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Journal

Critical reviews in oncogenesis, 5(5)

ISSN

0893-9675

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Publication Date

1994

DOI

10.1615/critrevoncog.v5.i5.10

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Alterations in Gene Expression and Signal Transductions in Human Melanocytes and Melanoma Cells

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ABSTRACT: The development of techniques to cultivate human primary melanocytes *in vitro* has provided the technical foundation for understanding the biology of this cell. Human melanocytes require various growth factors and agents for proliferation *in vitro*. These compounds activate two major signal transduction pathways: a calcium- and phospholipid-dependent (protein kinase C or PKC) pathway and a cyclic AMP (cAMP)-dependent (protein kinase A or PKA) pathway. Alterations in these signal transduction pathways coupled with changes in specific genes (protooncogenes, growth factors, and tumor suppressor genes) have been observed in human melanoma cells compared with normal melanocytes. Our own work indicates that loss in the expression of the PKC β_{II} isotype is a common, if not universal, alteration that occurs early in human melanocyte transformation. In this review, we concentrate on alterations in the signal transduction pathways in human melanocytes and melanoma cells and delineate how an understanding of these changes may allow us to understand the molecular mechanisms involved in human melanocyte transformation.

KEY WORDS: melanocyte, melanoma, signal transduction.

I. INTRODUCTION

Cutaneous malignant melanoma (CMM) has plagued mankind since recorded history, and the first mention of this cancer was presented by Hippocrates in the fifth century (Urteaga and Pack [1966]). The examination of pre-Colombian Inca mummies (approximately 2400 years old) demonstrated metastases to the bones (Selby et al. [1956]). Melanoma was described as the "fatal black tumor with metastases and black fluid in the body" as encountered by Highmore (Urteaga and Pack [1966]). Despite the recognition and long documented history of this disease, the etiology of melanoma and the mechanisms leading to transformation remain largely unknown (Hecht [1989]). The incidence of CMM has been increasing at an average rate of about 4% per year in the U.S. (Longstreth [1988]). Those lesions that are not surgically cured are often fatal. The exact reason for the increase in the incidence of melanoma is

unknown but epidemiological evidence suggests that excess intermittent exposure to ultraviolet light (UV) radiation may be partly responsible (Longstreth [1988]).

Within the past decade, it has become possible to routinely culture human melanocytes *in vitro*. Melanocytes from epidermis can be cultured for a limited time and require media containing serum, phorbol esters (e.g., 12-*O*-tetradecanoylphorbol-13-acetate, TPA), agents that elevate cAMP levels, and bovine pituitary extracts (BPE) or basic fibroblast growth factor (bFGF) (Eisinger and Marko [1982]; Halaban et al. [1987]; and Kath et al. [1989]). Melanocytes grown in these conditions have a diploid karyotype, are nontumorigenic, and will not proliferate in anchorage-independent conditions.

The characterization of normal human primary melanocytes and cells from various stages of human melanocyte transformation (dysplastic nevi, primary and metastatic melanoma) has al-

lowed researchers to obtain information concerning genotypic and phenotypic events associated with melanocyte transformation. These studies have observed differences in human melanocytes and melanoma cells, including alterations in the expression levels of certain genes and their expressed proteins, growth factor requirements, and growth characteristics *in vitro* and *in vivo*. Previous reviews have described cellular (growth requirements), genetic (gene expression levels), phenotypic (protein expression levels), and clinical alterations in human melanocytes and melanoma cells (Herlyn [1993]; Albino and Fountain [1993]; Bennett [1993], and Clark [1991]). In this review we summarize recent studies on alterations in gene expression and signal transduction discovered in human melanocytes and melanoma cells and highlight those factors that may play a role in human melanocyte transformation and how these changes may interact with each other to produce the malignant phenotype.

II. CHROMOSOMAL CHANGES AND A GENETIC MODEL OF TRANSFORMATION

Chromosome alterations have been described in detail in several reviews of human melanoma (for reviews see Albino and Fountain [1993]; Bennett [1993]; Herlyn [1993]; and references therein). Cytogenetic studies have identified chromosomes 1, 6, 7, 9, 10, and 11 as frequently involved in human melanoma. Based on this information, a presumptive working model of acquired genetic change and phenotypic alterations as a melanocyte progresses to transformation has been proposed by a number of investigators, and this model is updated and presented in Figure 1 (Albino and Fountain [1993]).

Alterations have been observed in chromosome 1p in human melanoma tissue biopsies and cell lines. Bale et al. (1989) have noted either a loss of heterozygosity or cytogenetic alterations for chromosome 1p in human melanoma tissues and cell lines. This loss appears to occur late in human melanocyte transformation. There was a frequent loss of heterozygosity in 43% of melanoma tissue biopsies and 52% of melanoma cell lines on chromosome 1p36, a region that contains

genes encoding *c-jun*, RAB3B, VCAM1, TCL5, and p58^{clk-1}. Alterations in the expression of *c-jun* and p58^{clk-1} gene are discussed in more detail in this article.

The next most common alteration observed in human melanoma was in chromosome 6q12-25 (Trent [1983]; and Parmiter and Nowell [1993]). Several genes have been mapped to this region, including *c-myb*, *c-ros*, and others. The most definitive study to determine the gene involved in this region was initiated by Trent et al. (1990). Using microcell hybrids, the introduction of a normal chromosome 6 into melanoma cells resulted in the ability of the hybrids to form tumors *in vivo*. A possible gene located in this region of chromosome 6 is discussed later in this review. Welch et al. (1994) have determined that introduction of a normal chromosome 6 suppressed the metastatic but not the tumorigenic phenotype of human metastatic melanoma cells. An inverse correlation was also observed with nm23-H1 RNA transcript expression levels and the metastatic ability of the hybrid cell clones.

An increase in the ploidy of chromosome 7 (7p11-13) has also been observed in human melanoma and correlates with an increase in the copy number of the epidermal growth factor receptor (EGF-R). Alteration in a gene similar to EGF-R has been observed in a swordfish melanoma model. An experimental model for the formation of ultraviolet radiation induced melanoma using F1 progeny from a mating with swordtail (*Xiphophorus belleri*) and platyfish (*Xiphophorus maculatus*) has determined that there are two genes involved in the formation of tumors (Ahuja and Anders [1976]). One of the genes responsible was a tumor suppressor gene, Tu, which encodes Xmrk, a membrane receptor tyrosine kinase similar to the human EGF-R (Anders [1991]).

An alteration that may occur early in human melanocyte transformation is located on chromosome 9. Cytogenetic studies from several groups suggest the involvement of chromosome 9p21-p22 as a locus for familial melanoma susceptibility gene (Cannon-Albright et al. [1992]; Fountain et al. [1992]; Holland et al. [1994]; Isshiki et al. [1994]; and Coleman et al. [1994]). Fountain et al. (1992) determined that 85% of melanoma tissue biopsies and cell lines had alterations in this region. p16 has been proposed to be the gene

FIGURE 1. Genetic model of human melanocyte transformation. Proposed critical genetic alterations are listed in the upper part of the model. Those genetic alterations listed below the arrow are considered secondary changes. Features in () are supported by relatively less data.

altered in this region and is discussed later in this review.

Changes on chromosome 10 have also been associated with human melanocyte transformation as well. Parmiter and Nowell (1993) have observed alterations involving chromosome 10 in 10% (1/10) of dysplastic nevi, 67% (2/3) of primary melanomas, and 37% (19/51) of advanced melanomas. Using either loss of heterozygosity or cytogenetic studies, other chromosomes that show alterations include 11, 2, and 3, but these changes have been less frequent. The genes involved at the identified cytogenetic sites of abnormality are currently unknown, and studies are underway to define these regions.

III. ALTERATIONS IN GENE EXPRESSION

Alteration in the RNA transcript expression levels of various genes has been investigated using Northern blot hybridization analysis and reverse transcriptase in combination with polymerase chain reaction (RT-PCR). Researchers have observed changes in the relative expression levels of specific genes during human melanocyte transformation (Chenevix-Trench et al. [1990]; Herlyn [1993];

and Albino and Fountain [1993]. Some of these alterations include:

1. DNA transcription factors: AP-1, RAR family
2. Cell cycle genes: *p58*, *myc* family
3. Growth factors: bFGF
4. Membrane proteins: PKC family, EGF-R, Ras family
5. Tumor suppressor genes: *p53*

A. AP-1 (*jun* and *fos* Families)

The phorbol ester, TPA, affects the expression of many genes (for review see Karin and Herrlich [1989]). One of the transcription factors involved in modulating the expression levels of TPA-inducible genes is the c-jun protein, which in cellular form is one of several polypeptides that form a complex called AP-1 (a combination of the *jun* and *fos* families). The *c-jun* gene belongs to a gene family; the other members being *jun-B* and *jun-D*. *Jun-B* inhibits the transforming and transactivating activities of the *c-jun* protooncogene (Schutte et al. [1989]). This complex recognizes a specific DNA sequence that mediates the transcription response to phorbol es-

ters. Some of the TPA-inducible genes are proto-oncogenes (*c-fos* and *c-myc*), proteases (collagenase and stromelysin), and cytokines (interleukin 1 β and 2) (for review see Karin and Herrlich [1989]). Thus, change in the expression level of the AP-1 complex may induce the expression of genes that assist in melanocyte transformation.

Transcriptional activation of the *c-fos* gene by various agents has been investigated by many researchers. *c-fos* belongs to a family of genes, including *fos-b*, *fra-1*, and *fra-2*. *c-fos* whose mRNA levels increase during stimulation of cells with growth factors or mitogens and after DNA damage (Verma [1986]; Sassone-Corsi et al. [1988]). *c-fos* is a protooncogene whose elevated expression level can induce tumors *in vivo* (Jenumein et al. [1985]) and transformation of cells *in vitro* (Miller et al. [1984]). *c-fos* expression has been investigated in an immortalized, nontumorigenic, murine melanocyte cell line. Hart et al. (1989) observed an increase in *c-fos* mRNA following treatment of murine melanocytes with dibutyl cyclic AMP (dbcAMP). Investigators have also studied the inhibition of *c-fos* RNA transcript expression by antisense *fos* expression vectors. Both the proliferation of dividing (Holt et al. [1986]) and quiescent cells stimulated with mitogens (Nishikura and Murray [1987]) was blocked by antisense *fos*.

We have described alterations in the expression of *jun* and *fos* families in human melanocytes and melanoma cells (Yamanishi et al. [1991a]; Jiang et al. [1993]). We observed an increase in *c-fos* and a decrease in *c-jun* RNA transcript expression levels as melanocytes went from quiescent to proliferative growth states. A similar change in expression of *c-jun* (decreased), *c-fos* (increased), and *jun-B* (decreased) was observed in human melanoma cells compared with melanocytes. Changes in the expression levels of these genes may alter the ability of melanoma cells to undergo differentiation. The shift in the expression of the *c-jun* and *c-fos* RNA transcripts should shift AP-1 activity, resulting in the expression of other genes that are involved in cell proliferation rather than differentiation.

Other genes that have AP-1 binding sites located in their promoter are growth factors (e.g., bFGF), cell division cycle genes (e.g., *p58*), and cytokines. Jiang et al. have determined that mel-

noma cells induced to differentiate had an elevated *c-jun* and *jun-B* RNA transcript expression level (Jiang et al. [1993]). Alterations in the expression of these gene families may determine whether melanocytic cells proliferate or undergo differentiation. Future studies using expression vectors will determine what role these genes play in cell growth and differentiation and which genes are altered by overexpression of these transcriptional regulators.

