affinity, but not to the recently cloned vertebrate homolog of Drosophila Smoothened (vSmo); this was contrary to previous research suggesting that Smo was the receptor for Hh in Drosophila (79). Also, Ptc was found to form a physical complex with vSmo, suggesting that vSmo could be linked to Ptc in the signaling pathway, and that Shh, Ptc, and vSmo form a physical complex (85). A diagram depicting these findings is shown in Figure 4.

These results were important in the elucidation of the Ptc signaling pathway in the mouse; the same concepts may apply in humans as well. The possible application of information gained from the Drosophila and mouse systems could be especially important in light of the recent discovery that ptc acts as a tumor suppressor in basal cell carcinoma in humans.

4.2.2. *ptc* mutations and nevoid basal cell carcinoma syndrome

The human *ptc* gene was found to colocalize with the map location of nevoid basal cell carcinoma syndrome (NBCCS) on chromosome 9, at 9q22.3 (82). NBCCS, also called basal cell nevus syndrome or Gorlin's syndrome, is a rare autosomal dominant disorder characterized by multiple BCCs that appear at a young age. NBCCS patients are very susceptible to the development of these tumors; in the second decade of life, large numbers appear, mainly on sun-exposed areas of the skin. This disease also causes a number of developmental abnormalities, including rib, head and face alterations, and sometimes polydactyly, syndactyly, and spina bifida. They also develop a number of tumor types in addition to BCCs: fibromas of the ovaries and heart, cysts of the skin and jaws, and in the central nervous system, medulloblastomas and meningiomas. Studies of NBCCS patients show that they have both genomic and sporadic mutations in the *ptc* gene, suggesting that these mutations are the ultimate cause of this disease (86). The number, distribution, and early onset of the BCCs suggested that *ptc* may be a tumor suppressor gene (87).

The deregulation of the ptc signaling pathway may be a general feature of basal cell carcinomas caused by *ptc* mutations. Consistent overexpression of human *ptc* mRNA has been described in tumors of familial and sporadic BCCs, determined by *in situ* hybridization (88). Mutations that inactivate ptc may be expected to result in overexpression of mutant Ptc, because ptc displays negative autoregulation. It is also plausible that mutation of ptc might lead to tumorigenesis through overexpression of Hedgehog proteins, given the role of the hh gene described in Drosophila. That SHH has a role in tumorigenesis in the mouse has been suggested by recent research in which transgenic mice overexpressing SHH in the skin developed features of NBCCS, including multiple BCC-like epidermal proliferations over the entire skin surface, after only a few days of skin development (89, 90). A mutation in the Shh human gene from a BCC was also described (89); it was suggested that Shh or other Hh genes in humans could act as dominant oncogenes in humans.

4.2.3. UV-induced *ptc* mutations play a role in BCC development

NBCCS patients develop many BCCs early in life, mostly on sun-exposed sites; the degree of sun exposure and skin pigmentation also influence the age of onset and number of skin tumors (91). These observations suggest that UV exposure is involved in the development of the BCCs in these patients, who may be more susceptible due to the *ptc* mutations. Most genomic *ptc* mutations found in NBCCS patients lead to a truncated protein (92). A mutation in a sporadic BCC from an NBCCS patient, a GCAT transition characteristic for UV-induced mutations, caused a stop codon also leading to premature termination of the protein (86). Skin fibroblasts from NBCCS patients have been shown to have increased sensitivity to killing by UVB radiation, which was not due to defects in DNA repair (93). These results suggested a connection between the genetic susceptibility of NBCCS patients to skin cancer (due to *ptc* mutations) and sensitivity to UV radiation.

Sporadic *ptc* mutations have been described in BCCs from otherwise normal individuals, some of which are UV-signature mutations. In one recent study of sporadic BCCs, five UV-signature type mutations, either

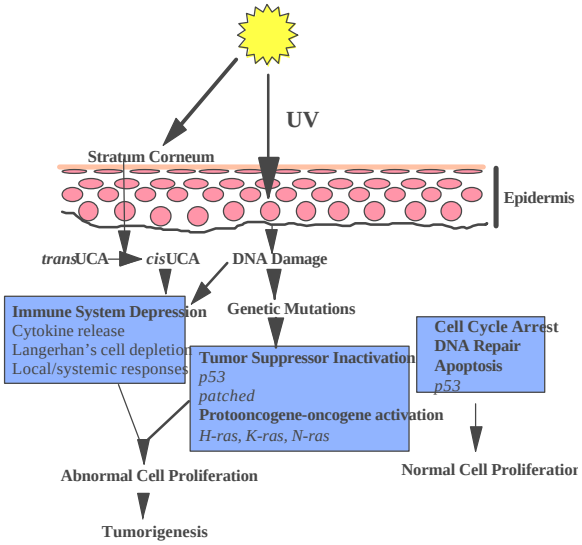

Figure 5. A model for induction of skin cancer by UV

CT or CCTT changes, were found out of fifteen tumors determined to contain *ptc* mutations (94). Another recent analysis of sporadic ptc mutations in BCCs and neuroectodermal tumors revealed one CT change in one of three *ptc* mutations found in the BCCs (95).

