The Origins of Human Cancer: Molecular Mechanisms of Carcinogenesis and Their Implications for Cancer Prevention and Treatment—Twenty-seventh G. H. A. Clowes Memorial Award Lecture

I. Bernard Weinstein

Abstract

Epidemiological studies provide evidence that environmental factors (external agents such as chemicals, radiation, and viruses) play a major role in the causation of the majority of human tumors. This is a highly optimistic message, since it implies that cancer is largely a preventable disease. To meet this challenge we must, however, understand the mechanisms of cancer causation at the cellular and molecular levels and, in a parallel effort, develop new laboratory methods that can be used to identify specific causative agents in humans. The approach must be comprehensive since it is likely that human cancers are due to complex interactions between multiple factors, including the combined actions of chemical and viral agents. This paper reviews recent studies from our laboratory and studies by other investigators related to these themes.

A major principle in studies on mechanisms of carcinogenesis is that the process proceeds through multiple discernible stages, including initiation, promotion, and progression. It is likely that the transition between these stages is driven by different environmental and endogenous factors and involves different biochemical mechanisms and genetic elements. Several types of chemicals initiate the carcinogenic process by yielding highly reactive species that bind covalently to cellular DNA. Our group has elucidated the details of this process with two groups of compounds, aromatic amines and polycyclic aromatic hydrocarbons, emphasizing how these agents distort the conformation of DNA and its functions during DNA replication and transcription. The implications of these findings with respect to oncogene activation, DNA amplification, gene transposition, and chromosome translocations are discussed. Our studies on tumor promotion have concentrated on the mechanisms of action of the potent tumor promoter 12-O-tetradecanoylphorbol-13-acetate. Studies from several laboratories indicate that this agent and related compounds produce their effects by activating a specific cellular enzyme, protein kinase C (PKC). This produces a cascade of events which include alterations in gene expression and, ultimately, changes in cellular differentiation and proliferation. Recent studies on the isolation and stable overexpression of a cloned DNA sequence that encodes PKC are described. The results obtained provide direct evidence that PKC plays a critical role in growth control. The possible role of PKC, and other mediators of signal transduction pathways, in the origin of certain human cancers is also discussed.

Finally, this paper discusses how insights into molecular mechanisms of carcinogenesis provide new approaches to the detection of specific causes of human cancer, using an approach termed "molecular cancer epidemiology," and also new strategies for cancer prevention and treatment.

Introduction

Maimonides, the famous 12th century Hebrew scholar and physician, said "honor your teachers because they have brought you into the world of the future." My career was molded by several wonderful teachers, beginning with my parents who, as immigrants from Czarist Russia, did not have a formal education but inspired a dedication to learning in their children. Other inspirational teachers included: William S. Middleton, who stimulated my interest in academic medicine when I was a medical student at the University of Wisconsin; Daniel Laszlo, whose compassion for the cancer patient and clinical insights stimulated my interest in oncology when I was a Medical Resident at Montefiore Hospital; Nathaniel Berlin, who guided me in clinical research when I was a Clinical Associate at the National Cancer Institute; Boris Magasanik, who taught me biochemistry and how to think at the cellular and molecular levels when I was a research fellow at MIT; Alfred Gellhorn, who fostered my career in cancer research and continues to provide a role model as a physician/scientist and humanitarian; and Jacob Furth, who shared with me his insights into tumor biology and served as a role model for commitment to research, students, and colleagues. I also want to acknowledge the inspiration of several leaders in the field of chemical carcinogenesis, especially Charles Heidelberger, Elizabeth and James Miller, Ross Boutwell, and Harold Rusch. It is curious that all five of these individuals are from Wisconsin, and so am I. There must be something in the Wisconsin environment that encourages cancer research.

I am also grateful to numerous colleagues and students with whom I have worked over the past 25 years. In particular, I am indebted to Dezider Grunberger with whom I have had a very productive collaboration and warm friendship for many years. I am also grateful to Columbia University and the Columbia-Presbyterian Medical Center, which has provided an intellectually rich environment and marvelous resources for the pursuit of my research. Finally, I am indebted to the National Cancer Institute, the American Cancer Society, and other funding sources for providing the funding required for our research.