B. Retinoic Acid Receptor (RAR) Isotypes

The nuclear RAR family has provided a mechanistic basis by which gene expression may be modulated by retinoids. Three RAR isotypes α , β , and γ have been identified in mammalian cells and are conserved between species (for review see Chambon et al. [1991] and Mangelsdorf and Evans [1992]). Variations in the expression of the RAR isotypes have been found in many tissues (Elder et al. [1991]; Chambon et al. [1991]; and Mangelsdorf and Evans [1992]). RAR α RNA transcripts are expressed in most tissues, while expression of RAR β RNA transcripts in tissues is variable and RAR γ RNA transcripts are expressed at high levels in the skin. A retinoic acid-responsive element has been found in the promoter of the RAR β isotype and is a direct repeat of the sequence AG(G/T)TCA separated by five nucleotides (De Luca [1991]). A target for retinoid-induced transcription activation is the RAR β isotype. While the RAR α and γ isotypes are constitutively expressed, the RAR β isotype is markedly inducible by all-trans retinoic acid (tRA).

We have determined the basal RNA transcript expression levels of RAR isotypes in human melanocytes and melanoma cells using Northern blot hybridization analyses. RAR α (2.8 and 3.6 kb) and RAR β (2.8 and 3.1 kb) RNA transcripts were detected in melanocytes. Expression of the RAR α , β , and γ RNA transcripts in melanocytes was only slightly affected by the addition of growth factors in the melanocyte medium. RAR α RNA transcript expression levels were decreased in melanocytes cultivated in complete medium and RAR β RNA transcript expression levels increased as the melanocytes

were cultivated in more complete medium. RAR γ RNA transcripts (2.8 kb) were detected in melanocytes cultivated in the various growth medium. Expression of the RAR isotypes in melanoma cells was different compared with melanocytes. RAR α and γ RNA transcripts were detected in all four melanoma cell strains, while RAR β RNA transcripts were detected only in the melanoma cell strains c81-46a and c81-46c. Expression of the RAR isotypes RNA transcripts in human melanocytes and melanoma cells as well as their cell response to 13-cis retinoic acid (13-cisRA) and tRA is summarized in Table 1.

Although tRA was previously shown to induce the expression of RAR β isotype in other cell types, we investigated if the undetectable RAR β RNA transcript expression levels in two of the melanoma cell strains was due to an altered cell responsiveness. In those melanoma cell lines that had basal expression of RAR β , tRA induced RAR β RNA transcripts (3- to 8-fold). RAR α and γ RNA transcripts expression levels were also induced following tRA treatment. However, in melanoma cell strains c81-61 and c83-2c that lacked onstitutive expression of the RAR β , neither of the RAR β RNA transcripts were induced following tRA treatment. Loss in the expression of the RAR β isotype may be one mechanism by which melanoma cells become resistant to tRA treatment. Future studies using RAR β expression vectors may allow us to determine the role of the

RAR β isotype and provide insight into the mechanisms involved in determining melanocyte response to tRA.

C. p58^{clk-1}

The protein complex that controls the onset of mitosis in normal cell cycle contains at least four members: p34^{cdc2}, cyclin B, p13^{suc1}, and cdc25 (for review see Maller et al. [1991]). The mitotic protein kinase p34^{cdc2} is required for both the G₂- to M-phase transition and the G₁- to S-phase transitions in the cell cycle. p58^{clk-1} was shown to be structurally and functionally related to p34^{cdc2} (Bunnell et al. [1990]) and has been localized to chromosome 1p36 (Bunnell et al. [1990]; Eipers et al. [1991, 1992]). Its abnormal expression alters the ability of eukaryotic cells to progress through the cell cycle and changes cell phenotype. p58^{clk-1} may function to negatively regulate normal cell cycle progression. Due to the chromosomal location of the *p58^{clk-1}* gene, we investigated its expression in four melanoma cell lines and were unable to detect its expression (Table 2), although the gene was expressed in normal melanocytes. We also observed a decrease in the expression level of the p58^{clk-1} in melanoma tissue biopsies. We have been unable to detect any gross rearrangements in the *p58^{clk-1}* gene using Southern blot hybridization analysis, which would suggest an alteration in the activity of the promoter.

TABLE 1
Retinoid Response and RNA Transcript Expression Levels of the RAR Isotypes in Human Melanocytes and Melanoma Cells

	13-cisRA IC ₅₀	tRA IC ₅₀	RAR α	RAR β	RAR β induction	RAR γ
Melanocytes	16.7	0.7	+	+	Both	+
c81-46a	1.7	>33.0	+	+ ^a	Both	+
c81-46c	2.3	1.7	+	+ ^a	Both	+
c81-61	10.0	16.7	+ ^a	—	—	+
c83-2c	20.0	20.0	+ ^a	—	—	+

Note: 13-cisRA and tRA IC50 is in micromolar concentration. RAR β induction was determined in cells treated with tRA. RNA transcript expression levels of RAR α , β , and γ are indicated as: present (+), or not detected (—).

^a Expression levels at least 3x greater than melanocytes.

TABLE 2
RNA Transcript Expression Levels of the p58 Gene in Human Primary Melanocytes, Tissue Biopsies, and Metastatic Melanoma Cell Lines

		Metastatic melanoma	
Melanocyte		Cell lines	Biopsies
p58	++	– (13/14)	– (9/10)

Note: ++ medium expression level; – low or undetectable expression.

D. myc Family

Alteration in the expression of the *myc* family (*c-myc* [chromosome 8q], *N-myc* [chromosome 2p], and *L-myc* [chromosome 1p]) have been observed in human tumor cell lines. *c-myc* RNA transcript expression levels have been shown to be tightly linked to the proliferate state of the cell, and the myc protein may serve to induce G₀/G₁ transition genes.

While Linnenbach et al. (1988) were unable to observe any gross alterations in the *myc* family from 28 melanoma cell lines, Bauer et al. (1990) observed an amplification of the *N-myc* gene in biopsies from two melanoma patients. The effect of the overexpression of the *c-myc* gene on human melanoma cells has been investigated by several groups. Versteeg et al. (1988) determined the expression levels of the *c-myc* gene and HLA class I antigens in 11 melanoma cell lines. A high level of *c-myc* expression was observed to correlate inversely with the expression of the HLA class I antigen (Versteeg, et al. [1989]; Schrier and Peltenburg [1993]). Transfection of melanoma cells (IGR 39 cells with a low *c-myc* and high HLA class I antigen expression level) with a *c-myc* expression vector resulted in a decrease in the HLA class I antigens and β_2 -microglobulin expression (Versteeg et al. [1988]). Lenardo et al. (1989) determined that *N-myc* may work by altering the factor binding to the HLA class I gene enhancers. The effect of myc expression on HLA class I antigens was determined to be through enhancer inactivation. There are two distinct elements in the MHC class I promoter that are susceptible to *N-myc* suppression (Lenardo et al.

[1989]). However, a consistent overexpression of the *myc* genes has not been observed in melanoma cell lines compared with melanocytes (Chenevix-Trench et al. [1990]).

E. Basic Fibroblast Growth Factor (bFGF)

bFGF (FGF-2) is a potent mitogen for many cell types of mesodermal and neuroectodermal origin (Gospodarowicz [1990]). This growth factor is a member of a gene family, including acidic fibroblast growth factor (aFGF, FGF-1), int-2 (FGF-3), Hst/Ks-FGF (FGF-4), FGF-5, FGF-6, and keratinocyte growth factor (KGF, FGF-7) (Gospodarowicz [1990]). Cloning of the bFGF gene has allowed its characterization at the DNA and RNA level and four RNA transcripts have been detected (Abraham et al. [1986]; Kurokawa et al. [1987]). Two major RNA transcripts (7.0 and 3.7 kb) are observed as well as several low-abundance RNA transcripts (1.0–1.8 kb) in several cell lines (Yamanishi et al. [1992]; Murphy et al. [1988]; Thompson et al. [1991]). Interestingly, the smallest RNA transcript (1 kb) is an antisense RNA transcript to the bFGF gene and may function in its regulation (Kimelman and Kirschner [1989]; Volk et al. [1989]).

bFGF protein has a basic pI (>9.0) and a high affinity for heparin and glycosaminoglycans. Using heparin-affinity chromatography and bFGF antibodies, the translation of bFGF has been investigated. Four bFGF polypeptides (18, 21, 22.5, and 24 kDa) have been detected in human cells. Utilization of non-AUG codons produced the three larger molecular weight bFGF isoforms (Florkiewicz and Sommer [1989]).

The role of bFGF in melanocyte cell growth has been studied widely, and this growth factor is a mitogen *in vitro* (Halaban et al. [1988]). However, the expression levels of bFGF in human melanocytes and melanoma cells varies. While there is some disagreement on whether human melanocytes express bFGF RNA transcripts, bFGF protein was not detected (Yamanishi et al. [1992]; Rodeck et al. [1991]; Halaban et al. [1988]). Our data on the absence of bFGF RNA transcript expression in melanocytes are supported by *in situ* hybridization studies. Nevertheless, bFGF RNA

transcripts are expressed at various stages of melanocyte progression and were detected in tissue from nevi, primary melanomas, and metastatic melanomas (Scott et al. [1991]). Interestingly, the expression level of bFGF RNA transcripts detected decreased with increasing progression toward malignant tissue (nevi (+2 to +3), primary (+2), and metastatic melanoma [+1]). On the other hand, our studies and those of others indicate that melanoma cells expressed very low levels of bFGF RNA transcripts and proteins (Yamanishi et al. [1992]; Halaban et al. [1988]). While three of four cell strains expressed bFGF RNA transcripts and protein isoforms, the cell strain c83-2c only expressed the 1.2-kb RNA transcript and did not synthesize any detectable levels of bFGF protein as measured by Western blot analysis.

The effect of bFGF neutralizing antibodies on human metastatic melanoma cell growth has been investigated also (Becker et al. [1992]). A decrease in melanoma proliferation was observed following incubation of cells with antibodies to bFGF protein. Inhibition of cell proliferation by antisense oligonucleotides to the bFGF gene also has been studied and using antisense oligodeoxynucleotides targeted against the bFGF gene, Becker et al. (1989) demonstrated a slight inhibition of cell proliferation in melanoma cells as well as inhibition of anchorage-independent growth. The use of either bFGF antisense oligonucleotides or bFGF antibodies had only a limited effect on cell growth, suggesting that there may be more than one fibroblast growth factor or bFGF-R involved in melanoma cell growth or that bFGF is of secondary importance in contributing to cellular proliferation of melanoma cells. These data suggest that although exogenous bFGF protein may be required for the proliferation of human primary melanocytes and the molecule is expressed frequently by melanoma cell strains, expression of the bFGF protein does not appear to be a consistent or necessary alteration in melanocyte transformation.

F. PKC Isotypes

PKC is a serine/threonine protein kinase that interacts with calcium ions, phospholipids, and diglycerides to form a complex associated with a cellular membrane structure (Nishizuka et al.

[1988]). PKC represents a multigene family and 10 different cDNA clones have been isolated to date (Nishizuka [1988]; Ohno [1991]). Three distinct groups of PKC genes have been isolated to date. The four conventional PKC (cPKC) cDNAs (α , β_I , β_{II} , and γ) are encoded by three distinct genes (α , β , and γ) with the β_I and β_{II} RNA transcripts formed by utilizing different 3' splice acceptor sites. In addition to the cPKC genes, four novel PKC (nPKC) cDNAs (δ , ϵ , η/L , and θ) and two atypical (aPKC) cDNAs (ζ and λ) have been identified and have been shown to have regions of high homology (conserved regions, C) and weak homology (variable regions, V) at the protein level. cPKC isotypes have four highly conserved (C_1 – C_4) regions surrounded by five variable (V_1 – V_5) regions, whereas the four nPKC and two aPKC isotypes lack the C_2 region. The aPKC isotypes are also missing one of the two cysteine-rich domains located in the C_1 region.

