Environmental Skin Cancer: Mechanisms, Models and Human Relevance

This meeting was held in Cleveland, OH, on October 16 and 17, 1992, to provide an opportunity for clinicians and experimental scientists working in cutaneous chemical, viral, and photocarcinogenesis to discuss the current status of knowledge regarding the mechanisms of the induction of cancer in the skin. Further aims of the meeting were to enhance interest in research using the skin for the study of carcinogenesis and to discuss the relevance of animal studies to the pathogenesis of human malignancy. The symposium was attended by over 150 speakers and participants and was sponsored by the Skin Diseases Research Center, University Hospitals of Cleveland, and Case Western Reserve University. The meeting was also funded by American Cancer Society of Cuyahoga County and the National Institute of Arthritis, Musculoskeletal and Skin Diseases. The highlights of the meeting were a keynote lecture, seven scientific sessions, and two panel discussion sessions which emphasized the current status of research accomplishments in cutaneous carcinogenesis and the future research needs in this field.

Keynote Lecture

The keynote lecture was given by Dr. Stuart H. Yuspa, Chief, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, Bethesda, MD, and was titled "Biochemical and Molecular Changes Relevant to the Pathogenesis of Skin Cancer." Dr. Yuspa indicated that we are on the threshold of an era in which we are beginning to understand, at the molecular level, the sequential order of various pathways leading to skin cancer induction.

He indicated that the introduction of the ras oncogene into normal mouse epidermal cells by transduction with a defective retrovirus converts the cells into a benign neoplastic phenotype. The neoplastic phenotype can be reproduced pharmacologically by costimulation of the epidermal growth factor receptor and phospholipase C activity in cultured normal keratinocytes. PKC contributes to induction of the benign phenotype since the phenotype can be reversed in vitro by PKC inhibitors. Data were also presented to show that progression from the benign to the malignant phenotype is marked by predictable changes in keratin gene expression, redistribution of integrins and loss of the homotypic cell adhesive phenotype can be reproduced pharmacologically by costimulation of the epidermal growth factor receptor and phospholipase C activity in cultured normal keratinocytes.

Session I: Mechanisms in Skin Cancer

Dr. Philip Hanawalt, Stanford University, Stanford, CA, discussed gene-specific DNA repair and its relevance for human skin carcinogenesis mechanisms, especially the differences that occur in patients with inherited predisposition to cutaneous malignancy such as xeroderma pigmentosum and Cockayne's syndrome. He emphasized that during multistage carcinogenesis in the skin, structural changes in DNA may be due to replication of DNA at unique sites in which damage has occurred in or near protooncogenes and/or tumor suppressor genes. A predominant source of DNA damage in the skin is solar UV radiation which causes a number of lesions, of which the principal ones are cyclobutane pyrimidine dimers and 6-4 pyrimidine- pyrimidone photoproducts. Such lesions are usually repaired but the efficiency of repair is much higher in expressed genes than in silent regions of the genome. He reported that techniques have been developed in his laboratory for measuring DNA lesions and their repair in specific DNA sequences and provided evidence that for some types of damage, such as cyclobutane pyrimidine dimers, repair is highly selective for the transcribed DNA strands in active genes. He suggested that sunlight-induced damage in certain sensitive target(s) in silent domains of the genome may be particularly important in the etiology of skin cancer. He emphasized that we now need to learn the nature of the relevant genomic domains and the types of genetic changes that are relevant to the multistage progression to cancer.

Dr. Frank Gonzalez of the National Cancer Institute, Bethesda, MD, spoke about P450 and environmental carcinogenesis. P450s are the principal enzymes involved in the metabolic activation of protoxins, promutagens, and procarcinogens. He discussed characterization of numerous forms of P450 in several animal models and humans leading to the findings of marked species differences in P450 catalytic activities and regulation and the description of important P450 genetic polymorphisms. In humans, P450s have been characterized, in large part, through complementary DNA cloning and expression. These studies are laying the foundation for new systems that can be used to estimate human risk assessment for exposure to foreign chemicals such as skin carcinogens. In his laboratory and elsewhere human P450 genes have been characterized in an effort to identify mutant genes and allelic variants with altered catalytic activities. He provided information about P4501A1, the enzyme responsible for metabolic activation of procarcinogenic chemicals to reactive metabolites. Expression of this gene in human and rodent skin occurs predominantly as a result of exposure to polycyclic aromatic hydrocarbons.