At the beginning of this century the major cause of death in the United States was infectious diseases. Due to a combination of basic research and prevention and treatment approaches, death from these diseases has been markedly reduced. Thus, as we approach the latter decade of the 20th century, cancer has become a leading cause of death in this country and in many other countries throughout the world (1). The challenge that currently faces cancer researchers is the following: how can we mount effective research and clinical approaches so that within a few decades we can achieve a decline in cancer mortality similar to that achieved during the earlier part of this century in the field of infectious diseases? Recently, there has been a good deal of rhetoric about whether or not we are "winning the war on cancer." This rhetoric, like war itself, can be destructive. Progress in the field of cancer will not come from a single short-sighted approach but rather from a long-term commitment to both fundamental laboratory and clinical research, and a pluralistic approach that includes research on cancer causa-
tion, prevention, and treatment, as well as skilled and compassionate care of patients with cancer. In fact, what makes the current period of cancer research so exciting is that advances in molecular genetics, cellular biology, carcinogenesis, virology, immunology, and therapeutics are providing unifying concepts and methods that bring together investigators from diverse fields of cancer research. The following quotation from H. L. Mencken is relevant to the need for a pluralistic approach to the cancer problem: "For any complex question there is almost certainly a simple answer that is almost always wrong."

In this paper I will provide an overview of recent advances in our understanding of the molecular and cellular mechanisms by which various chemical agents influence the multistage carcinogenic process, thus resulting in the evolution of malignant tumor cells. I will also provide examples of how advances in these areas of research are likely to provide new and effective strategies for both cancer prevention and treatment. Since this paper provides a broad overview, the reader is referred to more specific review articles for detailed references.

Multistage Carcinogenesis: Initiation

The Multistage Process Predicts Multiple Mechanisms and Genes. The development of a fully malignant tumor involves complex interactions between several factors, both environmental (i.e., exogenous) and endogenous (i.e., genetic, hormonal, etc.). In addition, carcinogenesis often proceeds through multiple discernible stages (initiation, promotion, progression) (Fig. 1) and the overall process can occupy a major fraction of the life span of the individual (2–6). The transitions between successive stages can be enhanced or inhibited by different types of agents. Thus, it appears that the individual stages may involve qualitatively different mechanisms at the cellular and genetic levels. These aspects predict that the establishment and maintenance of a malignant tumor involve multiple cellular genes and multiple types of changes in genomic structure and function.

Action of Initiating Agents. Agents that initiate the carcinogenic process often do so by damaging cellular DNA. Indeed this has become an axiom in the field of chemical carcinogenesis (5–7). This principle is well illustrated with the chemical benzo(a)pyrene, an ubiquitous carcinogen that is representative of a large number of polycyclic aromatic hydrocarbon carcinogens (5, 7, 8). The compound is not active as such but undergoes metabolism by the microsomal cytochrome P-450 monoxygenase system to yield the highly reactive metabolite BPDE. Several years ago, Dr. Alan Jeffrey in our research group at Columbia University provided evidence that a specific isomer of BPDE preferentially reacts with the 2-amino group of guanine residues present in nucleic acids to yield the structure shown in Fig. 2. We also obtained evidence that, when bound to double-stranded DNA, the bulky carcinogen residue lies in the minor groove of the DNA helix (Fig. 3). This is in contrast to the situation found with the aromatic amine carcinogen N-2-acetylaminofluorene, which, following metabolic activation,
becomes bound to the C-8 position of guanine residues and results in a major distortion of the DNA helix which we termed base-displacement (Fig. 3) (5, 9). The precise conformational changes in the structure of DNA produced by the covalent binding of various aromatic amine carcinogens have been elucidated by my colleague Dr. Dezider Grunberger (9). An important principle that has emerged is that the modification of specific sequences in DNA by certain carcinogens can induce the Z DNA conformation. The modification of DNA by less bulky carcinogens, i.e., methylating or ethylating agents, causes functional disturbances by directly interfering with base pairing, rather than through major changes in DNA conformation. Thus, we are beginning to understand, at the molecular level, the multiple ways in which initiating carcinogens attack DNA and the types of conformational and functional changes in DNA that result from these modifications (for a detailed review of this subject see Refs. 5 and 9).