PKC has been implicated in the regulation of many cellular processes, including growth, differentiation, neuronal function, and gene expression (Nishizuka [1988]; Nairn et al. [1985]). Although the role of each PKC isotype in cellular processes is unknown, investigations in the overexpression of PKC α gene (Housey et al. [1988]) or expression of a mutated PKC isotype in mouse fibroblasts (Megidish and Mazurek [1989]) have demonstrated either altered growth regulation or complete transformation of the transfected cells. In addition, PKC has been implicated in the *in vitro* transformation processes induced by the oncogenes *ras*, *sis*, *fms*, *srs*, *fps*, and *fes* (Jackowski et al. [1986]; Preiss et al. [1986]). Researchers have observed either elevated levels of sn-1,2-diacylglycerol or phosphorylation of a transformation-related protein and a PKC substrate in cells transformed by these oncogenes.

We have measured the expression of PKC isotypes in human melanocytes and melanoma cells (Yamanishi et al. [1991b]; unpublished data), due to their involvement in the TPA response (its membrane receptor being the PKC). The expression levels are summarized in Table 3. We and others have observed that melanocytes expressed PKC α , β , and ϵ isotypes, and that the PKC isotypes could be downregulated following TPA treatment (Yamanishi et al. [1991b]; Arita et al. [1992]; Powell et al. [1992]). Melanoma cells

expressed PKC α and PKC ϵ RNA transcripts at various levels. Alterations in the expression of PKC α (increased), PKC β_{II} (undetectable), and PKC ϵ (increased) were common to the melanoma cells (compared with melanocytes) screened. The most striking finding was that PKC β_{II} RNA transcripts were detected in only primary melanocytes or benign moles (Table 3).

Eighteen metastatic melanoma cell lines (cell lines from ATCC and those derived by Dr. Meyskens' laboratory) have been screened for PKC β_{II} RNA transcripts using Northern blot hybridization analysis and expression was undetectable in all of the cell lines (Table 3). Dysplastic nevi and metastatic melanoma biopsy tissue samples were also screened and were shown to have either decreased or undetectable PKC β_{II} RNA transcript expression levels in nevi and in melanoma cells and biopsies. We have also investigated the expression levels of the PKC isotypes in other human tumors. There was a consistent loss in the expression of the PKC β_{II} isotype in malignant tissue biopsies and cell lines derived from human brain, head and neck regions, breast, and hemapoietic tissues (unpublished data).

As measured by Southern blot hybridization, no gross alterations were detected in the PKC β gene in four melanoma cell strains using the restriction enzymes, BamHI, EcoRI, and PvuII. Our data clearly demonstrate a loss in the expression of the PKC β_{II} RNA transcripts early in human melanocyte transformation; we speculate this was

possibly due to an alteration in the transcriptional activity of the PKC β promoter.

In order to determine the functional role for the PKC β_{II} isotype in human melanocyte transformation, we have transfected human melanoma cells with an expression vector containing the PKC β_{II} isotype, pSR α -PKC β . Colonies were isolated in cells transfected with either the selection vector, pSV2-neo, alone or co-transfected with an expression vector containing the PKC α isotype (Table 4). However, a decrease in the number of colonies was observed with transfection of the PKC β_I or β_{II} isotypes. Melanoma cell growth was suppressed after 1 week following transfection with the expression vector containing the PKC β_I or β_{II} isotype, suggesting that the PKC β isotype can suppress tumor growth. Choi et al. (1990) also observed similar results when PKC β_I was transfected in colon cancer cells. The cells had reduced tumorigenicity and ability to grow in anchorage-independent conditions. Thus, PKC β_{II} may play a common role early in human transformation and alterations may lead to abnormal (i.e., tumor) cell growth. Although the gene for PKC β_{II} does not undergo genomic rearrangement, the expression of the PKC β_{II} isotype is downregulated. This may be another mechanism by which a cell can regulate cell growth and undergo cell transformation. We hypothesize that there is a common alteration during human melanocyte transformation, resulting in the loss of a transcription factor that induces the expression of the PKC β_{II} isotype.

TABLE 3
RNA Transcript Expression Levels of the PKC Isotypes in Human Primary Melanocytes, Tissue Biopsies, and Metastatic Melanoma Cell Lines

	Cells	Melanocyte		Melanoma	
		Normal	Dysplastic	Biopsies	Cells
		Nevi	Nevi		
PKC α	+	+ (3/3)	+ (5/5)	+ (14/14)	+ (18/18)
PkC β_{II}	+	+ (3/3)	- (5/5)	- (12/14)	- (18/18)
PKC γ	-	N.T.	N.T.	N.T.	- (4/4)
PKC δ	+	N.T.	N.T.	N.T.	+ (4/4)
PKC ϵ	+	N.T.	N.T.	N.T.	+ (4/4)
PKC ζ	+	N.T.	N.T.	N.T.	- (2/4), + (2/4)

Note: N.T. — not tested.

TABLE 4
Number of Colonies Observed After Transfection of
the Melanoma Cell Strains

	Metastatic melanoma cell strains		
	c81-46c	c81-61	c83-2c
pSV2-neo alone	20-25	20-30	20-30
pCD-SR α + pSV2-neo	20-30	20-30	20-30
pSR α -PKC β + pSV2-neo	0-2	0-2	0-1
PSR-PKC α + pSV2-neo	N.T. ^a	20-30	20-30
pSR-PKC β _I + pSV2-neo	N.T. ^a	4-7	6-9
pSR-PKC β _{II} + pSV2-neo	N.T. ^a	0-2	0-3

Note: Cells were cultivated until 50 to 70% confluent and then transfected with plasmids. Cells were rinsed and prewarmed fresh medium was added to the plates. Cells were allowed to recover for 2 d and then fresh medium containing 250 μ g/ml of G418 was added to the plates. Cells were fed twice per week with fresh medium containing G418.

^a N.T. — not tested.

The effect of tRA on the expression of PKC isotypes in murine melanoma cells, B16, has been investigated by many groups. tRA-induced differentiation of B16 induces cyclic AMP-dependent protein kinase and PKC (Ludwig et al. [1980]; Rogelj et al. [1984]). Selective increase in the expression levels of PKC α isotype with no detectable alteration in the expression of the PKC δ , ϵ , and ζ was observed in tRA-treated B16 cells (Oka, M. et al. [1993]). Transfection of the PKC α isotype induced longer doubling times, reduced anchorage-independent growth, longer tumor formation *in vivo*, and increased melanin production (Gruber, J. R. et al. [1992]).

Interestingly, Park et al. (1993) has pursued the role of the PKC β _{II} isotype in pigmentation in human melanoma cells and determined that human melanoma cells were depigmented due to a decrease in the phosphorylation of the tyrosinase protein by PKC β . Pigmentation in this cell line could be recovered by transfecting the PKC β isotype into the melanoma cells. We have also observed that transfection of melanoma cells with a constitutive PKC β expression vector induced pigmentation in two amelanotic melanoma cell lines, and a decrease in cell growth [unpublished data]. However, we also have a melanoma cell line that does not express PKC β RNA tran-

scripts and is pigmented. It may be that other PKC isotypes can substitute for the PKC β and activate the tyrosinase protein. Thus, alteration in the expression of the PKC isotypes may play a role in melanocyte transformation through a number of mechanisms.

G. Epidermal Growth Factor Receptor (EGF-R)

EGF-R is a member of a famiyl of growth factor receptor tyrosine kinases (Carpenter [1987]). These receptors have an extracellular domain that contains a site to which growth factor or ligand binds, an intracellular domain encoding a tyrosine kinase and connected by a transmembrane domain of hydrophobic amino acids. Intracellular portion of the EGF-R has three tyrosinase residues that are autophosphorylated after binding of EGF to the receptor. Activation of PKC will result in the phosphorylation of the threonine 654 residue that contributes to the conversion of EGF-R from the high- to low-affinity state (called transmodulation), which results in a form of desensitization of the receptor. Ligands for the EGF-R include TGF- α and EGF. Increased levels of the EGF-R have been found in breast cancer, gliomas, and carcinomas.

An elevated expression level of the EGF-R in melanoma cells has been correlated to an increase in the copy number of human chromosome 7. An increase in the expression level also has been used as a marker of tumor progression by *in situ* studies. The EGF-R is detected in vertical growth phase primary (>80%) and metastatic (>80%) melanomas compared with normal melanocytes and common nevi (0%), and dysplastic nevi and radial growth phase primary melanomas (20%) (De Wit et al. [1992]).

H. Ras Family

There are three members of the ras family: *Ha-ras* (chromosome 11p), *Ki-ras* (chromosome 12p), and *N-ras* (chromosome 1p). These genes code for 21-kDa protein (p21), which functions as a GTP/GDP binding protein with a GTPase activity and plays a role in signal transduction, cell proliferation, and differentiation. Mutations in the first, second, and third codons activate the *ras* genes with transformation potential.

van t'Veer et al. (1989) used PCR and oligonucleotide hybridization to detect point mutations in the *N-ras* gene in 7/37 cutaneous melanomas (primary, metastatic, and cell lines). Interestingly, the tumors with an activated *N-ras* oncogene were found in sites with continuous sunlight exposure. The site of mutations (codons 12, 13, and 61) were not always at a thymidine dimer but mostly at a thymidine-cytidine dimer site. In a more detailed study by Shukla et al. (1989) using PCR and oligonucleotide hybridization, activated *N*-, *Ki*-, and *H-ras* oncogenes were detected. In benign nevi samples, 2/4 samples had a codon 12 *Ki-ras* mutations. In primary melanoma, 2/22 had a codon 12 *ki-ras* mutation, 1/22 had a codon 61 *N-ras* mutation, and 1/22 had a codon 12 *Ha-ras* and a codon 12 *Ki-ras* mutations. In lymph node metastases, 2/12 had a codon 12 *Ki-ras* mutation, 1/12 had two different codon 12 *Ki-ras* mutations, and 1/12 had both a codon 12 *Ki-ras* and a codon 61 *N-ras* mutation. In systemic metastases, 0/2 had no activated *ras* gene mutations.

Albino et al. (1989) have detected mutations in the *ras* genes in melanoma using PCR and oligonucleotide hybridization. They were unable to detect an activated *ras* gene in biopsies from

nevi tissue. In primary melanomas, 5% of the samples had an activated *N-ras* gene at the codon 61st. In metastatic melanoma biopsies, 6% of the samples had a mutation in the 61st codon of the *N-ras* gene, while cultured metastatic melanomas had mutations in the 61st codon (22% or the codon 13th of the *N-ras* gene and in the 61st (2%) codon of the *H-ras* gene. Interestingly, the melanomas with an activated *ras* gene were representative of early or intermediate stages of differentiation as determined by the expression of a large number of EGF receptors, class II histocompatibility antigens (IA), nonpigmented, and with a morphology that was epithelioid/spindle type.

The expression of the *ras* gene was investigated using a monoclonal antibody against Ha- and Ki-ras protein (Yasuda et al [1989]). A highly positive reaction was observed in melanocytic nevi located in the dermal region, while low reactivity or no detectable response was detected in compound nevi and junctional nevi. High reactivity was also detected in nodular melanoma and metastatic melanoma. Medium reactivity was observed in acral lentiginous melanoma and superficial spreading melanoma. The authors comment that "the different p21 expression levels among the type of tumors may represent the state of tumor cell differentiation with greater p21 expression with the more immaturity in the melanocyte lineage".

Ball et al. (1994) also have observed *ras* mutations in a subset of melanomas from sun-exposed skin. *Ras* mutations were observed in 56% of tumors from continuously exposed skin sites compared with 21% of tumors from intermittent or non-sun-exposed skin sites. Most of the mutations in the *ras* genes occurred as primary melanomas progressed from Clark's level II to III; their results suggest that the *ras* mutations occur during the phase that melanomas acquire a more aggressive phenotypic behavior. They propose that "activated *ras* contributes to the growth advantage of melanomas in the vertical growth phase of dermal invasion" and may play a role in tumor progression in a subset of melanoma.