Dr. Peter Cerutti, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland, reported on the role of oxidant stress in cutaneous carcinogenesis. Evidence from his laboratory indicates that UVB-induced cancer is related, at least in part, to the generation of reactive oxidant intermediates. He raised the question whether UVB and oxidants have the capacity to introduce activating mutations into protooncogenes and inactivating mutations into tumor suppressor genes. In his laboratory, for the detection of base pair changes in a minute minority of cells without the selection of phenotypically altered cells a restriction fragment length polymorphism/polymerase chain reaction protocol has been developed. This procedure measures mutations in restriction endonuclease recognition sequences. He presented data indicating the application of these detection systems for UV- and oxidant-induced mutagenesis of cancer-related genes in human cells. He proposed that two phases (an early peak and a second broader peak) of c-fos induction by UVB occur by quite different mechanisms. He emphasized that the carcinogenic action of UVB can be conceptualized as due to specific mutational changes in cancer-related genes, general genotoxicity, or growth factor-like epigenetic effects. Ongoing work is attempting to clarify these possibilities.

Session II: Transgenic Mice and Skin Cancer

Dr. Beatrice Mintz, Fox Chase Cancer Center, Philadelphia, PA, reported on her studies on UV-induced melanomas in transgenic mice. In her laboratory transgenic animals with SV40 oncogenes sequences...
have been developed. These mice, with oncogenic sequences under the transcriptional control of the tyrosinase promoter expressed in pigment cells, are found to develop melanomas. She reported on a technique where melanocytes from young transgenic animals without skin lesions were explanted and irradiated with a single low dose (0.7 mJ/cm²) of UVB. Transgenic cells formed foci but cells from the foci were not tumorigenic when grafted into hosts. After 1.75 mJ/cm², many more foci appeared and these cells gave rise to malignant melanomas. Thus melanoma-susceptible cells become tumorigenic at UVB intensities that are innocuous for wild-type cells of the same inbred strain. She also concluded that multiple genetic changes are likely to be leading to these UVB-induced malignancies.

Dr. Dennis Roop, Baylor College of Medicine, Houston, TX, reported on the development of transgenic mouse models of skin carcinogenesis. A major interest in his laboratory is to stably introduce genes into skin cells for study of skin diseases. At the outset Dr. Roop emphasized the need for such animal models as a strategy for the development of novel pharmaceuticals which may be designed to specifically inhibit expression of human viruses or counteract the effect of mutated genes that occur in human cancers. He reported the development of a vector which specifically targets gene expression to the epidermis of transgenic mice. Using this vector, he has targeted the expression of several genes which have been implicated in epithelial carcinogenesis (ras², fos, TGFα, and E6/E7 genes of human papillomavirus 18) and observed preneoplastic and neoplastic changes in the epidermis of these animals. Benign papillomas were developed 3–4 months later. He provided data that malignant conversion of these papillomas has not been observed and that the papillomas usually regressed, suggesting that additional genetic events are required for malignant conversion. Data from mating experiments was presented to validate this hypothesis. To determine if loss of a tumor suppressor gene is required for malignant conversion, Dr. Roop described experiments for mating these mice with transgenic animals that are null (as the result of a knock-out experiment) for the tumor suppressor gene, p53.

**Session III: Viral, Melanoma, and UV Cancer**

Dr. Ronald Ley of the Lovelace Medical Foundation, Albuquerque, NM, described models for studies of melanoma. He indicated that various investigators have used chemical carcinogens for the induction of cutaneous melanoma in animal models including guinea pigs, Syrian and Chinese hamsters, Mongolian gerbils, and mice. In none of these animals, however, was melanoma induced by UVR alone. He presented studies from his laboratory, in which the skin of the marsupial *Monodelphis domestica* was used for assessing the role of UVR in melanoma induction. Areas of dermal melanocytic hyperplasia occurred. Following termination of exposures, a number of these lesions continued to increase in size and were histologically classified as melanoma. These studies indicate that (a) UVR can act as a complete carcinogen for the induction of melanoma and (b) pyrimidine dimers in DNA are involved in the induction process.