Critical Genetic Targets: Oncogenes, Tumor Suppressor Genes, Transcriptional Regulatory Sequences. As advances in the above area of research were proceeding, studies with RNA tumor viruses revealed the existence of a set of tumor-causing genes, the oncogenes (for review see Refs. 10–13). This was followed by the finding that a set of related genes, termed “protooncogenes,” normally exists in the genomes of higher organisms and the accumulating evidence that the latter genes code for components of signal transduction pathways that play a role in controlling normal growth and differentiation. These findings led to the concept that initiating carcinogens might act by mutating protooncogenes to produce “activated” oncogenes and thus lead to abnormalities in growth control and differentiation (Fig. 4). Indeed, there is accumulating evidence that several types of tumors induced in rodents by chemical carcinogens and certain tumors in humans are associated with base substitutions at specific sites in the c-ras oncogenes (10–13). As this data base expands, however, it is apparent that there is considerable variation between tumors in the same or different tissues in terms of whether or not they carry a mutated ras gene and the type of mutation seen, even when the tumors were induced by the same carcinogen. Furthermore, the types of base substitutions seen do not always correlate with the known chemistry of carcinogen-DNA modifications, and in some cases it appears that the ras mutation occurred after the initiating event. Therefore, I am not convinced that these mutations represent the major mechanism of initiation. Elsewhere (13), I have predicted that, given the multiplicity of signal transduction pathways and the overall complexity of growth control and differentiation, there probably exist more than 300 protooncogenes in the mammalian genome. As probes for these genes become available, I suspect that we will discover numerous other oncogene mutations in carcinogen-induced tumors.

In addition to protooncogenes, I believe that two other classes of DNA sequences that are normally present in the mammalian genome, tumor suppressor genes and transcriptional regulatory sequences, may also be critical targets during chemical carcinogenesis (Fig. 4) (13). The probable roles of these two classes of DNA elements in carcinogenesis are discussed in greater detail below.

Complex Genomic Changes and Inducible Responses to DNA Damage. Although the above-mentioned base substitution mutations in c-ras oncogenes are of great interest, they represent only a limited glimpse of the complex types of DNA changes seen in carcinogen-induced tumors. Tumors can also display deletions, chromosome translocations, amplifications, and transpositions of protooncogenes and other genes (10–13). In addition, in certain experimental systems the early steps in the carcinogenic process occur with a much higher frequency than would be expected for random point mutations (14).

For these reasons, our laboratory has been interested in the possibility that DNA damage in mammalian cells not only causes targeted point mutations but also induces the accumulation of factors which produce more complex genomic changes. We have been examining this possibility in a model system in which a rat fibroblast cell line (H3) carries an integrated copy of polyoma virus DNA which normally replicates synchronously with the host DNA (15–19). This system is of interest since if these cells are exposed to either a chemical carcinogen or UV, both of which damage DNA, then the polyoma DNA replicates asynchronously, producing thousands of copies of viral DNA. We found that if we treated normal rat cells (that lack polyoma DNA) with BPDE or UV irradiation and then fused these cells with untreated H3 cells, then this also induced polyoma DNA replication. The latter studies provided evidence that this induction is due to the formation of a trans-acting factor in the carcinogen-treated cells. Direct evidence for this putative factor has been obtained using extracts of UV-irradiated cells (19). Since this factor can be induced in normal cells, we believe it may play a role in the amplification of specific cellular genes or cause other types of structural changes in cellular DNA.

Studies are under way to identify more precisely this factor and its mechanism of action. Other investigators have demonstrated that DNA damaging agents can also induce the replication of SV40 viral DNA and that this effect may also be mediated by a trans-acting factor (20–23). We believe that the results obtained in both the polyoma and SV40 virus DNA systems may be of interest for several reasons. (a) They may serve as useful models for understanding mechanisms underlying the amplification of cellular genes during the carcinogenic process. (b) They might explain the well known phenomenon that chemical carcinogens can act synergistically with certain viruses to enhance cell transformation (5). Since certain human tumors may be caused by the combined effects of viruses and environmental chemicals, this phenomenon could be of direct relevance to the origin of certain human tumors (5). (c) They
may provide assays for detecting potential environmental carcinogens, thus complementing the current battery of short-term tests for mutagens.

Table 1 summarizes evidence that DNA damage in mammalian cells not only enhances the replication of certain viral DNAs but also induces other complex responses. The diversity of these responses indicates that the concept of "genomic stress," first elucidated in lower organisms, applies to mammalian cells (5, 15, 22, 23). Genomic stress responses may play a role not only in carcinogenesis but also in the complex effects of radiation therapy and chemotherapy on tumors. Thus, a better understanding of genomic stress responses may suggest ways to enhance the therapeutic effects of radiation and chemotherapy.