I. p53

Mutations in the *p53* gene are the most common alterations in human cancers (Harris [1994], and

references therein). Loss of the wild-type *p53* gene has been observed in a high proportion of lung, brain, breast, colon, ovary, and bladder tumors.

Alterations in the *p53* gene have been observed in human skin cancer also. An elevated expression level of the p53 protein was detected in malignant melanomas compared with primary melanocytes and dysplastic nevi using immunofluorescence and flow cytometry (Stretch et al. [1991]; Akslen and Mørkve [1992]; Lassam et al. [1993]). However, Volkenandt et al. (1991) were able to detect a mutated *p53* gene in only one out of ten melanoma cell lines. Montano et al. (1994) observed a differential increase in nuclear expression of p53 protein in melanoma cells but were not able to detect a mutation in the *p53* gene. Our studies on the expression of the *p53* gene were similar (Table 5). Although we were able to detect an alteration in the RNA transcript expression level of the *p53* gene in four human melanoma cell lines and two metastatic melanoma tissue biopsies, we were unable to detect any point mutations in the entire coding regions of the *p53* gene in these tissue biopsies (unpublished data).

IV. PROPOSED MOLECULAR MODEL OF PROLIFERATION AND DIFFERENTIATION PATHWAYS

Notwithstanding the large number of molecular changes that have been detected in human melanomas, we proposed that a few alterations are key and at the heart of regulation of melanocyte growth and maturation (Figure 2). These hypotheses include

1. The interaction of the cAMP transduction pathway with bFGF through the c-fos/AP-1 mechanism
2. The effect of TPA (may act as a surrogate for UV radiation) on the PKC pathway and its interaction with c-jun/AP-1 mechanism
3. The modulation of the AP-1 mechanism via interaction with the RAR family of transcription factors

Several studies have investigated the role of the transcription factor AP-1 and the steroid receptors in cell pathway programming (Ponta et al. [1993] and references therein). The steroid hormone receptor family includes the retinoic acid, thyroid hormone, and vitamin D receptors. Although there is crosstalk between the various signal transduction pathways, a reductionist model would suggest that AP-1 is involved in the proliferation program, while the steroid hormone receptors are involved in the differentiation program (Figure 2). Several studies have investigated how these two pathways interfere and interface with each other, and they probably modulate the growth of human melanocytes in a critical manner.

In one aspect, AP-1 may be a key pathway producing a proliferative response in melanocytes. Quiescent melanocytes have a low level of c-fos with a high level of c-jun protein. Stimulation of human melanocytes to proliferate with bFGF, serum, and cAMP induces the expression of c-fos protein levels. An increase in c-fos protein levels will stimulate the formation of AP-1 heterodimers and result in a subsequent decrease in c-jun RNA transcript expression levels (induction of other genes rather than inducing its own expression). The activation of AP-1 heterodimers may induce

TABLE 5
RNA Transcript Expression Levels of the *p53* Gene in Human Primary Melanocytes, Tissue Biopsies, and Metastatic Melanoma Cell Lines

Melanocytes		Metastatic melanoma	
		Cell lines	Biopsies
<i>p53</i>	++	+++ (2/4), + (2/4)	+++ (1/2), + (1/2)

Note: +++ high; ++ medium; + low expression levels.

FIGURE 2. Schematic illustration of a molecular model of proliferation and differentiation pathways in human primary melanocytes. (+) phosphorylated; (-) dephosphorylated.

the expression of specific genes involved in cell proliferation through TPA-responsive elements (TRE). Also, TPA or UV affects PKC with downstream modulation of c-jun/AP-1.

On the other hand, RARs largely act on the differentiation pathway. Treatment of cells with RA or other differentiation hormones may transmit signals to the nucleus to induce cell differentiation. Alterations in the expression of the RARs may change the ability of the melanocytes to undergo differentiation. A decrease in RAR RNA transcript expression levels and an inability to induce the expression of the RAR β isotype was observed in tRA-resistant human melanoma cells. This situation may also occur in human squamous cell carcinomas and other tumor types: a loss in the expression of the RARs may allow a cell to escape from normal cell programming.

In this model, we have proposed how two major signal transduction pathways may modulate the ability of a cell to undergo a proliferation or differentiation response to external stimuli. In normal melanocytes, there exists a homeostatic interaction in the proliferative and differentiation

signals. Alterations in this balance (treatment with growth factors or retinoids) may shift the focus of the cell. Transformation of the human melanocyte would likewise result in inhibiting or altering the ability of the cell to undergo differentiation. There may be other "partners" in the scheme of human melanocyte proliferation/differentiation that are not addressed in this model; the role of the AP-1 regulator, IP-1, and the retinoic acid-related receptors are reasonable candidates. With future studies, the interaction of these genes and others will be investigated and may allow a clearer picture to develop at the molecular level.

V. INVOLVEMENT OF UV RADIATION IN TRANSFORMATION

The epidemiological evidence that suggests a causative role of nonionizing radiation in the formation of melanoma include (Longstreth [1988]):

1. The incidence of CMM is higher in lighter pigmented people

2. Freckles and nevi are induced by sunlight exposure
3. Epidemiological correlation between decreasing latitude and increasing sunlight exposure and higher CMM rates
4. The higher incidence of CMM in patients who cannot repair UV radiation DNA damage
5. The indication of excess sunlight exposure at an early age and higher CMM incidence

Experimentally, UV radiation has been shown to induce cellular and molecular alterations *in vivo* and *in vitro* (Kripke [1990]; Ambach and Blumthaler [1993]; Kaina et al. [1989]). Besides its well-known mutagenic response in all cellular systems (Hanawalt [1991]), UV radiation, particularly at low doses, induces a transient mitogenic response and alters the expression of genes. The mitogenic and mutagenic responses have been demonstrated in many cells and are either immediate or early alterations induced in cells following UV radiation exposure. In contrast, the late events following UV irradiation, particularly at high doses, are cell death, cell mutagenesis, and cell transformation (Longstreth [1988]; Ananthaswamy and Piercell [1990]).

In general, the ozone layer in the upper stratosphere blocks out shorter wavelengths of UV radiation (less than 290 nm) so that UV-C probably does not play a role in melanocyte transformation. Whether this is true with a depleted ozone layer is less certain (Jones et al. [1987]; Van der Lubbe et al. [1988]). An increase in the incidence of basal and squamous cell carcinomas (utilizing data from dose-response models) suggest that for each 1% increase in UV-B (290 to 320 nm), a 1.0 to 2.8% long-term increase in skin cancer is predicted (Fears et al. [1987]). To date, no dose-response model for malignant melanoma has been determined and thus the estimates of the increase in the incidence of cutaneous cancers are on the conservative side (Rogers and Gilchrest [1990]).

UV-B has been thought to be involved in skin cancer in both its initiation (due to its mutagenic effect) and promotion (induction of select growth factors and other effects, e.g., immunosuppressive response). The evidence that links UV radiation and melanocyte transformation in animal systems include studies in mice (Kripke et al.

[1979]; Epstein [1992]; Berkelhammer et al. [1987]; Hussain et al. [1991]), fish (Anders [1991]; Setlow et al. [1989]), and other animal model systems (Ley et al. [1989]). Using a single treatment of 7, 12-dimethylbenz(a)anthracene followed by multiple UV-A or B, Husain et al. (1990) induced blue nevi (80 to 100%), melanomas (20 to 30%), and lymphomas (44 to 63%) in pigmented hairless mice (SKH-hr2). A second model has been developed that uses the South American opossum, *monodelphis domestica*, as the animal lacked the mechanism to repair UV-induced pyrimidine dimers (Ley et al. [1989]). After 70 weeks of UV treatment, 25% of the opossum develop melanocytic tumors. The third animal model involves a fish crossed between platyfish and sword-tail fish (Anders [1991]; Anders and Setlow [1984]). Fish exposed to multiple UV-B irradiation show 20 to 40% tumors.

Exposure to UV radiation also has the potential to interfere with the immunological response to tumors. Two immune responses impaired by high doses of UV radiation are contact hypersensitivity to skin sensitizers and delayed hypersensitivity to complex antigens that may be regulated by antigen-specific suppressor T lymphocytes (Kripke [1990]). Kripke et al. have observed an UV-induced immunosuppressive effect on mice and the inability of UV-irradiated mice to reject transplanted tumor cells (melanoma and other skin cancer cells) as well as the development of primary skin cancer. Thus, UV radiation may not only directly induce skin cancer but also interferes with the body's immune responses to the altered phenotype.

What role UV radiation plays in melanoma causation is still not totally understood at the basic mechanistic level. However, UV radiation has been shown to induce several alterations *in vitro* and *in vivo*, including

1. Induced DNA damage
2. Altered gene expression
3. Changed cell membrane components
4. Inhibition of the antioxidant system

The mutagenic effect of UV radiation has been well characterized. The two types of DNA damage that are considered to be primarily responsible for the lethal and mutagenic effects of UV radiation

are the formation of cyclobutane pyrimidine dimer and (6-4) pyrimidine-pyrimidine photoproducts. Transition and transversion mutations have been observed following *in vitro* UV irradiation (Vrieling et al. [1989]). An assay to measure the repair of UV-induced photoproducts in genes using the enzyme T4 endonuclease V has been developed by Hanawalt (Bohr et al. [1989]). Investigators have shown that the repair of photoproduct adducts is dependent on gene transcription and DNA methylation levels (Bohr et al. [1989]). While cell survival of UV mammalian cells does not correlate with overall genomic DNA repair, it does correlate with repair of essential genes (Bohr et al. [1987]).

Decreased repair of UV radiation-induced pyrimidine dimers has been observed in patients with basal cell carcinomas (Alcalay et al. [1990]). Cancer patients and healthy volunteers were treated with a single dose of solar-simulated radiation (dose equal to one minimal erythema dose), and DNA repair was measured using the T4 endonuclease V assay. There was a decrease in the DNA repair of UV-induced photoproducts in cancer patients compared with healthy volunteers (22 to 33%, respectively). Cell lines derived from patients with hereditary dysplastic nevus syndrome (DNS) were also shown to be hypermutable following exposure to UV radiation (Perera et al. [1986]). These DNS cell lines had similar cell survival values following exposure to UV-C but had a two- to threefold increase in frequency of induced mutants (6-thioguanine resistance assay) compared with cell lines derived from control individuals. To examine the mechanism involved in this UV radiation hypermutability, Seetharam et al. (1989) used a transient shuttle mutagenesis assay. There was an increase in frequency of single base mutations in UV-irradiated plasmids isolated from DNA cell line compared with a normal cell line. Conceivably, a similar situation may occur with melanomas because dysplastic nevi are a precursor to this cancer.

Schothorst et al. (1991) have investigated the induction of pyrimidine dimers and DNA repair in cultured human keratinocytes and melanocytes following UV irradiation. Using monochromatic UV radiation of 254, 297, 302, and a light source emitting predominantly 312 nm, the number of T4 endonuclease V-sensitive sites (ESS) in genomic DNA was determined. The action spectra

for dimer induction in keratinocytes and melanocytes was similar for other cultured mammalian cells. The kinetics and overall genomic DNA repair was found to be similar for both cell types. Nine hours after UV irradiation, 55% of ESS were removed and after 24 h 70% of ESS were removed. Melanocytes were irradiated with a higher dose (250 J/m²) compared with keratinocytes (200 J/m²) to obtain a similar amount of ESS in the cells, so melanin may have some UV radiation protective effect in melanocytes.

