CANCER BIOLOGY. I
Induction, Regulation, Immunology and Therapy

Edited by

Robert R. Pascal, M.D.
Fred Silva, M.D.
Donald West King, M.D.

ADVANCES IN PATHOBIOLOGY

CANCER BIOLOGY.
Induction, Regulation, Immunology and Therapy

Edited by

Robert R. Pascal, M.D.
Fred Silva, M.D.
Donald West King, M.D.

College of Physicians and Surgeons of Columbia University
New York

Based on a series of lectures presented at the
Given Institute of Pathobiology in
Aspen, Colorado, July 1974

Stratton Intercontinental Medical Book Corp. / New York

Courses Sponsored by the Given Institute, 1975

July 6–11	Developmental Genetics Seminar
July 13–18	Chromosome Banding Laboratory Workshop
July 20–25	Cancer Biology I Seminar—Etiology and Therapy
July 27–August 1	*In Vitro* Chemical Transformation Laboratory Workshop
August 3–8	Cancer Biology II Seminar—Herpes Virus
August 10–15	Cell Fusion Laboratory Workshop
August 17–22	Messenger RNA Laboratory Workshop

ADVANCES IN PATHOBIOLOGY is published
under the general Series Editorship of
Dr. Donald West King.

Stratton Intercontinental Medical Book Corp.
381 Park Avenue South
New York, N.Y. 10016

LC 75-3356. ISBN 0-913258-35-0
Printed in U.S.A.

CONTENTS

Contributors: Cancer Biology Seminar
July 22-26, 1974

Nathaniel I. Berlin, M.D., Ph.D., Division of Cancer Biology and Diagnosis, National Cancer Institute

Joseph R. Bertino, M.D., Department of Pharmacology, Yale University School of Medicine

Paul Carbone, M.D., Medical Oncology, National Cancer Institute

Maurice Green, Ph.D., Institute for Molecular Virology, St. Louis University School of Medicine

John J. Marchalonis, Ph.D., Walter and Eliza Hall Institute for Medical Research

Bert W. O'Malley, M.D., Department of Cell Biology, Baylor College of Medicine

Henry C. Pitot, M.D., Ph.D., McArdle Laboratory for Cancer Research, University of Wisconsin

Gordon M. Tomkins, M.D., Ph.D., Department of Biochemistry and Biophysics, University of California at San Francisco (†)

I. Bernard Weinstein, M.D., Institute of Cancer Research, College of Physicians and Surgeons of Columbia University

Foreword

This volume is a continuation of a series of monographs on biology and medicine sponsored by the Given Institute of Pathobiology.

Although we hope to preserve the informality and spirit of small group discussions of the Pathobiology conferences, which have been held in Aspen for over ten years, the rapid advances in knowledge and the need for interdisciplinary exchange make it important to establish a written record of the seminars.

This volume on "Cancer Biology" is described in the following Introduction. It is identified on the title page as number "I" of the topic, dealing with specific aspects, since, among other subjects to be dealt with in forthcoming volumes of this series, the same basic topic will be represented in several, concerned with other aspects and identified accordingly as "Cancer Biology, II," "III," etc.

Donald West King

Introduction

A microcosm has been defined as anything regarded as a world in miniature, and also as man as the epitome of the universe. In many ways, the study of cancer is the examination of a microcosm. Molecules of small or large size interact with macromolecules of RNA, DNA protein, or as yet undiscovered cellular chemicals, and this in turn, through some series of complex events, results in the transformation of a cell to a state of uncontrolled replication and abnormal activity Through another series of poorly understood mechanisms, these cells spread in a human body, destroying normal tissues, producing the disease (diseases?) we call cancer.

There is also the extension of the study of cancer to the macrocosm, for there are epidemiologic factors associated with neoplasia; the foods we eat, the regions in which we live, the amount of sunlight we receive, the animals and people with whom we associate, all have some statistical correlation with the incidence of tumor formation. Beyond our own world and our solar system, what additional influences we are receiving are as yet subjects for a fantasist.