Disturbances in Transcriptional Control and Retrotransposons. As illustrated in Fig. 4 it seems likely that carcinogenesis involves not only the activation of cellular protooncogenes but also disturbances in the function of DNA sequences that play a role in controlling gene transcription, including cis-acting promoter and enhancer sequences. With respect to the latter types of DNA sequences, we have been intrigued with the fact that the genomes of higher organisms, including humans, contain thousands of copies of retrovirus-like elements (6, 16, 24). These elements are bounded by long terminal repeat sequences that contain specific promoter and enhancer sequences that control gene transcription. Moreover, in lower organisms (notably Drosophila and yeast) similar endogenous retrovirus-like elements function as mobile elements which can insert into new sites in the cellular genome, thus deleting or activating specific genes (24). There now exist several examples in which an endogenous mammalian retrovirus-like element, the intracisternal A particle sequence, has undergone transposition in murine tumors (25–28), leading in some cases to activation of the c-mos oncogene or causing the constitutive expression of the interleukin 3 gene. In yeast and Drosophila the transposition of similar retrovirus-like elements occurs via the reverse transcription of RNA transcripts of these elements, hence the term "retrotransposition" (24). We have found that murine and rat tumors that were induced by chemical carcinogens display a high level of constitutive expression of specific endogenous retrovirus-like sequences (29–33). The increased expression of these sequences is seen in both benign and malignant tumors and is often more striking than alterations in the expression of the c-ras or c-myc oncogenes. This phenomenon provides evidence for a fundamental disturbance in gene transcription in tumor cells. Studies are in progress to determine whether or not it predisposes to gene transposition during the process of carcinogenesis.

Tumor Suppressor Genes. There is increasing recognition of the fact that in addition to genes that can code for components of pathways that stimulate cell growth (protooncogenes), there exists a reciprocal set of genes the products of which inhibit cell growth and/or induce cells to undergo terminal differentiation (for review see Refs. 13, 34, and 35). These so-called "tumor suppressor genes" or "antioncogenes" could inhibit the evolution and growth of tumor cells. Mutations that delete their function would be expected to enhance tumor formation. Indeed the deletion of such genes appears to be involved in certain human tumors including retinoblastoma, Wilm's tumor, lung cancer, colon cancer, and other cancers (34–37). In view of the ability of chemical carcinogens to induce not only point mutations but also gene deletions and various types of chromosomal anomalies, it seems likely that tumor suppressor genes also represent critical targets in the action of chemical carcinogens (Fig. 4). Hopefully, the cloning of tumor suppressor genes will provide probes for examining this possibility. One approach which our laboratory has explored is to isolate from a cDNA library DNA sequences the expression of which is turned off rather than on in cells undergoing a response to a mitogenic stimulus (38). Possible biochemical functions of these suppressor genes are discussed elsewhere (13).

Tumor Promotion

Tumor Promoters Produce Epigenetic Effects. I now want to turn to studies on tumor promotion, emphasizing recent advances in our understanding of the mechanism of action of the potent tumor promoter TPA. Tumor promoters can be defined as compounds which have very weak or no carcinogenic activity when tested alone but markedly enhance tumor yield when applied repeatedly following a low or suboptimal dose of a carcinogen (initiator) (3, 4, 39, 40). Early studies indicated that, in contrast to initiating agents, the phorbol ester tumor promoters do not bind to DNA but instead act by binding to membrane-associated receptors and thus produce their initial effects at the epigenetic level. Studies from a number of laboratories, including our own, indicated that TPA induces a number of phenotypic effects in cell culture systems which could be grouped into three categories: mimicry of the transformed phenotype; modulation (either inhibition or induction) of differentiation; and membrane effects (39, 40). The discovery in 1982 by Castagna et al. (41) that TPA activates the enzyme PKC and subsequent studies indicating that PKC is the major cellular receptor for TPA have merged research on tumor promotion with that on growth factors, signal transduction, and the action of specific oncogenes (13, 42, 43).