4.3. The role of *ras* oncogenes in skin cancer

The protooncogenes H-*ras*, K-*ras*, and N-*ras* encode 21-kDa proteins that share about 70% sequence homology (96). Located at the inner cell surface, ras proteins participate in signal transduction by binding to GTP. Signals involved in growth control, such as binding of activators to cell surface receptors, are transferred from the cell surface to the nucleus. Recent study has added to the understanding of this signalling pathway (97, 98). Mutations in the *ras* genes that cause continuous activation have been found mainly in codons 12, 13 and 61 for all members of the *ras* family (99). The *ras* genes are active when bound to GTP; mutations may cause activation by reducing the rate of hydrolysis of GTP to GDP, as has been found for a mutation to valine in codon 12 (100).

Mutations that activate *ras* genes occur less frequently in human skin cancers than mutations in the *p53* gene. *ras* mutations have been reported to occur at 10-40% frequency in human skin cancers (reviewed in 101). However, skin cancers from XP patients harbor mutation in *ras* genes at a high frequency (53%) as compared to skin cancers from the general population which occured at 22% frequency (102). In contrast to this report, Ishizaki *et al.*, (103) and Sato *et al.* (104) found a relatively low frequency of *ras* mutation in skin cancers from XP patients. This discrepancy could be due to differences in XP complementation groups between the Japanese and European patients. Analogous to human skin skin cancers, mutations in N-*ras* codon 61 of the N-*ras* gene have been reported in UV-induced mouse skin cancers (105).

5. A MODEL FOR UV-INDUCTION OF SKIN CANCER

The epidermal keratinocytes of the skin are the most susceptible to damage from UV exposure, due to their localization relative to the skin surface; therefore, most skin cancers in humans arise from the epidermis. The cellular and molecular events that contribute to the development of UV-induced skin cancer is a complex process involving at least two distinct pathways that interact or converge to cause skin cancer (Figure 5). One pathway involves the action of UV on target cells (keratinocytes) for neoplastic transformation, and the other involves the effects of UV on the host's immune system. There is evidence to indicate that UV-induced DNA damage plays an important role in both pathways. In the normal human epidermis, cells are constantly turning over, about once a month. During this period, stem cells in the basal layer undergo cell division, and the keratinocytes differentiate into squamous cells producing keratin and other proteins, and finally desquamate (106, 107). Chronic exposure to sunlight causes damage to the skin including erythema, edema, hyperplasia, formation of sunburn cells, photoaging, suppression of the immune system and skin cancer. Some of the molecular events that occur in cells following UV exposure are, DNA damage, induction of *p53* and *p53*-regulated proteins, cell cycle arrest, errors in DNA repair and/or replication, mutations in *p53* and other genes (Figure 5). In addition, UV-induced DNA damage causes the production of immunosuppressive cytokines and some types of immunosuppression that may contribute to the emergence of skin cancer.

The *p53* tumor suppressor gene appears to be one of the key UV-responsive genes, and mutations in this gene is thought to initiate the process of skin carcinogenesis. Jonason *et al*, (59) used a scanning confocal microscope to reconstruct a three-dimensional immunofluorescent cone of mutant *p53*-positive keratinocytes from an epidermal whole mount of sun-exposed skin. They found the apex of the cone at the dermal-epidermal junction, indicating the location of initiating stem cells and suggesting *p53* mutation as a marker for the progeny of a single stem cell. It seems likely that UV radiation targets particularly those original cells at the basal layer and damages the *p53* gene by inducing cyclobutane pyrimidine dimers and (6-4) photoproducts at sites of adjacent pyrimidines. Photodamage to DNA induces the expression of *p53* protein and cause cell cycle arrest, thereby permitting the repair of the damage. If the DNA damage is too much and left unrepaired, the cell undergoes apoptosis. In addition, UV-induced pyrimidine dimers may inhibit transcription factor binding thereby interfering with other important DNA-dependent processess (12). Thus, UV radiation may inhibit *p53* binding and transcriptional activities leading to the deregulation of its function, such as DNA repair and apoptosis (108-111). The deregulation of *p53* functions and slow repair of UV-induced photoproducts at particular codons (112) may lead to the induction and accumulation of *p53* mutations, particularly C→T or tandem CC→TT transitions thereby initiating the molecular process of carcinogenesis. The location of these mutations in the *p53*