Viruses have received much attention by students of carcinogenesis. Dr. Maurice Green has summarized much of our knowledge of viral carcinogenesis. Little bits of genetic information pervading our atmosphere, they are likely suspects on which to blame anything that goes wrong in our bodies. It might be best for all of us if cancer turned out to be an infectious disease—as easily prevented as poliomyelitis. But this does not seem to be the case. There are the complex relationships among viruses, chemicals, hormones, radiation and other organisms in carcinogenesis. There are even the more annoying disclosures that our evolution has in part been the product of the incorporation of viruses into our natural genetic makeup, and that some of our own DNA can, at any moment, turn against us.

Even if our genes are in perfect order, and each bit of chromatin is working to preserve our lives, there is still the rest of our cellular material to worry about. We now know that we have enzymes which can bring information from the cytoplasm to the nucleus, and that the government of a cell is not one of nuclear totalitarianism. The induction of cancer might involve an aberration of cytoplasmic protein, with the DNA having its abnormal replication imposed upon it. The studies of Drs. I. B. Weinstein and Henry Pitot lend heavy support to this concept.

Then there are the hormones of our microcosm—a variety of chemicals manufactured at one place and having their influences at many distant places. As elaborated by Drs. Gordon Tomkins and Bert O'Malley, they

alter the functions of our cell membranes, enter our nuclei, change the genetic expression of our DNA and maybe even change its structure. There are so many of these molecules regularly at work within our bodies that the influence of "outside" hormones is probably superfluous in any theory of hormonal carcinogenesis. We don't really know how they work, but we trust them to regulate and be regulated properly.

However it comes into being, a transformed cell is still not necessarily a cancer. It must divide, form a population and take hold. Since the time of our early metazoan existence, we have had the facilities to deal with cells that did not belong in our bodies. Cells capable of recognizing foreign cells and eliminating them protect small multicellular organisms from invasion by parasites. Our own T-lymphocytes or macrophages seem to be there for that purpose, and they will kill neoplastic cells in the laboratory. An overview of the interactions between cancer cells and our immune mechanisms is given by Dr. John Marchalonis. A cancer cell is, in many ways, a parasite, different from our other cells. Many have, on their surfaces, foreign molecules, recognizable as such by our highly trained T-cells. Yet they survive. Perhaps they hide until their number is overwhelming; perhaps they shield or disguise themselves with the products of the B-lymphocytes cells we have acquired much later in evolution. The educational system of our microcosm might need some reorganization after we discover which cells are being duped and how.

When a single organism ablates a malignancy it is called spontaneous regression. When one or more organisms are involved in getting rid of a cancer, it is called therapy. When a large number of people join forces to treat cancer in other people, it is called a program. In several areas of cancer diagnosis and therapy, we have formed groups of diagnosticians, chemotherapists, radiotherapists, surgeons, immunologists—many minds attempting to act as a larger, unified mind directed to a particular problem. Drs. Nathaniel Berlin, Paul Carbone and Joseph Bertino, in this symposium, present some of the modern and rational approaches to the early detection and effective treatment of cancer. These programs exist in our cancer centers and other medical centers. They also interact with each other, sending and receiving bits of information, sorting and evaluating data, and even forming larger groups such as the N.I.H. and W.H.O.

Such programs also exist among researchers in carcinogenesis, but often they are highly specialized, centering about a certain theory, such as viral carcinogenesis or chemical carcinogenesis. Communication among these specialized groups is difficult. They speak different languages, use different symbols, and are too often insular. It is only when they get together and make an attempt to interact with one another that we begin to simulate the physical and chemical interactions that are so freely taking place in our

cells. If this can be done often and effectively, we will drastically reduce the time it will take to understand cancer.

The Editors

ACKNOWLEDGMENTS

We are indebted to Anne Anderson and Karin Peterson for their secretarial aid and invaluable editorial assistance. We would also like to acknowledge the aid of Dr. Gerda Netta and Ida Nathan who helped with the photographic work.