Cloning of DNA Sequences That Encode PKC. Because of the central role of PKC in signal transduction, growth control, and tumor promotion our laboratory has done extensive studies on the function of PKC and has also cloned DNA sequences that encode this enzyme. The enzyme has two domains, a catalytic domain, containing an ATP-binding site and the region to which protein substrates bind, and a regulatory domain which is controlled by allosteric cofactors, i.e., lipid, Ca2+, and DAG, or lipid and TPA. We hypothesize that the usual function of the regulatory domain is to inactivate the enzyme by "closing" the catalytic site and that the binding of cofactors to the regulatory domain induces a conformational change that opens the catalytic site and thus activates enzyme function (Fig. 5) (13). Consistent with this scheme is evidence that limited proteolysis of the enzyme yields a fragment with a molecular weight of about 66,000 which has catalytic activity in the absence of lipid and other allosteric cofactors (42). In addition, there exist inhibitors of PKC that appear to act preferentially on the regulatory domain or the catalytic domain of the enzyme (44). A recent paper by House and Kemp (45) is also consistent with this model. Indeed, the latter authors provide evidence that the regulatory domain contains a peptide sequence that acts as a pseudosubstrate inhibitor of the catalytic domain when the enzyme is in the inactive configuration.

Our laboratory (46, 47) and other laboratories (48–52) have recently reported the isolation of cDNA clones that encode

Table 1 Inducible responses to DNA damage in mammalian cells*

<table>
<thead>
<tr>
<th>No.</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Virus reactivation</td>
</tr>
<tr>
<td>2</td>
<td>Induction of plasminogen activator, ornithine decarboxylase, metallothionein I, c-myc, c-fos, CIN 1, etc.</td>
</tr>
<tr>
<td>3</td>
<td>Induction of DNA amplification and virus replication</td>
</tr>
<tr>
<td>4</td>
<td>Induction of endogenous retroviruses (transposition)</td>
</tr>
</tbody>
</table>

* For details see Refs. 15–23.
PKC. The results indicate that PKC belongs to a multigene family, with at least 6 separate genes that encode distinct isozymes of PKC. The deduced amino acid sequences of the isolated cDNA clones indicate remarkable homologies between the individual forms of PKC and also appreciable homologies with other protein kinases, especially the cyclic AMP-dependent protein kinase (Fig. 6). The carboxyl-terminal portion comprises the catalytic domain, whereas the amino-terminal portion is the regulatory domain. The latter region contains two repeats of a cysteine-rich consensus sequence found in certain metal-binding proteins and in several DNA-binding proteins. We think that this may induce a specific conformation that is required for the interaction of this region of the enzyme with phospholipid and DAG, or TPA. TPA, teleocidin, and aplysia-toxin, three chemically diverse skin tumor promoters that are also potent activators of PKC, share certain common stereochemical features (6, 53). We suspect that this allows them to bind to a specific conformation in the cysteine-rich region of PKC. The development of variant PKCs by site-directed mutagenesis of cloned cDNAs may clarify these aspects and thus lead to the rational design of PKC inhibitors.

The existence of multiple genes for PKC, the preservation of these multiple forms during evolution, and the accumulating evidence that they are differentially expressed in different tissues (46–52) suggest that the individual forms may have subtle functional differences, but this requires further study.

Overexpression of PKC Alters Growth Control. To better define the function of a specific form of PKC we have inserted the cDNA sequence for the θ1 form of PKC into a retrovirus-derived vector developed in our laboratory (pMV7) (54), encapsidated the corresponding RNA into defective murine leukemia virus particles, and then infected the Rat 6 fibroblast cell line with these viral particles. This yielded several cell lines that stably overexpress 20 to 53 times the normal level of PKC enzyme activity (47). These cell lines also have an increase in high affinity phorbol ester receptors when compared to control cells. Phosphorylation studies indicate that they display a marked increase in a M, 76,000 32P-labeled protein, reflecting autophosphorylation of the overproduced PKC, as well as an increase in other phosphorylated proteins. The cell lines that overproduce PKC exhibit a dramatic change in morphology when exposed to TPA. Unlike control cells, which become refractory to the effects of TPA due to down-regulation of PKC, the overproducer cell lines continue to respond to repeated TPA treatments. In monolayer culture, these cell lines have a shorter exponential doubling time and grow to a higher saturation density and, when maintained at postconfluence, develop small, dense foci. In contrast to the control cells, which display complete anchorage dependence in the absence or presence of TPA, the cell lines that overproduce PKC form small colonies in soft agar in the absence of TPA and larger and more frequent colonies in the presence of TPA. Thus, overproduction of a single form of PKC is sufficient to confer anchorage-independent growth and other growth abnormalities in rat fibroblasts (47).