There is also evidence that UV radiation may induce photoproducts in the *ras* gene, which is mutated in 10 to 25% of human melanomas. Activated *ras* oncogenes have been found in human squamous cell carcinomas, basal cell carcinomas, and metastatic melanomas. Activated *c-Ha-ras* oncogenes were found in four out of eight human basal and squamous cell carcinomas that occurred on sun-exposed body sites (Anathaswamy et al. [1988]; Anathaswamy and Pierceall [1990]). Activated *ras* (*N*-, *Ki*-, and *Ha*-) oncogenes have also been demonstrated in benign atypical nevi, primary, and metastatic melanomas (Van't Veer et al. [1989]; Shukla et al. [1989]). UV-induced photoproducts may be involved in the activation of these oncogenes. Interestingly, the sites where the point mutations were located in the *ras* genes were at potential pyrimidine dimer sites.

The UV-induced effect at the molecular level may not only be induction of point mutations but also alteration of the expression of various genes in mammalian cells. Induction of the *c-fos* gene following UV irradiation has been observed in HeLa cells (Buscher et al. [1988]; Stein et al. [1989]), CHO cells (Hollander and Fornace, Jr. [1989]; Fornace, Jr. [1988]), and keratinocytes and fibroblasts (Ronai et al. [1988]). Fornace et al. (1988) have isolated DNA damage-inducible transcripts in mammalian cells using a subtractive hybridization procedure following UV irradiation. Some of the genes induced in mammalian cells following UV irradiation are *c-fos*, *c-Ha-ras*, *c-myc*, *c-jun*, collagenase, metallothionein IIa, PKC, ornithine decarboxylase, TGF- α , bFGF, IL-1, IL-6, IGF-1, NGF-R, and *p53* (Ellem et al. [1988]; Kaina et al. [1989]; Rosen et al. [1990]; Matsui and DeLeo [1990]; Mai et al. [1989]; Ronai et al. [1988]; Devany et al. [1991]).

UV irradiation at low doses produces a proliferative response in human melanocytes. Three studies have reported the effect of UV-B on melanogenesis and proliferation (Friedmann and Gilchrest [1987]; Libow et al. [1988]; Schothorst et al. [1991]). We also have investigated the role of UV-B on cell growth and gene expression levels in human melanocytes and have observed an increase in DNA synthesis following UV irradiation with low to moderate doses of UV-B. We also observed an increase in the expression of the *c-jun* RNA transcripts that returned to baseline after 2 h. A slight delay in the induction of *c-fos* RNA transcripts was observed that peaked 2 h after UV irradiation. We are also investigating the ability of chemopreventive agents to act as modulators of UV-induced alterations.

VI. A SYNTHETIC MODEL OF HUMAN MELANOCYTE TRANSFORMATION

We have summarized recent studies on the molecular events that accompany human melanocyte transformation. The use of molecular, cytogenetic, immunological, and biochemical analyses has led to the detection of genetic alterations that may play a role in the transformation of human melanocytes. A probable etiological agent involved in human melanocyte transformation is UV radiation. Based on this information, we propose the following model as one heuristic vehicle by which to investigate melanocyte transformation. We recognize that several aspects of this model are speculative, but the approaches allow experimental solutions.

The first question we address is, Is there a specific group of alterations that are required to obtain the transformed melanocyte? Cytogenetists and molecular biologists have observed specific alterations that occur during human melanocyte transformation. The current model involves alterations on chromosomes 9p, 10q, 6q, 11, 1p, 2, 3, and 7 as well as altered expression levels of the *MGSA*, *EGF-R*, *Ha-ras*, *N-ras*, *bFGF*, *c-kit*, *TGF- α* , *TGF- β 2*, *PDGF-A*, and *c-fos* (Albino and Fountain [1993]). We have added our data involving *c-jun*, *PKC- β _{II}*, *p58^{cl_k-1}*, and *RAR- β* to this model and expanded the involvement of certain genes (Figure 1).

One of the first required alterations may be a deletion of a gene(s) to reduce or inhibit the ability of the primary melanocyte to undergo terminal differentiation. As human melanocytes undergo changes to become dysplastic nevi, a loss in the ability of these cells to undergo normal programmed cell differentiation has been proposed (Clark [1991]). Cytogenetic and molecular research have pointed to a region of chromosome 9p as one that may contain a tumor suppressor gene involved in the earliest stage of human melanocyte transformation (Albino and Fountain [1993]) and *p16* has been proposed to be the candidate gene (Kamb et al. [1994a]; and Nobori et al. [1994]). *p16* (MTS/CDKN2) binds to cyclin-dependent kinase 4 (*cdk4*) and inhibits the ability of *cdk4* to interact with cyclin D, which has been shown to be involved in the cell cycle and to stimulate the passage of cells through the cell cycle (Musgrove et al. [1994]; Serrano et al. [1993]). The gene encoding *p16* is deleted in many tumor cell lines (brain, breast, renal, bone, and melanoma) and contain point mutations in a few melanoma-prone families (Kamb et al. [1994a, 1994b]). As the *p16* protein functions as a negative regulator of cell cycle progression, its gene has the potential to be the melanoma susceptibility locus. However, another group has determined that the *p16* gene was neither deleted nor mutated in a high percentage of their melanoma-prone families (Hussussian et al. [1994]). Further studies will be required to address whether *p16* is the melanoma susceptibility locus on chromosome 9 involved in a significant subset of melanoma or involved in the growth of human tumor cell lines *in vitro*.

A second required alteration may induce the activity of protein kinases resulting in the abnormal phosphorylation of various proteins (e.g., transcription factors, membrane receptors, etc.). Loganzo et al. (1993) found an elevated expression levels of the *p62^{c-yes}* protein and tyrosine kinase activity in melanoma cells (18/20 melanoma cell lines). Easty et al. (1993) also observed that melanoma cells preferentially expressed two families of tyrosine kinases: Eph (Tyro-6 and ECK) and FGF-R (FGF-R4 and Tyro-9). A decrease or loss in the expression of *c-kit* and *c-met* has been observed in melanoma cells also (Natali et al. [1992]; Lassam and Bickford [1992];

Halaban et al. [1992]). c-kit has been thought to play an important role in melanocyte differentiation and growth. Although lost late in melanocyte transformation, loss in the response to the c-kit ligand may allow a melanoma cell to undergo a proliferative response rather than a differentiative response. The expression of bFGF and EGF-R protein levels, which are frequently abnormally expressed, would activate bFGF-R and EGF-R (including other protein kinases), resulting in a proliferate response.

Alterations in protein kinase activities may lead to an increase in cell proliferation and an inability to undergo differentiation. This may result via the activation of growth signals from exogenous and endogenous factors. The protein kinases probably activate transcription factors (e.g., AP-1 and SP-1), which induce the expression of various protooncogenes (e.g., c-fos and c-myc), growth factors (e.g., bFGF and cytokines), and immunosuppressive factors (e.g., TGF- α).

A third required alteration may be the loss in the ability of human melanocytes to protect itself from oxygen-free radicals. Melanocytes are highly susceptible to damage induced by hydrogen peroxide and endogenous reactive oxygen species compared with human fibroblasts and keratinocytes (Yohn et al. [1991]). Melanocytes have decreased peroxidase, catalase, glutathione peroxidase, and superoxide dismutase enzyme activities compared with keratinocytes and fibroblasts. One of the regions involved in melanocyte transformation is chromosome 6q, a region that has been shown to include the gene manganese superoxide dismutase (MnSOD) (Church et al. [1993]). Loss of this gene may result in nonspecific damage to the cell membrane and genomic DNA, which could result in alterations leading to a transformed phenotype.

We propose that one pathway to melanoma is via multiple exposures to UV radiation. We provide the following model in Figure 3 as a heuristic vehicle from which to explore this hypothesis.

FIGURE 3. A synthetic model of human melanocyte transformation.

Figure 3 displays some of the changes that UV radiation may induce in irradiated melanocytes and the surrounding cells. UV irradiation can affect the target cell, the primary melanocyte, as well as the surrounding cells. Following UV irradiation, the target melanocyte may be altered by UV-induced DNA damage and UV-induced gene expression, resulting in various phenotypic effects, including production of growth factors. UV irradiation of the surrounding cells will induce host cell death, resulting in the release of growth factors and a localized area of concentrated growth factors. In some cases, due to the protective effect of melanin as well as being quiescent, the melanocyte will be induced to proliferate rather than cell death being the result. These UV-induced changes may parallel the proliferative response observed in melanocytes *in vitro* (Friedmann and Gilchrest [1987]; Libow et al. [1988]). This proliferative response would "fix" the damaged DNA and allow pyrimidine dimers and single strand breaks to result in point mutations, deletions, and rearrangements. With multiple UV irradiations (e.g., intermittent severe sunburns), several mutations may occur in the target melanocyte and allow receptivity to endogenous and exogenous signals that lead to abnormal cell growth.

Also, UV irradiation will induce a regional immunosuppressive response resulting in loss in ability of the host to detect the mutated melanocyte. As the altered melanocyte is exposed to multiple UV irradiations, the cell will become progressively more abnormal and eventually develop to a fully transformed cell that will have lost the ability to respond to growth control signals. It will also become less immunologically detectable with the self-production of cytokins (to decrease local immunosurveillance from the immune system) and a decrease in the expression levels of histocompatibility antigens.

This model is based on several changes occurring in the same cell involving gene mutations, the abnormal production of growth factors *in situ* by damaged host cells and a decrease in immunosurveillance. Together these effects allow the growth of an altered subpopulation of melanocytes. With multiple UV exposures, this subpopulation of cells may undergo unscheduled DNA replication resulting in the formation of a melanocyte with cumulative molecular abnormalities. Several alterations

would have had to occur in the same cell; however, a parallel model may already exist to study this phenomenon. Albino et al. (1993) have observed multiple changes in melanocytes infected with an activated ras and cultured for several passages. Growth of these transfected melanocytes led to the formation of a select cell population that had lost specific chromosomes (after 6 months) and became fully transformed.

VII. SUMMARY

This review has provided information on alterations in gene expression and the signal transduction pathways in human melanocytes and melanoma cells. The development of an *in vitro* cell culture system was the first step in unraveling the genetic alterations that occur as a human melanocyte transforms and progresses to malignant melanoma. Using biochemical, cytogenetic, and molecular techniques to develop an understanding of the changes that occur, we may yet explain the relevant etiological genetic events in human melanocyte transformation.

Signal transduction pathways do not function as independent units but rather form an interactive network of pathways. This interaction may be synergistic as observed with activators of PKC and PKA in melanocyte proliferation or antagonistic as observed with activators of RAR and other members of the steroid family. It is this combination of proliferation and differentiation signals (Figure 2) that regulates human melanocyte cell growth. Ongoing studies will increase our knowledge of this balance and allow us to develop a further understanding of the key linkages in the different pathways involved as well as the control of their interactive nature. Understanding human melanocyte transformation will allow the accumulation and assimilation of information that will assist clinicians with new diagnostic tools and development of innovative therapies.

ACKNOWLEDGMENTS

We would like to thank the doctors and nurses at Long Beach Memorial Hospital for providing access to human neonatal foreskin (approved by

University of California at Irvine IRB). We are grateful to several colleagues (Drs. I. Verma, R. Tjian, P. Chambon, V. J. Kidd, J. A. Abraham, S. Ohno, and E. H. Stanbridge) for providing plasmids to accomplish our studies.

These studies were supported in part by grants from the National Institutes of Health (CA 27502 and CA 51610).