The sponsorship of Dr. Fred Silva by the Frank Mariani Traineeship in Surgical Pathology is gratefully acknowledged.

Molecular and Cellular Mechanisms of Chemical Carcinogenesis

I. Bernard Weinstein, M.D.

Environmental and physical agents have been known to be important in carcinogenesis since the mid-18th century. Yet the mechanisms of chemical carcinogenesis have not been clearly defined. Among the possible mechanisms are the following:

1. *Somatic mutation.* Proposed in 1910 by Boveri, this is a very popular hypothesis which has received tacit acceptance without hard evidence. The chemical produces a change in DNA, resulting in a hereditable mutation.

2. *Cell selection.* This depends upon the presence of dormant tumor cells and an environmental change, such as suppression of the immune system, which allows them to grow. It does not explain the inception of the tumor cell. Furthermore, we know that chemicals can directly transform cells *in vitro.*

3. *Virus activation or enhancement.* This hypothesis, also popular, presupposes a latent or concurrent oncogenic virus.

4. *Aberrant differentiation.* The carcinogen produces an epigenetic change which alters the stability of the differentiated state, with defects in growth control.

The evidence presented in support of the last mechanism begins with the basic biochemical facts of chemical carcinogens.

There is a great diversity of structure among chemical carcinogens, with no apparent common features. This suggests that they have limited specificity when they attack cells. They range from simple metals to complex polycyclic hydrocarbons. They usually are, or generate via metabolism, highly reactive electrophiles which will attack nucleophilic residues in cellular proteins and nucleic acids.

They bind most extensively to cell proteins, and with some selectivity specific cytoplasmic and nuclear proteins have a high affinity for the carcinogen. Binding to nucleic acids, both DNA and RNA, occurs at a lower level, with a ratio of carcinogen to nucleotide residue of $1/10^4$–$1/10^5$ All RNA species will bind with carcinogens. Several carcinogens

From the Institute of Cancer Research, College of Physicians & Surgeons of Columbia University, New York. These studies were supported by National Cancer Institute Research Grant CA-02332 and Contract E-72-3234.

preferentially attack guanine residues in RNA and DNA. Early effects on biosynthesis include: inhibition of transcription, with preferential inhibition of 45S ribosomal RNA precursor synthesis, inhibition of translation, and stimulation of DNA repair. They produce tumors with a variety of "private" antigens and diverse biochemical phenotypes.

N-2-ACETYLAMINOFLUORENE—A MODEL FOR CHEMICAL CARCINOGENESIS

A model for chemical carcinogenesis is N-2-acetylaminofluorene (AAF) (Fig. 1). This compound must be metabolized in the liver, where it can either be detoxified by ring hydroxylation or activated to an electrophile via the sulfate ester (Fig. 1). Activated AAF can attach to the C-8 position of any guanine-containing nucleic acid. It is believed to do so by displacing the guanine from its normal position in the helix.

In Figure 2 the heavy line represents AAF in a nucleic acid lying coplanar to the neighboring base. The displaced guanine is now unavailable for base pairing. The center illustration shows that this could lead to a frame shift mutation during DNA replication which might occur during carcinogenesis if the somatic mutation hypothesis is correct. Base displacement might apply to other planar chemical carcinogens, such as the polycyclic hydrocarbons. These compounds are hydroxylated by microsomal enzymes via an epoxide intermediate. Recent studies indicate that DMBA epoxide binds preferentially to guanine residues in nucleic acids. Similar results are