Taken together, the above results provide direct evidence that PKC plays a critical role in normal cellular growth control and that it mediates several of the cellular effects of the phorbol ester tumor promoters. These results also provide evidence that disturbances in the activation or down-regulation of PKC may be critical events in tumor promotion and multistage carcinogenesis. The exaggerated phosphorylation of specific cellular proteins in the cell lines that overproduce PKC may provide a
useful tool for identifying these target proteins and their role in signal transduction. In recent studies we have found that these cells are also much more susceptible to transformation by an activated c-H-ras oncogene than control cells. Studies are in progress to determine whether they are also more susceptible to malignant transformation by chemical carcinogens.

**Isolation of Phorbin, a Gene Induced by PKC Activation.** TPA induces the expression of a number of cellular genes (38–40). We are also interested in the role of PKC activation in mediating these effects and the underlying molecular mechanisms. To identify specific genes that are regulated by TPA, we prepared a cDNA library using polyadenylate-containing RNA obtained from quiescent C3H 10T½ murine fibroblasts 4 h after treatment with TPA and then screened this library for induced sequences by differential hybridization. We have isolated and characterized a cDNA clone (TPA-S1) the corresponding mRNA of which is induced up to 20-fold in response to the treatment of cells with TPA, PDGF, epidermal growth factor, serum, or diacylglycerol (38). This effect is apparently at the level of transcription and does not require de novo protein synthesis. We have designated the gene corresponding to TPA S-1 “phorbin” (phorbol ester induced). The role of PKC in the induction of phorbin is supported by the following lines of evidence: (a) agents that activate PKC, such as TPA, mezerein, PDGF, serum, and 1-oleyl-2-acetylgllycerol, also increase phorbin mRNA levels; (b) protein kinase inhibitors with differential effects on PKC activity demonstrate the same relative inhibitory effects on the induction of phorbin by TPA; (c) down-regulation of PKC activity, by treatment of 10T½ cells with TPA for 24 h, results in a loss of responsiveness to phorbin induction by subsequent TPA treatment; and (d) the above-described cell lines that overexpress PKC are extremely sensitive to TPA induction of phorbin RNA and also yield an exaggerated and prolonged response (44).

Complete sequence analysis of the phorbin cDNA (730 base pairs) predicts a cysteine-rich, secreted protein with a molecular weight of 22,600. The sequence of phorbin exhibits homology with two previously isolated cDNA sequences, designated “EPA” (erythroid-potentiating activity) and “TIMP” (tissue inhibitor of metalloproteinase) (38). The significance of these homologies and the possible role of phorbin in cell proliferation and tumour promotion are under investigation.

**Relevance of the Above Studies to Tumor Promotion in Various Tissues.** Although the studies described above are concerned with the molecular mechanisms of action of TPA, a potent tumor promoter on mouse skin, we think that they have relevance to other types of tumour promoters and to tumour promotion in other tissues and species, including the process of multistage carcinogenesis in humans. For example, we have found that certain bile acids that are implicated in colon carcinogenesis can activate PKC, induce translocation of PKC to the membrane fraction of cells, and induce phorbin expression (55, 56). Although it is unlikely that all classes of tumour promoters function by directly activating PKC, it is possible that they activate PKC indirectly by enhancing DAG production or by distorting related pathways of signal transduction (Fig. 1). This merits further study, particularly with certain halogenated organic compounds that have tumour-promoting activity in rodent liver. In recent studies on patterns of gene expression during rat liver regeneration and carcinogenesis (33, 57), we have discovered that in both systems there is an early decrease in the abundance of epidermal growth factor-receptor mRNA. This may be an important clue to an underlying change in signal transduction associated with hepatocyte proliferation.

**Some Major Unresolved Aspects of Multistage Carcinogenesis**

Despite the astounding progress that has been made in our understanding of the mechanisms by which initiating carcinogens modify the structure and conformation of DNA, the recent explosion of information on tumor promotion, PKC and signal transduction pathways, and the revelation of cellular oncogenes and tumor suppressor genes, we still lack a comprehensive understanding of the cellular and molecular events involved in multistage carcinogenesis. As discussed earlier in this paper, it is not known with certainty whether carcinogens that act as initiators produce a heritable event in somatic cells by causing point mutations that activate cellular oncogenes, whether they cause deletions in critical growth suppressor genes, whether they act on elements that specifically regulate transcription, whether they act by inducing complex changes in the genome (e.g., DNA amplification or transposition), or whether they act by combinations of such mechanisms. The possible role of alterations in DNA methylation in initiation or in later stages of carcinogenesis also requires clarification (for review see Ref. 58). A further consideration is that although most of the emphasis in studies on chemical carcinogens has focused on nuclear DNA and nuclear genes, we and others have demonstrated that the mitochondrial DNA of cells is also a major target for covalent binding by certain carcinogens (59). Whether or not carcinogen-induced alterations in mitochondrial DNA play a critical role in carcinogenesis remains to be determined.