Dr. Douglas R. Lowy of National Cancer Institute, Bethesda, MD, reported on the role of papillomaviruses in epithelial carcinogenesis. He indicated that papillomaviruses are associated with benign and malignant epithelial tumors, including cancer of the uterine cervix. The E5, E6, and E7 papillomavirus proteins have *in vitro* transforming activity. E6 and E7, which are preferentially retained and expressed in cervical cancers, cooperate to efficiently immortalize normal human keratinocytes *in vitro*. This activity is correlated with the ability of E6 and E7 to inactivate the tumor suppressor proteins p53 and Rb, respectively. He presented experiments from his laboratory which determined that the major viral protein (L1), when expressed in the absence of other viral proteins, can self-assemble to form empty particles that closely resemble authentic virus particles suggesting that a self-assembled L1 preparation might be a candidate for a subunit vaccine to prevent papillomavirus infection.

**Session IV: Immunology and Skin Cancer**

Dr. Thomas Luger, University of Munster, Germany, summarized the role of cytokines in malignant melanoma induction. He pointed to several recent investigations that demonstrated, at the transcriptional as well as translational level, that normal human melanocytes as well as melanoma cells are capable of producing distinct cytokines and growth factors such as IL-1α, IL-1β, IL-3, IL-6, IL-8, tumor necrosis factor α, granulocyte macrophage colony-stimulating factor, basic fibroblast growth factor, platelet-derived growth factor, TGFα, and TGFβ. He provided evidence that melanocyte-stimulating hormones (α and β forms) which are known for their ability to stimulate pigmentation have been recognized as potent stimuli of cytokine production by melanoma cells; they up-regulate the synthesis and release of IL-1, IL-6, and tumor necrosis factor α. Their role in tumor induction remains unclear.

Dr. J. Wayne Streilein of University of Miami, Miami, FL, described the role of cutaneous immunity in the development of murine and human skin cancer. He indicated that the emergence of skin cancers bearing neoantigens implies that afferent and/or efferent limbs of the cutaneous immune response have been compromised. He reported that UVB in sunlight is the major etiological factor in human skin cancer, and chronic exposure of mice to UVB elicits skin tumors that are associated with systemic immune deficits. He presented data indicating that whereas approximately 40% of normal human beings are UVB susceptible, virtually all patients with basal/squamous cell cancer are UVB susceptible. He emphasized recent data, gathered from experiments in mice and humans, indicating that UVB contributes to the immune pathogenesis of skin cancer (α) by impairing the functional integrity of cutaneous antigen presenting cells that are required for induction of antitumor immunity and (b) by creating a local immunosuppressive microenvironment that thwarts expression of destructive antitumor immunity.

**Session V: Tumor Promotion and Progression**

Dr. Thomas Slaga of University of Texas System Cancer Center, Smithville, TX, summarized cellular and molecular mechanisms involved in skin tumor promotion and progression pathways. Dr. Slaga pointed out that all known skin tumor promoters can induce a sustained hyperplastic response and the major effect of tumor promoters is the specific expansion of the initiated stem cells in the skin. He presented data indicating that the appearance of GGT and keratin 13 (K13) and the lack of expression of K1 and K10 are good markers for skin tumor progression in mice. These alterations occur at a time when papillomas change from a diploid to an aneuploid state which is mainly due to trisomies of chromosomes 6 and 7. To evaluate a causal role for GGT in skin tumor progression he described experiments in which a functional GGT complementary DNA was transfected into two cell lines which normally induce papillomas when grafted into the skin of nude mice. When injected s.c. all of the GGT transfected clones formed malignant tumors while only 24% of the vector (control) transfected cells produced tumors. He showed that glutathione levels were very low in the tumor cells compared to normal keratinocytes suggesting that GGT has a functional role in skin tumor progression.

Dr. Friedrich Marks of the German Cancer Research Institute, Heidelberg, Germany, discussed the mechanism of skin tumor promotion. He summarized that hormones (TGFα, TGFβ) provide promoting stimuli during cellular hyperproliferative changes that occur during tumor promotion. He indicated that a tissue reaction which is closely related to the wounding response is evoked by practically all skin tumor-promoting agents as the result of either nonspecific tissue
damage or more specific interactions with key events of intracellular signal transduction such as protein phosphorylation. He described experiments indicating that like the wound response, tumor development in initiated skin depends on both the induction of prolonged epidermal hyperproliferation and supporting stromal reactions such as rearrangement of the intercellular matrix, leukocyte infiltration, and factors such as TGFα and TGFβ.