REFERENCES

- Abraham, J. A., J. L. Whang, A. Tumolo, A. Mergia, J. Friedman, D. Gospodarowicz, and J. C. Fiddes: Human basic fibroblast growth factor: nucleotide sequence and genomic organization. *EMBO J.*, **5**:2523-2528 (1992).
- Ahuja, M. R. and F. Anders: A genetic concept of the origin of cancer, based in part on studies of neoplasms in fishes. *Prog. Exp. Tumor Res.*, **20**:380-397 (1976).
- Akslen, L. A. and O. Mørkve: Expression of p53 protein in cutaneous melanoma. *Int. J. Cancer*, **52**:13-16 (1992).
- Albino, A. P. and J. W. Fountain: Molecular genetics of human malignant melanoma. In: *Current Research and Clinical Management of Melanoma* (L. Nathanson, Ed.) Kluwer Academic Publishers, Norwell, MA, 201-254 (1993).
- Albino, A. P., D. M. Nanus, I. R. Mentle, C. Cordon-Cardo, N. S. McNutt, J. Bressler, and M. Andreeff: Analysis of ras oncogenes in malignant melanoma and precursor lesions: correlation of point mutations with differentiation phenotype. *Oncogene*, **4**:1363-1374 (1989).
- Alcalay, J., S. E. Freeman, L. H. Goldberg, and J. E. Wolf: Excision repair of pyrimidine dimers induced by simulated solar radiation in the skin of patients with basal cell carcinomas. *J. Invest. Dermatol.*, **95**:506-509 (1990).
- Ambach, W. and M. Blumtyhaler: Biological effectiveness of solar UV radiation in humans. *Experientia*, **49**:747-753 (1993).
- Ananthaswamy, H. N. and W. E. Pierceall: Molecular mechanisms of ultraviolet radiation carcinogenesis. *Photochem. Photobiol.*, **52**:1119-1136 (1990).
- Ananthaswamy, H. N., J. E. Price, L. H. Goldberg, and E. S. Bales: Detection and identification of activated oncogenes in human skin cancers occurring on sun-exposed body sites. *Cancer Res.*, **48**:3341-3346 (1988).
- Anders, F.: Contributions of the Gordon-Kosswig melanoma system to the present concept of neoplasia. *Pigment Cell Res.*, **4**:7-29 (1991).
- Arita, Y., K. R. O'Driscoll, and I. B. Weinstein: Growth of human melanocyte cultures supported by 12-*O*-tetradecanoyl phorbol 13-acetate is mediated through protein kinase C activation. *Cancer Res.*, **52**:4514-4521 (1992).
- Bale, S. J., N. C. Dracopli, M. A. Tucker, W. H. Clark, Jr., M. C. Fraser, B. Z. Stanger, P. Green, H. Donis-Keller, D. E. Housman, and M. H. Greene: Mapping the gene for hereditary cutaneous malignant melanoma-dysplastic nevus to chromosome 1p. *N. Engl. J. Med.*, **320**:1367-1372 (1989).
- Ball, N. J., J. J. Yohn, J. G. Morelli, D. A. Norris, L. E. Golitz, and J. P. Hoeffler: Ras mutations in human melanoma: a marker of malignant progression. *J. Invest. Dermatol.*, **102**:285-290 (1994).
- Bauer, J., L. Sokol, J. Stríbmá, M. Kremen, Krajsová, P. Hausner, and P. Hejnar: Amplification of N-myc oncogene in human melanoma cells. *Neoplasia*, **37**:233-238 (1990).
- Becker, D., C. B. Meier, and M. Herlyn: Proliferation of human malignant melanomas is inhibited by antisense oligodeoxynucleotides targeted against basic fibroblast growth factor. *EMBO J.*, **8**:3685-3691 (1989).
- Becker, D., P. L. Lee, U. Rodeck, and M. Herlyn: Inhibition of the fibroblast growth factor receptor 1 (FGFR-1) gene in human melanocytes and malignant melanomas leads to inhibition of proliferation and signs indicative of differentiation. *Oncogene*, **7**:2303-2313 (1992).
- Bennett, D. C.: Genetics, development, and malignancy of melanocytes. In: *International Review of Cytology: A Survey of Cell Biology* (K. W. Jean, J. Jarvik, and M. Friedlander, Eds.) Academic Press, San Diego, CA, 191-260 (1993).
- Berkelhammer, J. and R. W. Oxenhandler: Evaluation of premalignant and malignant lesions during the induction of mouse melanomas. *Cancer Res.*, **47**:1251-1254 (1987).
- Bohr, V. A. and P. C. Hanawalt: Demethylation enhances removal of pyrimidine dimers from the overall genome and from specific DNA sequences in Chinese hamster ovary cells. *Mol. Cell. Biol.*, **9**:1594-1603 (1989).
- Bohr, V. A.: Differential DNA repair within the genome. *Cancer Rev.*, **7**:28-55 (1987).
- Bunnell, B. A., L. S. Heath, D. E. Adams, J. M. Lahti, and V. J. Kidd: Increased expression of a 58-kDa protein kinase leads to changes in the cell cycle. *Proc. Natl. Acad. Sci. U.S.A.*, **87**:7467-7471 (1990).
- Buscher, M., H. J. Rahmsdorf, M. Litfin, M. Karin, and P. Herrlich: Activation of the c-fos gene by UV and phorbol ester: different signal transduction pathways converge to the same enhancer element. *Oncogene*, **3**:301-311 (1988).
- Cannon-Albright, L. A., D. E. Goldgar, L. J. Meyer, C. M. Lewis, D. E. Anderson, J. W. Fountain, M. E. Hegi, R. W. Wiseman, F. M. Petty, A. E. Bale, O. I. Olopade, M. O. Diaz, D. J. Kwiatkowski, M. W. Piepkorn, J. J. Zane, and M. H. Skolnik: Assignment of a locus for familial melanoma, MLM, to chromosome 9p13-p22. *Science*, **258**:1148-1152 (1992).
- Carpenter, G.: Receptors for epidermal growth factor and other polypeptide mitogens. *Ann. Rev. Biochem.*, **56**:881-914 (1987).
- Chambon, P., A. Zelent, M. Petkovich, C. Mendelsohn, P. Leroy, A. Krust, P. Kastner, and N. Brand: The family of retinoic acid nuclear receptors. In: *Retinoids: 10 Years On* (J. -H. Saurat, Ed.) Karger, Basel, 10-27 (1991).

- Chenevix-Trench, G., N. G. Martin, and K. A. O. Ellem: Gene expression in melanoma cell lines and cultured melanocytes: correlation between levels of c-Src-1, c-Myc, and p53. *Oncogene*, **5**:1187-1193 (1990).
- Choi, P. M., K. M. Tchou, and I. B. Weinstein: Overexpression of protein kinase-C in HT29 colon cancer cells causes growth inhibition and tumor suppression. *Mol. Cell. Biol.*, **10**:4650-4657 (1990).
- Church, S. L., J. W. Grant, L. A. Ridnour, L. W. Oberley, P. E. Swanson, P. S. Meltzer, and J. W. Trent: Increased manganese superoxide dismutase expression suppresses the malignant phenotype of human melanoma cells. *Proc. Natl. Acad. Sci. U.S.A.*, **90**:3113-3117 (1993).
- Clark, W. H.: Tumor progression and the nature of cancer. *Br. J. Cancer*, **64**:631-644 (1991).
- Coleman, A., J. W. Fountain, T. Nobori, O. I. Olopade, G. Robertson, D. E. Housman, and T. G. Lugo: Distinct deletions of chromosome 9p associated with melanoma versus glioma, lung cancer, and leukemia. *Cancer Res.*, **54**:344-348 (1994).
- Copeman, M. C. and H. Harris: The extracellular matrix of hybrids between melanoma cells and normal fibroblasts. *J. Cell Sci.*, **91**:281-286 (1988).
- Devary, Y., R. A. Gottlieb, L. F. Lau, and M. Karin: Rapid and preferential activation of the c-jun gene during the mammalian UV response. *Mol. Cell. Biol.*, **11**:2804-2811 (1991).
- De Wit, P. E. J., S. Moretti, P. G. Koenders, M. A. J. Weterman, G. N. P. van Muigen, B. Gianotti, and D. J. Ruiter: Increasing epidermal growth factor receptor expression in human melanocyte tumor progression. *J. Invest. Dermatol.*, **99**: 166-173 (1992).
- De Luca, L.: Retinoids and their receptors in differentiation, embryogenesis, and neoplasia. *FASEB J.*, **5**:2924-2933 (1991).
- Easty, D. J., S. E. Ganz, C. J. Farr, C. Lai, M. Herlyn, and D. C. Bennett: Novel and known protein tyrosine kinases and their abnormal expression in human melanoma. *J. Invest. Dermatol.*, **101**:679-684 (1993).
- Eipers, P. G., B. L. Barnoski, J. Han, A. J. Carroll, and V. J. Kidd: Localization of the expressed human p58 protein kinase chromosomal gene to chromosome 1p36 and a highly related sequence to chromosome 15. *Genomics*, **11**:621-629 (1991).
- Eipers, P. G., J. M. Lahti, and V. J. Kidd: Structure and expression of the human p58^{cl^k-1} protein kinase chromosomal gene. *Genomics*, **13**:613-621 (1992).
- Eisinger, M. and O. Marko: Selective proliferation of normal human melanocytes in vitro in the presence of phorbol ester and cholera toxin. *Proc. Natl. Acad. Sci. U.S.A.*, **79**:2018-2022 (1982).
- Elder, J. T., G. J. Fisher, Q. -Y. Zhang, D. Eisen, A. Krust, P. Kastner, P. Chambon, and J. J. Voorhees: Retinoic acid receptor gene expression in human skin. *J. Invest. Dermatol.*, **96**:425-433 (1991).
- Ellem, K. A., M. Cullinan, K. C. Baumann, and A. Dunstan: UVR induction of TGF- α : a possible autocrine mechanism for the epidermal melanocytic response and for promotion of epidermal carcinogenesis. *Carcinogenesis*, **9**:797-801 (1988).
- Epstein, J. H.: Experimental models for primary melanoma. *Photodermatol. Photoimmunol., Photomed.*, **9**:91-98 (1992).
- Fears, T., J. Scotto, and M. A. Schneiderman: Mathematical models of age and ultraviolet effects on the incidence of skin cancer among whites in the United States. *Am. J. Epidemiol.*, **105**:420-427 (1987).
- Florkiewicz, R. Z. and A. Sommer: Human basic fibroblast growth factor gene encodes four polypeptides: three initiate translation from non-AUG codons. *Proc. Natl. Acad. Sci. U.S.A.*, **86**:3978-3981 (1989).
- Fornace, A. J., Jr., I. Alamo, Jr., and M. C. Hollander: DNA damage-inducible transcripts in mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.*, **85**:8800-8804 (1988).
- Fountain, J. W., M. Karayiorgou, M. S. Ernstoff, J. M. Kirkwood, D. R. Vlock, L. Titus-Ernstoff, B. Bouchard, S. Vihayasaradhi, A. N. Houghton, J. Lahti, V. J. Kidd, D. E. Housman, and N. C. Dracopoli: Chromosome 9p involvement in melanoma. *Proc. Natl. Acad. Sci. U.S.A.*, **89**:10557-10561 (1992).
- Friedmann, P. S. and B. A. Gilchrest: Ultraviolet radiation directly induces pigment production by cultured human melanocytes. *J. Cell. Physiol.*, **133**: 88-94 (1987).
- Gospodarowicz, D.: Biological activities of fibroblast growth factors. In: *The Fibroblast Growth Factor Family*. (A. Baird and M. Klagsbrun, Eds.) New York Academy of Sciences, New York, 1-8 (1991).
- Gruber, J. R., S. Ohno, and R. M. Niles: Increased expression of protein kinase C α plays a key role in retinoic acid-induced melanoma differentiation. *J. Biol. Chem.*, **267**:13356-13360 (1992).
- Halaban, R., S. Ghosh, and A. Baird: bFGF is the putative natural growth factor for human melanocytes. *In Vitro Cell Dev. Biol.*, **23**:47-52 (1987).
- Halaban, R., B. S. Kwon, S. Ghosh, P. Delli Bovi, and A. Baird: bFGF as an autocrine growth factor for human melanomas. *Oncogene Res.*, **3**:177-186 (1988).
- Halaban, R., J. S. Rubin, Y. Funasaka, M. Cobb, T. Boulton, D. Faletto, E. Rosen, A. Chan, K. Yoko, W. White, C. Cook, and G. Moellmann: Met and hepatocyte growth factor/scatter factor signal transduction in normal melanocytes and melanoma cells. *Oncogene*, **7**:2195-2206 (1992).
- Hanawalt, P. C.: Heterogeneity of DNA repair at the gene level. *Mutat. Res.*, **247**:203-211 (1991).
- Hart, I. R., J. Rao, and R. E. Wilson: c-AMP-induced c-fos expression in cells of melanocyte origin. *Biochem. Biophys. Res. Commun.*, **159**:408-413 (1989).
- Harris, C. C.: p53: at the crossroads of molecular carcinogenesis and risk assessment. *Science*, **262**:1980-1981 (1994).
- Hecht, F.: The annals of cancer genetics: the description by Norris of hereditary malignant melanoma of the skin in 1820. *Cancer Genet. Cytogenet.*, **42**:153-156 (1989).
- Herlyn, M.: *Molecular and Cellular Biology of Melanoma*, R. G. Landes Co., Austin, Texas (1993).
- Holland, E. A., S. C. Beaton, B. G. Edwards, R. F. Kefford, and G. J. Mann: Loss of heterozygosity and homozy-