Although the fields of chemical and viral carcinogenesis have evolved as two rather separate disciplines, human populations are often exposed to both types of agents. I think it is likely, therefore, that certain forms of human cancer are due to synergistic interactions between specific viruses, which on their own have little or no tumorigenic potential, and chemicals that have initiating (genotoxic) or tumor-promoting activity (5, 6). There are a number of such examples in experimental models (5, 6, 60). I would encourage, therefore, further studies on the mechanisms that underly chemical-viral synergy and their possible roles in the causation of specific forms of human cancer. Another unresolved question is how certain initiating agents can act as “complete” carcinogens, particularly with high or repeated doses. Some of these chemicals can produce phenotypic effects that mimic the action of tumor promoters (61). They might, therefore, act as both initiators and promoters (61).

With respect to tumor promotion, I have described exciting progress in studies on PKC, but we still know very little of how, following PKC activation, signals are carried from the cytoplasm to the nucleus to alter patterns of gene expression. Further studies on the phorbin gene (38) and on various interacting regulatory elements and the corresponding activator proteins that control the transcription of specific genes (62) may clarify these aspects. At the present time we do not know why tumor promoters appear to act preferentially on the initiated cell to cause its clonal expansion. I suspect that the critical effects of tumor promoters involve not just clonal expansion of the initiated cell but also some type of heritable “imprinting.” Why, for example, is it that on mouse skin (4) and in certain tissue culture systems (60, 63–66), some of the effects of TPA are irreversible, since even after cessation of repeated treatments with TPA some skin papillomas do not regress, and transformed foci in cell culture remain stably transformed? There is

---

3 W. Hsiao and I. B. Weinstein, unpublished data.  
4 Unpublished studies.
also evidence that tumor promotion on mouse skin can be subdivided into two phases (4, 40), but we do not understand at a mechanistic level the differences between these two phases. Some investigators have emphasized that since in certain cell systems TPA can induce the formation of activated forms of oxygen and also cause sister chromatid exchange, tumor promotion might involve indirect damage to cellular DNA (67). I doubt that these effects explain the early effects of TPA during tumor promotion, since these effects are usually reversible, but indirect DNA damage may explain some of the later and irreversible effects of TPA.

We understand even less about the molecular mechanisms responsible for the process of tumor progression, i.e., the conversion of benign tumors to malignant tumors. Since there is evidence that the process is enhanced by genotoxic agents (68), progression may require further DNA damage. Amplification of specific DNA sequences and instability of the karyotype appear to play important roles in tumor progression and the tendency of malignant tumors to display increasing degrees of autonomy and heterogeneity. Peyton Rous (69) described tumor progression as “the process whereby tumors go from bad to worse.” Obviously the mechanisms that underlie tumor progression require much more intensive study, particularly in view of the fact that tumor invasion, metastasis, and heterogeneity present the major clinical challenges in terms of cancer treatment.

Finally, I would emphasize that some of the current seemingly divergent hypotheses used to explain carcinogenesis need not be mutually exclusive because of the complex multistage nature of the carcinogenic process, the diversity of causative factors, and the heterogeneity of tumor cell phenotypes.

Implications of Molecular Carcinogenesis Research with Respect to Cancer Treatment and Prevention

Fig. 7 provides a schematic diagram of a cell showing various pathways of signal transduction. Several growth factors, e.g., PDGF, act through membrane-associated receptors to activate tyrosine protein kinases, whereas certain hormones act through membrane-associated receptors to increase cellular levels of cyclic AMP, which then activate the cyclic AMP-dependent protein kinase. The central role of PKC in signal transduction has already been discussed in detail. A number of oncogenes act by disturbing specific aspects of these signal transduction pathways. Carcinogenesis can, therefore, be viewed as a “distortion in signal transduction.” This concept suggests a variety of strategies for developing novel drugs that might be useful both in cancer chemoprevention and in the therapy of fully established tumors. These strategies are outlined in Table 2. Thus, research on multitarget carcinogenesis, growth factors, and protein kinases should provide clinicians with a variety of new pharmacological approaches to cancer control.