Dr. Toshio Kuroki of the University of Tokyo, Tokyo, Japan, reported on the roles of PKC in epidermal growth, differentiation, and carcinogenesis. Studies from his laboratory have found that among the nine known members of the PKC family, nPKCζ (eta), a Ca²⁺-independent isoform, is expressed predominantly in epidermis of the skin and other epithelia. Expression of nPKCh mRNA in these epithelial tissues was 3–10 times higher than that in brain, and was especially high in squamous epithelium. Two other PKC isoforms, PKCa and nPKCσ, were also expressed in these tissues, but no PKCγ was detected. He presented data suggesting that nPKCζ is closely linked to epidermal differentiation.

Dr. Thomas Kessler of the Johns Hopkins University, Baltimore, MD, reported on the metabolism of skin tumor promoters to reactive intermediates. He indicated that many tumor promoters, including the phorbol esters, do not require biotransformation to stimulate cell growth; metabolism of these compounds typically leads to their inactivation. By contrast, some promoters, notably organic peroxides and hydroperoxides, must be metabolized to reactive intermediates to trigger signal transduction pathways for mitogenesis. He concluded that like the phorbol esters, peroxides and hydroperoxides lead to a genetic reprogramming manifested by the induction of immediate early response genes such as c-fos and late response genes such as ornithine decarboxylase, suggesting convergence in the molecular signalling processes among different classes of promoters.

Session VI: Epidemiology/Prevention

Dr. Paul Strickland of Johns Hopkins University, Baltimore, MD, reported on the epidemiology of skin cancer in Maryland watermen. He described data examining the relationship between solar UVB radiation exposure and non-melanoma skin cancer in this population. In a survey of 808 white, male watermen 30 years of age and older working on the Chesapeake Bay, cases of SCC, BCC, and AK were identified. Among this population, 35 subjects had a total of 47 SCC, 33 subjects had 60 BCC, and 202 subjects had 344 AK. Thus, the ratio of subjects with BCC to subjects with SCC was approximately 1:1, whereas the ratio of BCC to SCC lesions was 1.3:1 since BCC cases were more prone to multiple lesions. He provided data showing that at relatively high solar UVB exposure levels, the risk of SCC increases with increasing exposure, whereas the risk of BCC and AK may plateau. In general, in this population the risk of BCC was greater than that for AK or SCC.

Dr. Kenneth Kraemer of National Cancer Institute, Bethesda, MD, summarized studies from his laboratory concerning xeroderma pigmentosum, which is a prototype of environmental-genetic interactions in skin cancer. Xeroderma pigmentosum is a autosomal recessive disorder in which patients are at increased risk for skin cancer in sun-exposed areas of the body. He described genetic changes that occur in this disease and contrasted them with those occurring in Cockayne’s syndrome where similar to xeroderma pigmentosum the patients have sun exposure sensitivity but do not develop skin cancers. He also presented evidence that high doses of retinoids are useful agents for the prevention of skin cancer in xeroderma pigmentosum patients.

Dr. Hasan Mukhtar of Case Western Reserve University, Cleveland, OH, reported about the chemoprevention of skin cancer. Using multistage murine skin carcinogenesis as a model system his laboratory has been studying the usefulness of inhibitors of metabolic events, considered important for the development of neoplasia as cancer chemopreventive agents. He presented data showing the anticarcinogenic effects of a polyphenolic fraction isolated from green tea. Topical application or p.o. administration of green tea polyphenolic fraction to mice inhibited covalent binding of radiolabeled polycyclic aromatic hydrocarbons to epidermal DNA and skin tumor initiation. Topical application of green tea polyphenolic fraction to mice in a dose-dependent manner inhibited tumor promoter-caused induction of epidermal edema, hyperplasia, myeloperoxidase, cylooxygenase, lipoygenase, and ornithine decarboxylase activities and skin tumor promotion. These studies indicate that green tea polyphenolic fraction possesses both anti-tumor-initiating as well as anti-tumor-promoting effects. He concluded that the anti-tumor-promoting effects were more pronounced. Topical application or p.o. feeding of green tea polyphenolic fraction also was found to afford protection against UVB-induced photocarcinogenesis.

Dr. Mukhtar indicated that inhibitors of the tumor progression stage of murine skin carcinogenesis are not well defined. He concluded that the identification of nontoxic inhibitors may help in developing strategies to reduce human skin cancer risk.