gene in human NMSC is not random; there are 9 known hotspot mutations in the *p53* gene that result in amino acid sequence changes (53, 54) inactivating the critical functions of the p53 protein. This is taken as evidence that these mutations do indeed offer some selective growth advantage to the initiated cells. Therefore, chronic exposure to sunlight can cause massive suicide of normal cells containing wild-type p53 protein by apoptosis (cellular proof reading) leaving more space for the *p53*-mutant cell to clonally expand and replace the dying sunburned cells (promotion) thus leading to abnormal precancerous cells. Sunlight is, therefore, acting as an initiator and a promoter; this phenomenon has been termed "double punch of sunlight" (76). However, even though 4% of the normal sun-exposed skin cells have *p53* mutations, very few develop into actinic keratoses (AK) or cancer. In addition, AK, which is a premalignant lesion have a high frequency of UV-specific *p53* mutations and allelic loss of many genes, including *p53*, rarely (1:1000) progress to SCC (106).

It appears that this is only a part of the story. Based on the hypothetical model of the multistep process of carcinogenesis, it has been suggested that alterations in two different gatekeeper genes may lead to cancer development (113). The development of NMSC may then involve *p53* as the first gatekeeper gene (114), and the predisposed clones described above may acquire an alteration of another gatekeeper gene. The tumor suppressor gene *ptc* is suspected to play such a role in UV-induction of BCC (94, 95), and the ESS1 gene localized on chromosome 9q31 in the induction of SCC (115).

UV radiation plays a dual role in the development of skin cancer. On one hand, UV radiation induces genetic alterations in keratinocytes, leading to their neoplastic transformation. On the other hand, UV radiation depresses the immune responses in the skin, which can permit the growth of emerging tumors produced by the effects of UV-induced DNA damage (reviewed in ref. 5). Studies by Kripke and coworkers (5) have shown that a majority of UV-induced mouse skin tumors are highly antigenic in that they are rejected when transplanted into normal syngeneic hosts, but they grow progressively in mice exposed to subcarcinogenic doses of UV. The systemic suppression results from the induction of suppressor T cells, either by damaged Langerhans cells or inflammatory macrophages that enter the skin following UV exposure (5). Another mechanism may be the release of soluble factors at the site of UV irradiation that act to suppress the immune system (reviewed in 116). These factors include cytokines such as IL-10, TNF-alpha, and IL-1alpha that can suppress the immune system and prevent T-cell mediated responses; these are known to be secreted by keratinocytes after UV damage (116). In addition, UV irradiation can also convert normal skin chromophores into agents that are immunosuppressive, such as the conversion of *trans*-urocanic acid to *cis*-urocanic acid (117). Analogous to the model, immunosuppressed patients have a higher risk for development of NMSC, as in renal transplant patients who develop an increased number of SCCs, BCCs, virus-associated skin tumors, and keratoacanthomas, mostly on sun-exposed areas (118, 119).

Recent studies have shown that UV-induced DNA damage plays an important role in the suppression of specific immune responses. In studies to investigate the effects of enhanced repair of UV-induced pyrimidine dimers on UV-induced immunosuppression, it was found that topical application of liposomes containing T4N5 endonuclease or DNA photolyase to mouse skin following UV irradiation abrogated UV-induced suppression of contact hypersensitivity (reviewed in 120). Thus, carcinogenesis by UV radiation appears to operate by the distinct mechanisms of genetic mutation and immunosuppression.

6. PERSPECTIVE

Recent research has aided in the understanding of the mechanisms by which UV radiation induces skin cancer. Work in other systems, including insect as well as mammalian systems, has added useful information for the elucidation of the complex effects of UV radiation on humans. Continued efforts will result in a greater understanding of the genetic and immune suppression pathways involved in the function of tumor suppressor and oncogenes that are now known, as well as genes (caretakers and gatekeepers) yet to be discovered. The efforts of research in skin cancer may help to increase overall awareness of the harmful effects of UV exposure and result in better methods of skin cancer prevention and treatment.

7. ACKNOWLEGMENTS

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