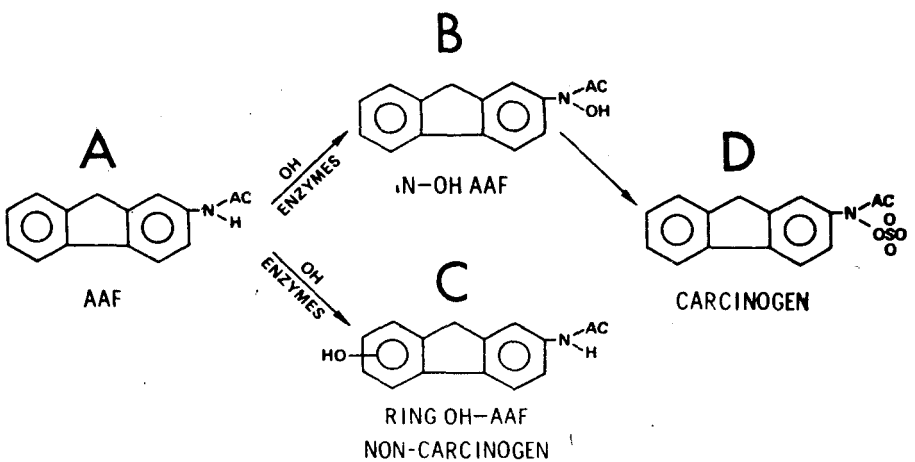

FIG. 1. Hydroxylation of acetylaminofluorene (AAF) by liver enzymes to a carcinogenic form or to inactive compounds. (Adapted from Miller JA [reference 1].)

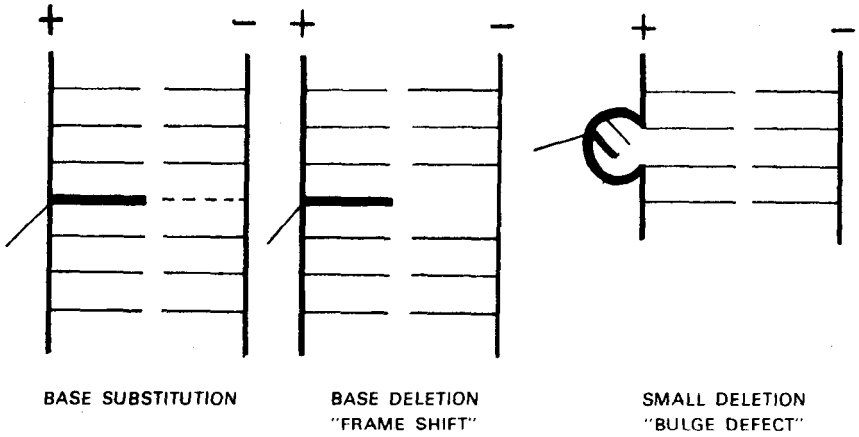

FIG. 2. Diagrammatic representation of how activated AAF (heavy line) might displace guanine in a nucleic acid molecule and thus alter base pairing. (From Levine et al. [3] with permission.)

obtained in the enzyme-mediated binding of DMBA to nucleic acids, and studies are in progress to see if this leads to base displacement.

CELL CULTURE STUDIES IN CHEMICAL CARCINOGENESIS

In cell cultures one can transform normal cells into malignant cells which will grow as a tumor when reimplanted into the proper host. Since most human cancers are epithelial (i.e., carcinomas) it is important to develop epithelial cell cultures. However, in epithelial cell culture, many of the criteria of transformation used in fibroblast cultures (such as contact inhibition) are not valid. Cell lines from adult rat liver have an epithelial morphology and stop dividing when the culture reaches a confluent monolayer. Cells divide not only at the expanding colony edge but throughout the colony. Although the ability to grow as a tumor *in vivo* is the ultimate cri-

TABLE 1. Predications of Base Displacement Model

a) Modified G residue will be unavailable for base pairing.
b) The stacking interaction between AAF and adjacent bases may interrupt the base pairing capacity of the adjacent bases.
c) G-residues in single-stranded regions in nucleic acids may be more susceptible to AAF modification than those in double-stranded regions.
d) AAF modification of double-stranded nucleic acids may produce localized denaturation.

teria for cell transformation, growth in agar (0.4%) was found to be a convenient and highly reliable cell culture criterion. Transformed cells have a 10-40% cloning efficiency in agar, whereas an inoculum of as many as 10^6 normal cells will not grow in agar.