- gous deletions on 9p21-22 in melanoma. *Oncogene*, **9**:1361-1365 (1994).
- Hollander, M. C. and A. J. Fornace, Jr., Induction of fos RNA by DNA-damaging agents. *Cancer Res.*, **49**:1687-1692 (1989).
- Holt, J. T., T. V. Gopal, A. D. Moulton, and A. W. Nienhuis: Inducible production of c-fos antisense RNA inhibits 3T3 proliferation. *Proc. Natl. Acad. Sci. U.S.A.*, **83**:4794-4798 (1986).
- Housey, G. M., M. D. Johnson, W. L. W. Hsiao, C. A. O'Brian, J. P. Murphy, P. Kirschmeier, and I. B. Weinstein: Overproduction of protein kinase C causes disordered growth control in rat fibroblasts. *Cell*, **52**:343-354 (1988).
- Hussain, Z., M. A. Pathak, T. Flotte, and M. M. Wick: Role of ultraviolet radiation on the induction of melanocytic tumors in hairless mice following 7,12-dimethylbenz (a)anthracene application and ultraviolet irradiation. *Cancer Res.*, **51**:4964-4970 (1991).
- Hussussian, C. J., J. P. Struewing, A. M. Goldstein, P. A. Higgins, D. S. Ally, M. D. Sheahan, W. H. Clark, Jr., M. A. Tucker, and N. C. Dracopoli: Germline p16 mutations in familial melanoma. *Nature Genet.*, **8**:15-21 (1994).
- Isshiki, K., B. A. Seng, D. E. Elder, D. Guerry, and A. J. Linnenbach: Chromosome 9 deletion in sporadic and familial melanomas *in vivo*. *Oncogene*, **9**:1649-1653 (1994).
- Jackowski, S., C. W. Rettenmier, C. J. Sherr, and C. O. Rock: A guanine nucleotide-dependent phosphatidylinositol-4,5-diphosphate phospholipase C in cells transformed by the v-fms and v-fes oncogenes. *J. Biol. Chem.*, **261**:4978-4985 (1986).
- Jenumein, T., D. Muller, T. Curran, and R. Muller: Extended lifespan and tumorigenicity of nonestablished mouse connective tissue cells transformed by the fos oncogene of FBR-MuSV. *Cell*, **41**:629-637 (1985).
- Jiang, H., S. Waxman, and P. B. Fisher: Regulation of c-fos, c-jun, and jun-B gene expression in human melanoma cells induced to terminally differentiate. *Mol. Cell. Diff.*, **1**:197-214 (1993).
- Jones, C. A., E. Huberman, M. L. Cunningham, and M. J. Peak: Mutagenesis and cytotoxicity in human epithelial cells by far- and near-ultraviolet radiations: action spectra. *Radiat. Res.*, **110**:244-254 (1987).
- Kaina, B., B. Stein, A. Schöenthal, H. J. Rahmsdorf, H. Ponta, and P. Herrlich: An update of the mammalian UV response: gene regulation and induction of a protective function. In: *DNA Repair Mechanisms and Their Biological Implications in Mammalian Cells* (M. W. Lambert and J. Laval, Eds.) Plenum Press, New York, 149-165 (1989).
- Kamb, A., N. A. Gruis, J. Weaver-Feldhaus, Q. Liu, K. Harshman, S. V. Tavtigian, E. Stockert, R. S. Day, III, B. E. Johnson, and M. H. Skolnick: A cell cycle regulator potentially involved in genesis of many tumor types. *Science*, **264**:436-440 (1994a).
- Kamb, A., D. Shattuck-Eidens, R. Eeles, N. A. Gruis, Q. Liu, N. A. Gruis, W. Ding, C. Hussey, T. Tran, Y. Miki, J. Weaver-Feldhaus, M. McClure, J. F. Aitken, D. E. Anderson, W. Bergman, R. Frants, D. E. Goldgar, A. Green, R. MacLennan, N. G. Martin, L. J. Meyer, P. Youl, J. J. Zone, M. H. Skolnick, and L. A. Cannon-Albright: Analysis of the p16 gene (CDKN2) as a candidate for the chromosome 9p melanoma susceptibility locus. *Nature Genet.*, **8**:22-26 (1994b).
- Karin, M. and P. Herrlich: Cis- and trans-acting genetic elements responsible for induction of specific genes by tumor promoters, serum factors, and stress. In: *Genes and Signal Transduction in Multistage Carcinogenesis*. (N. H. Colburn, Ed.) Marcel Dekker, New York, 415-440 (1989).
- Kath, R., U. Rodeck, H. D. Menssen, M-L. Mancianti, A. J. Linnenbach, D. E. Elder, and M. Herlyn: Tumor progression in the human melanocytic system. *Anticancer Res.*, **9**:865-872 (1989).
- Kimelman, D. and M. W. Kirschner: An antisense mRNA directs the covalent modification of the transcript encoding fibroblast growth factor in *Xenopus* oocytes. *Cell*, **59**:687-696 (1989).
- Kripke, M. L.: Speculations on the role of ultraviolet radiation in the development of malignant melanoma. *J. Natl. Cancer Inst.*, **63**:541-548 (1979).
- Kripke, M. L.: Effects of UV radiation on tumor immunity. *J. Natl. Cancer Inst.*, **82**:1392-1396 (1990).
- Kurokawa, T., R. Sasada, M. Iwane, and K. Igarashi: Cloning and expression of cDNA encoding human basic fibroblast growth factor, *FEBS Lett.*, **213**:189-194 (1987).
- Lassam, N. and S. Bickford: Loss of c-Kit expression in cultured melanoma cells. *Oncogene*, **7**:51-56 (1992).
- Lassam, N. J., L. From, and H. J. Kahn: Overexpression of p53 is a late event in the development of malignant melanoma. *Cancer Res.*, **53**:2235-2238 (1993).
- Lee, S. -T., K. M. Struck, and R. A. Spritz: A survey of protein tyrosine kinase mRNAs expressed in normal human melanocytes. *Oncogene*, **8**:3403-3410 (1993).
- Lenardo, M., A. K. Rustgi, A. R. Schievella, and R. Bernards: Suppression of MHC class I gene expression by N-myc through enhancer inactivation. *EMBO J.*, **8**:3351-3355 (1989).
- Ley, R. D., L. A. Applegate, R. S. Padilla, and T. D. Stuart: Ultraviolet radiation-induced malignant melanoma in *Monodelphis domestica*. *Photochem. Photobiol.*, **50**:1-5 (1989).
- Libow, L. F., S. Scheide, and V. A. DeLeo: Ultraviolet radiation acts as an independent mitogen for normal human melanocytes in culture. *Carcinogenesis*, **1**:397-401 (1988).
- Linnenbach, A. J., K. Huebner, E. P. Reddy, M. Herlyn, A. H. Parmiter, P. C. Nowell, and H. Koprowski: Structural alteration in the MYB protooncogene and deletion in the MYB protooncogene and deletion within the gene encoding alpha-type protein kinase C in human melanoma cell lines. *Proc. Natl. Acad. Sci. U.S.A.*, **85**:74-78 (1988).
- Loganzo, F., Jr., J. S. Dosik, Y. Zhao, M. J. Vidal, D. M. Nanus, M. Sudol, and A. P. Albino: Elevated expression of protein tyrosine kinase c-Yes, but not c-Src, in