Session VII: Oncogenes/Suppressor Genes

Dr. H. N. Ananthaswamy of M. D. Anderson Cancer Center, Houston, TX, reported on the role of oncogenes and tumor suppressor genes in UV carcinogenesis. He summarized the work from his laboratory on specific mutations in ras and p53 genes identified in human skin cancers originating on sun-exposed body sites and in C3H murine skin. He showed that 40% of human skin cancers display mutations preferentially at codon 12 of the Ha-ras oncogene, whereas 20% of UV-induced murine skin tumors contained mutations exclusively at codon 61 of the N-ras oncogene. In addition 2 of 10 human skin cancers displayed unusual single-stranded conformation polymorphism alleles at exon 7 of the p53 gene; only 5 of 11 UV-induced mouse skin tumors displayed unusual alleles at exon 5 or 8 of the p53 gene. Most mutations occurred at dipyrimidine sequences (C—C and T—T), which suggest that UV-induced pyrimidine dimers and possibly (6–4) photoproducts are the precursor lesions for these mutations. He concluded that specific mutations in ras and/or p53 genes may contribute to the development of UV-induced skin tumors.

Dr. Allan Balmain of Beatson Institute for Cancer Research, Glasgow, United Kingdom, described sequential genetic and biological changes during skin carcinogenesis. He presented evidence that initiation frequently involves mutation of the H-ras gene on chromosome 7, and this is followed by trisomy of this chromosome in papillomas. Further alterations at the H-ras locus occur during progression to squamous and spindle cell carcinomas. In at least 30–40% of cases, progression involves mutation and loss of heterozygosity at the p53 locus on chromosome 11. Other genetic alterations in skin cancers were found on chromosome 6 and at lower levels on chromosomes 4 and 1. He concluded that the stages of mouse skin carcinogenesis are caused by distinct genetic changes which result in altered differentiation control. The end result of this process is the spindle cell carcinoma which has lost many of the properties of epidermal cells, including the ability to synthesize keratins and to express cell adhesion molecules.

Dr. Douglas Brash of Yale University, New Haven, CT, reported on tumor suppressor gene mutations in human cutaneous cancer. He emphasized that since tumor suppressor genes lead to cancer due to inactivation, mutations in these genes provide a window to the initial carcinogenic events that occurred long before the observable tumor appeared. He reported that in patients, 91% of invasive squamous cell carcinomas of the skin contain mutations in the p53 tumor suppressor gene indicated by CC→TT double-base changes, a high frequency of C→T substitutions, and the occurrence of mutations exclusively at...
adjacent pyrimidines. All mutations were UV-like. He concluded that mutation of the p53 gene is often a sunlight-related step in human skin cancers.

Dr. Ervin Epstein, Jr., of the University of California, San Francisco, CA, reported on a casual relationship of tumor suppressor genes and the basal cell nevus syndrome. In persons with basal cell nevus syndrome, an autosomal dominant disease, BCCs arise as early as the second decade, and dozens or even hundreds of tumors may appear. These BCCs are reminiscent of retinoblastomas, in which mutations inactivating a single tumor suppressor gene may be acquired somatically and/or inherited. Epstein used linkage analysis to map the gene with mutations that underlie the basal cell nevus syndrome to chromosome 9. He concluded that this gene may be important in the development of BCCs in both sporadic and hereditary cases and perhaps in the development of extracutaneous malignancies as well.

Exciting Areas of Progress

Knowledge concerning the role of multiple oncogenes, including ras and fos, in UV and chemically induced cutaneous cancer, are beginning to show that it may be possible to link events at the gene level with the phenotypic expression of the stages of initiation, promotion, and progression. The availability of transgenic mice with targeted expression of several genes implicated in epithelial carcinogenesis (Ha-ras, fos, TGFα, and the E6/E7 genes of human papillomavirus 18 in which papillomas develop) suggest that cooperation among these genes may be critical for neoplastic growth. It was a general impression that multistage murine skin carcinogenesis remains the most appropriate model for studies of human skin cancer.

Future Research Needs

The symposium had two panel discussion sessions in which the relevance of animal data to human skin was discussed. Exciting new data regarding genetic alterations in the skin of animals and of humans exposed to various chemical, physical, and viral carcinogens suggest that additional research focused around mechanisms of oncogene activation and tumor suppressor gene inactivation could lead to important new insights into the pathogenesis of cutaneous malignancies. Additional studies are needed to clarify the disease repair processes for structural DNA damage that are present in the skin and to find novel ways of augmenting their effectiveness. The ability of ubiquitous nontoxic foodstuffs, such as green tea, to inhibit carcinogenesis in animal skin suggests that a search for other such agents could prove to exert anticarcinogenic effects in skin and, perhaps, other organs as well.

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