Liver cell cultures exposed to the N-acetoxy derivative of AAF are transformed so that they will grow in agar and produce tumors *in vivo*. To help in deciding between the various hypotheses of carcinogenesis, mutants of chemically transformed epithelial cells which are temperature-sensitive (TS) in the maintenance of transformation were isolated by mutagenesis and selection. The TS mutant 223, like the wild type transformed cell (W-8), grows in agar at 36°C, but unlike W-8 it does not grow in agar at 40°C. Growth curves indicated that TS 223 has the same exponential doubling time at 36° and 40°. With TS 223, however, at 40°, growth plateaus shortly after the culture is confluent, but at 36° it continues, leading to a higher saturation density and patches of piled up cells. If, in the plateau phase, the temperature of the 40° culture is dropped to 36°, growth increases after a one-day lag and the 36° saturation density is reached. If the temperature of the culture is raised to 40°, the reverse occurs (Fig. 3). Thus the defect is modulated by temperature, and since growth in the first few days at 40° is similar to that at 36°, it is not simply a TS defect in cell division per se. The TS mutation appears to specifically affect the saturation density of these cells and their ability to grow in agar.

A striking morphologic difference was noted between TS cells grown at 40° and at 36° by scanning electron microscopy. At 40° the cells are flat and smooth. At 36° one sees patches of piled up spherical cells with a rough surface. The significance of these cell surface changes is not known at the present time.

VIRAL INTERACTION

Although we do not believe that a virus acts as an intermediate in the transformation of cells by AAF, cultures established from hepatomas induced in the rat with aromatic amine carcinogens contain large numbers of budding C-type particles and intracytoplasmic A-type particles. Thus far no biologic activity has been discovered for these viral particles. They are believed to be symptomatic, i.e., defects in the control of gene expression occurring during chemical carcinogenesis which are associated with derepression of latent viruses. Nevertheless two other possibilities have not been excluded. Firstly, the virus may actually play a causative role. Secondly, it may be a cofactor which *enhances* transformation. Viruses have been recovered from other chemically induced tumors but only rarely have these viruses been demonstrated to cause the same type of tumor when injected into normal animals.

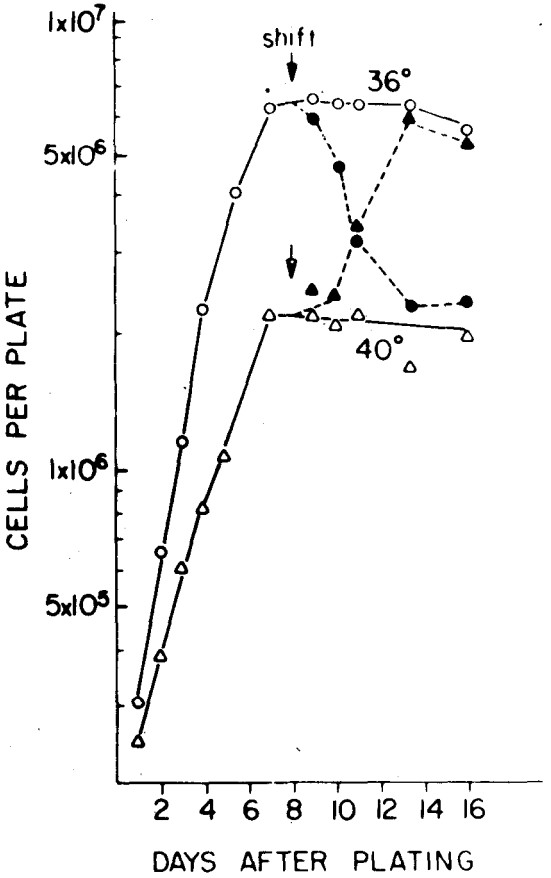

FIG. 3. Growth of TS 223 liver cell mutants. Open circles indicate cells grown at 36°, open triangles, those grown at 40°. Closed circles and triangles show growth when temperature is shifted. (From Yamaguchi, N. and Weinstein, I. B.: Proc Natl Acad Sci USA 72: 216, 1975, with permission.)