- human malignant melanoma. *Oncogene*, **8**:2637–2644 (1993).
- Longstreth, J.: Cutaneous malignant melanoma and ultraviolet radiation: a review. *Cancer Metastasis Rev.*, **7**:321–333 (1988).
- Ludwig, K. W., B. Loewy, and R. M. Niles: Retinoic acid increases cyclic AMP-dependent protein kinase activity in mure melanoma cells. *J. Biol. Chem.*, **255**:5999–6002 (1980).
- Mai, S., B. Stein, S. Van den Berg, B. Kaina, C. Lucke-Huhle, H. Ponta, H. J. Rahmsdorf, M. Kraemer, S. Gebel, and P. Herrlich: Mechanisms of the ultraviolet light response in mammalian cells. *J. Cell Sci.*, **94**:609–615 (1989).
- Maller, J. L.: Mitotic control. *Current Opin. Cell Biol.*, **3**:269–275 (1991).
- Mangelsdorf, D. J. and R. M. Evans: Vitamin A receptors: new insights on retinoid control of transcription. In: *Retinoids in Normal Development and Teratogenesis* (G. Morriss-Kay, Ed.) Oxford University Press, Oxford, England (1992).
- Matsui, M. S. and V. A. DeLeo: Induction of protein kinase C activity by ultraviolet radiation. *Carcinogenesis*, **11**:229–234 (1990).
- Megidish, T. and N. Mazurek: A mutant protein kinase C that can transform fibroblasts. *Nature*, **342**:807–811 (1989).
- Miller, A. D., T. Curran, and I. M. Verma: c-fos protein can induce cellular transformation: a novel mechanism of activation of a cellular oncogene. *Cell*, **36**:51–60 (1984).
- Montano, X., M. Shamsher, P. Whitehead, K. Dawson, and J. Newton: Analysis of p53 in human cutaneous melanoma cell lines. *Oncogene*, **9**:1455–1459 (1994).
- Murphy, P. R., Y. Sato, R. Sato, and H. G. Friesen: Regulation of multiple basic fibroblast growth factor messenger ribonucleic acid transcripts by protein kinase C activators. *Mol. Endocrinol.*, **2**:1196–1201 (1988).
- Nairn, A. C. H., H. C. Hemmings, and P. Greengard: Protein kinases in the brain. *Annu. Rev. Biochem.*, **54**:931–976 (1985).
- Natali, P. G., M. R. Nicotra, A. B. Winkler, R. Cavaliere, A. Bigotti, and A. Ullrich: Progression of human cutaneous melanoma is associated with loss of expression of c-kit protooncogene receptor. *Int. J. Cancer*, **52**:197–201 (1992).
- Nishikura, K. and J. M. Murray: Antisense RNA of protooncogene c-fos blocks renewed growth of quiescent 3T3 cells. *Mol. Cell. Biol.*, **7**:639–649 (1987).
- Nishizuka, Y.: The molecular heterogeneity of protein kinase C and its implications for cellular regulation. *Nature*, **334**:661–665 (1988).
- Nishikura, K. and J. M. Murray: Antisense RNA of protooncogene c-fos blocks renewed growth of quiescent 3T3 cells. *Mol. Cell. Biol.*, **7**:639–649 (1987).
- Nobori, T., K. Miura, D. J. Wu, A. Lois, K. Takabayashi, and D. A. Carson: Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. *Nature*, **368**:753–756 (1994).
- Ohno, S., Y. Akita, A. Hata, S. I. Osada, K. Kubo, Y. Konno, K. Akimoto, K. Mizuno, T. Saido, T. Kuroki, and K. Suzuki: Structural and functional diversities of a family of signal transducing protein kinases, protein kinase C family; two distinct classes of PKC, conventional and novel nPKC. *Adv. Enzyme Regul.*, **31**:287–303 (1991).
- Oka, M., K. Ogita, N. Saito, and Y. Mishima: Selective increase of the α subspecies of protein kinase c and inhibition of melanogenesis induced by retinoic acid in melanoma cells. *J. Invest. Dermatol.*, **100**:204S–208S (1993).
- Park, H. -Y., V. Russakovsky, S. Ohno, and B. A. Gilchrist: The isoform of protein kinase C stimulated human melanogenesis by activating tyrosinase in pigment cells. *J. Biol. Chem.*, **268**:11742–11749 (1993).
- Parmiter, A. H. and P. C. Nowell: Cytogenetics of melanocytic tumors. *J. Invest. Dermatol.*, **100**:354S–258S (1993).
- Perera, M. I. R., K. I. I. Um, M. H. Greene, H. L. Waters, A. Bredberg, and K. H. Kraemer: Hereditary dysplastic nevus syndrome: lymphoid cell ultraviolet hypermutability in association with increased melanoma susceptibility. *Cancer Res.*, **46**:1005–1009 (1986).
- Ponta, H., A. C. B. Cato, and P. Herrlich: Interference of pathway specific transcription factors. *Biochim. Biophys. Acta*, **1129**:255–261 (1992).
- Powell, M. B., R. K. Rosenberg, M. J. Graham, M. L. Birch, D. T. Yamanishi, J. A. Buckmeier, and F. L. Meyskens, Jr.: PKC β expression in melanoma cells and melanocytes: differential expression correlates with biological responses to TPA. *J. Cancer Res. Clin. Oncol.*, **119**:199–206 (1992).
- Preiss, J., C. Loomis, W. Bishop, R. Stein, J. Nidel, and R. M. Bell: Quantitative measurement of sn-1,2-diacylglycerols present in platelets, hepatocytes, and ras- and sis-transformed normal kidney cells. *J. Biol. Chem.*, **261**:8597–8600 (1986).
- Rodeck, U., K. Melber, R. Kath, H. -D. Menssen, M. Varello, B. Atkinson, and M. Herlyn: Constitutive expression of multiple growth factor genes by melanoma cells but not normal melanocytes. *J. Invest. Dermatol.*, **97**:20–26 (1991).
- Rogeljs, S., B. Loewy, and R. M. Niles: The effect of retinoic acid on cyclic AMP-binding protein in mouse melanoma cells. *Eur. J. Biochem.*, **139**:351–357 (1984).
- Rogers, G. S. and B. A. Gilchrist: The senile epidermis: environmental influences on skin aging and cutaneous carcinogenesis. *Br. J. Dermatol.*, **122**:55–60 (1990).
- Ronai, Z. A., E. Okin, and I. B. Weinstein: Ultraviolet light induces the expression of oncogenes in rat fibroblast and human keratinocyte cells. *Oncogene*, **2**:201–204 (1988).
- Rosen, C. F., D. Gajic, and D. J. Drucker: Ultraviolet radiation induction of ornithine decarboxylase in rat keratinocytes. *Cancer Res.*, **50**:2631–2635 (1990).
- Sassone-Corsi, P., W. W. Lamph, and I. M. Verma: Regulation of fos gene: a paradigm for nuclear oncogenes. *Cold Spring Harbor Symp. Quant. Biol.*, **53**:749–760 (1988).
- Schrier, P. I. and L. T. C. Peltenburg: Relationship between myc oncogene activation and MHC class I expression.

- In: *Advances in Cancer Research*** (G. F. Vande Woude and G. Klein, Eds.) Academic Press, New York, 181–246 (1993).
- Schothorst, A. A., L. M. Evers, K. C. Noz, R. Filon, and A. A. van Zeeland: Pyrimidine dimer induction and repair in cultured human skin keratinocytes or melanocytes after irradiation with monochromatic ultraviolet radiation. *J. Invest. Dermatol.*, **96**:916–920 (1991).
- Schutte, J., J. Viallet, M. Nau, S. Segal, J. Fedorko, and J. Minna: Jun-B inhibits and c-fos stimulates the transforming and trans-activating activities of c-jun. *Cell*, **59**:987–997 (1989).
- Scott, G., M. Stoler, S. Sarkar, and R. Halaban: Localization of basic fibroblast growth factor mRNA in melanocytic lesions by in situ hybridization. *J. Invest. Dermatol.*, **96**:318–322 (1991).
- Seetharam, S., H. L. Waters, M. M. Seidman, and K. H. Kraemer: Ultraviolet mutagenesis in a plasmid vector replicated in lymphoid cells from a patient with the melanoma-prone disorder dysplastic nevus syndrome. *Cancer Res.*, **49**:5918–5921 (1989).
- Selby, H. M., R. S. Sherman, and G. T. Pack: A roentgen study of bone metastases from melanoma. *Radiology*, **67**:224–228 (1956).
- Setlow, R. B., A. D. Woodhead, and E. Grist: Animal model for ultraviolet radiation-induced melanoma: platyfish-swordtail hybrid. *Proc. Natl. Acad. Sci. U.S.A.*, **86**:8922–8926 (1989).
- Serrano, M., G. J. Hannon, and D. Beach: A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature*, **366**:704–707 (1993).
- Shukla, V. K., D. C. Hughes, L. E. Hghes, F. McCormick, and R. A. Padua: Ras mutations in human melanotic lesions: K-ras activation is a frequent and early event in melanoma development. *Oncogene Res.*, **5**:121–127 (1989).
- Stein, B., H. J. Rahmsdorf, A. Steffen, M. Litfin, and P. Herrlich: UV-induced DNA damage is an intermediate step in UV-induced expression of human immunodeficiency virus type I, collagenase, c-fos, and methallothionein. *Mol. Cell. Biol.*, **9**:5169–5181 (1989).
- Stretch, J. R., K. C. Gatter, E. Ralfkiaer, D. P. Lane, and A. L. Harris: Expression of mutant p53 in melanoma. *Cancer Res.*, **51**:5976–5979 (1991).
- Thompson, S. A., A. A. Protter, L. Bitting, J. C. Fiddes, and J. A. Abraham: Cloning, recombinant expression, and characterization of basic fibroblast growth factor. **In: *Methods in Enzymology*** (D. Barnes, J. P. Mather, and G. H. Sato, Eds.) Academic Press, New York, 96–116 (1991).
- Trent, J. M., S. B. Rosenfeld, and F. L. Meyskens: Chromosome 6q involvement in human malignant melanoma. *Cancer Genet. Cytogenet.*, **9**:177–180 (1983).
- Trent, J. M., E. J. Stanbridge, H. L. McBride, E. U. Meese, G. Casey, D. E. Araugo, C. M. Witkowski, and R. B. Nagle: Tumorigenicity of human melanoma cell lines controlled by introduction of human chromosome 6. *Science*, **247**:568–571 (1990).
- Urteaga, B., Oscar, and G. T. Pack: On the antiquity of melanoma. *Cancer*, **5**:607–610 (1966).
- Van der Lubbe, J. L., H. J. Rosdoff, J. L. Bos, A. J. Vander Eb, and H. Witschi: Active ozone, nitrogen dioxide, and lung cancer: a review of some recent issues and problems. *Toxicity*, **48**:1–20 (1988).
- Van't Veer, L. J., B. M. T. Burgering, R. Versteeg, A. J. M. Boot, D. J. Ruiter, S. Osanto, P. I. Schrier, and J. L. Bos: N-ras mutations in human cutaneous melanoma from sun-exposed body sites. *Mol. Cell. Biol.*, **9**:3114–3116 (1989).
- Verma, I.: Protooncogene fos: a multifaceted gene. *Trends Genet.*, **2**:93–96 (1986).
- Versteeg, R., K. M. Krusen-Walters, A. C. Plomb, A. A. A. van Leeuwen, N. J. Stam, H. L. Pleogh, D. J. Ruiter, and P. I. Schrier: Suppression of class I human histocompatibility leukocyte antigen by c-myc in locus specific. *J. Exp. Med.*, **170**:621–635 (1989).
- Versteeg, R., I. A. Noordermeer, M. Krüse-Wolters, D. J. Ruiter, and P. I. Schrier: c-myc down-regulates class I HLA expression in human melanomas. *EMBO J.*, **7**:1023–1029 (1988).
- Volk, R., M. Koster, A. Poting, L. Hartmann, and W. Knochel: Antisense transcript from the *Xenopus laevis* bFGF gene coding for an evolutionary conserved 24 kD protein. *EMBO J.*, **8**:2983–2988 (1989).
- Volkenandt, M., U. Schlegel, D. M. Nanus, and A. P. Albino: Mutational analysis of the human p53 gene in malignant melanoma. *Pigment Cell Res.*, **4**:35–40 (1991).
- Vrieling, H., M. L. Van Rooijen, N. A. Groen, M. Z. Zdzienicka, J. W. I. M. Simons, P. H. M. Lohman, and A. A. Van Zeeland: DNA strand specificity for UV-induced mutations in mammalian cells. *Mol. Cell. Biol.*, **9**:1277–1283 (1989).
- Welch, D. R., P. Chen, M. E. Miele, C. T. McGary, J. M. Bower, E. J. Stanbridge, and B. E. Weissman: Micro-mediated transfer of chromosome 6 into metastatic human C8161 melanoma cells suppresses metastasis but does not inhibit tumorigenicity. *Oncogene*, **9**:255–262 (1994).
- Yamanishi, D. T., J. A. Buckmeier, and F. L. Meyskens, Jr.: Expression of c-jun, jun-B, and c-fos oncogenes in human primary melanocytes and metastatic melanomas. *J. Invest. Dermatol.*, **97**:349–353 (1991a).
- Yamanishi, D. T., M. Graham, J. A. Buckmeier, and F. L. Meyskens, Jr.: The differential expression of protein kinase C in normal human neonatal melanocytes and metastatic melanomas. *Carcinogenesis*, **12**:105–109 (1991b).
- Yamanishi, D. T., R. Z. Florkiewicz, J. A. Buckmeier, M. J. Graham, and F. L. Meyskens, Jr.: Expression of bFGF in normal human neonatal melanocytes and metastatic melanomas. *Cancer Res.*, **52**:5024–5029 (1992).
- Yasuda, H., H. Kobayashi, A. Ohkawara, and N. Kuzumaki: Differential expression of ras oncogene products among the types of human melanomas and melanocytic nevi. *J. Invest. Dermatol.*, **93**:54–59 (1989).
- Yohn, J. J., D. A. Norris, D. G. Yrastorza, I. J. Buno, J. A. Leff, S. S. Hake, and J. E. Repine: Disparate antioxidant enzyme activities in cultured human cutaneous fibroblasts, keratinocytes, and melanocytes. *J. Invest. Dermatol.*, **97**:405–409 (1991).