Our laboratory has been particularly interested in developing inhibitors of PKC, in view of the central role of this enzyme in tumor promotion and growth control (47, 70). In principle, one could design inhibitors that act on either the catalytic or the regulatory (amino-terminal) domain of the enzyme (Fig. 5). We have concentrated on the latter approach since we believe that such compounds would have greater specificity in view of the unique structure of this region of PKC. By screening a number of compounds, we discovered that the antiestrogen tamoxifen (and certain structurally related triphenylethylenes) are rather potent inhibitors of PKC and have obtained evidence that they do so by acting at the regulatory domain (44, 70). Tamoxifen is of interest because of its efficacy in the treatment of breast cancer. Although it is known to compete with estrogens for binding to the estrogen receptor, there is reason to believe that its antitumor effects might also be due to action on other cellular targets (44). Tamoxifen may, therefore, be a useful prototype for the design of specific inhibitors of PKC. As stressed earlier, the development of such inhibitors could provide a powerful new approach to both cancer prevention and treatment.

Finally, let me discuss the implications of studies on the mechanism of action of chemical carcinogens with respect to research on human cancer causation, emphasizing an approach which I and my colleagues call “molecular epidemiology” (71, 72). At the present time there exist three distinct methods for detecting potential human chemical carcinogens, epidemiological studies, long-term rodent bioassays, and short-term assays, (e.g., the Ames bacterial mutagenesis assay). Although the short-term assays are relatively simple and highly sensitive it is difficult to extrapolate the results obtained to humans. At the same time, conventional epidemiological methods have serious limitations since they are generally retrospective and are not very sensitive. Insights into the molecular mechanisms involved in carcinogenesis provide new laboratory methods which can
be used in epidemiological studies, to more precisely identify the causes of human cancers. Since, as I stressed at the beginning of this article, a number of carcinogens act by binding to DNA, one can now use highly sensitive techniques to detect and quantify the possible presence of specific carcinogens bound to the DNA in human tissues. Toward this end, we and others have developed monoclonal antibodies and highly sensitive immunoassays that can detect very low levels of benzo(a)pyrene and other carcinogens on human DNA (71, 72).

Foundry workers are exposed to high concentrations of polycyclic aromatic hydrocarbons and they are known to be at increased risk of developing lung cancer. Our group at Columbia University (73) and others (74) have found that these workers have increased levels of polycyclic aromatic hydrocarbon-DNA adducts, when compared to control subjects. A variety of methods are now available, and others are being developed, which will make it possible to determine whether other specific chemicals encountered in the work place, the diet, or other environmental sources cause DNA damage in humans (71, 72). Current research on the roles of protein kinases and altered gene expression during tumor promotion, which I have briefly reviewed in this paper, should also provide novel methods for detecting the action of tumor promoters in humans, thus broadening our understanding of human cancer causation.

In summary, as we approach the latter years of the 20th century, cancer research has reached an exciting crescendo. Advances in molecular and cellular biology have merged with research on growth control and the mechanisms that underlie multistage carcinogenesis. I am confident, therefore, that even before the year 2000 these advances will provide more precise information on the causes of specific human cancers and also lead to novel and more effective strategies for cancer prevention and treatment.

Acknowledgments

This lecture is dedicated to Drs. Elisabeth Miller and Charlotte Friend, both of whom passed away during the year 1987. These two women were inspiring and dedicated leaders in cancer research and had a profound influence on the author's own career. They made outstanding contributions, Dr. Elisabeth Miller in the areas of cancer biology and chemical carcinogenesis and Dr. Charlotte Friend in the areas of tumor virology and cellular differentiation. The author is also indebted to numerous colleagues, students, and collaborators for the invaluable contributions they have made to his research. He is grateful to the National Cancer Institute, the American Cancer Society, the Alma Toorock Memorial for Cancer Research, and the National Foundation for Cancer Research for providing the financial support required to carry out this research.

References

The Origins of Human Cancer: Molecular Mechanisms of Carcinogenesis and Their Implications for Cancer Prevention and Treatment—Twenty-seventh G. H. A. Clowes Memorial Award Lecture

I. Bernard Weinstein


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/15/4135

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/48/15/4135. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.