SUMMARY

Clearly, the technics of transformation in epithelial cell cultures bring us closer to understanding chemical carcinogenesis. Also clear is the need to develop new technics to evaluate the significance of viral particles found in tumors and to answer the basic question as to whether mutation or aberrant differentiation is the mechanism of chemical carcinogenesis.

REFERENCES

1. Miller JA: Carcinogenesis by chemicals: an overview—G.H.A. Clowes Memorial Lecture. Cancer Res 30: 559–576, 1970.
2. Nelson JH, Grunberger D, Cantor CR, Weinstein IB: Modification of ribonucleic acid by chemical carcinogens. IV. Circular dichroism and proton magnetic resonance studies of oligonucleotides modified with N-2-acetylaminofluorene. J Mol Biol 62: 331–346, 1971.
3. Levine AF, Fink LM, Weinstein IB, Grunberger D: Effect of N-2-acetylaminofluorene modification on the conformation of nucleic acids. Cancer Res 34: 319–327, 1974.
4. Weinstein IB, Gebert R, Stadler UC, et al: Type C virus from cell cultures of chemically induced rat hepatomas. Science 178: 1098–1100, 1972.
5. Grunberger D, Blobstein SH, Weinstein IB: Modification of ribonucleic acid by chemical carcinogens. VI. Effect of N-2-acetylaminofluorene modification of guanosine on the codon function of adjacent nucleosides in oligonucleotides. J Mol Biol 82: 459–468, 1974.

DNA and RNA Tumor Viruses—Molecular Events of Virus Replication and Cell Transformation and Role in Human Cancer

Maurice Green, Ph.D.

DNA and RNA tumor viruses hold the key to understanding mammalian cell function and growth control in molecular terms. (1) They provide some of the best experimental systems for studying mechanisms of growth control and the conversion of a normal to a malignant cell; by analyzing cell transformation by tumor viruses, we can learn how the functions of one or a few viral genes control oncogenesis. (2) Cells infected and transformed by tumor viruses provide models to analyze the cellular mechanisms that regulate DNA replication and gene expression in mammalian cells. (3) The study of tumor viruses will provide the answers to the question of what role, if any, viruses play in the pathogenesis of human cancer.

Tumor viruses are of two types, those with DNA as their genetic material and those with RNA (Table 1). There are three major groups of DNA tumor viruses—the papovaviruses, adenoviruses, and herpesviruses. The papovaviruses include two subgroups, the papillomaviruses, which cause warts in man and a variety of animals, and the polyomaviruses, which include the murine polyoma virus and simian SV40 virus. Little is known about the replication of and cell transformation by papillomaviruses since they do not grow in or transform cultured cells. In contrast, polyoma virus and SV40 have been studied in detail. Their DNA genome has a molecular weight of about 3×10^6 and codes for 3 major proteins, two of which are components of the virus particle and the third, the tumor (T) antigen, is required to initiate viral DNA synthesis and to maintain the transformed cell. In the case of papovaviruses, as well as the adenoviruses and

From the Institute for Molecular Biology, St. Louis University School of Medicine, St. Louis, Mo. These studies were supported by Contract NOI CP 43359 from the Virus Cancer Program of the National Cancer Institute, and Public Health Service Grant AI-01725 from the National Institute of Allergy and Infectious Disease. The author is a recipient of a National Institutes of Health Research Career Award 5K6-AI-4739. The editorial assistance of Michael R. Green is acknowledged.

TABLE 1. Oncogenic Viruses

RNA tumor viruses
Avian leukemia-sarcoma viruses
Murine leukemia-sarcoma viruses
Murine mammary tumor viruses
Primate type C particles
Leukemia-sarcoma virus of cat, hamster, rat, and guinea pig
Human milk virus (?)
Human leukemia virus (?)
DNA tumor viruses (*> 100*)
Papilloma viruses—producing warts in man, dog, cow, rabbit, and other species
Polyomaviruses—SV40 (monkey), polyoma (mouse), SV40-related viruses of humans (BK, JC, and PML-2 viruses)
Adenoviruses—(> 50)-human (31 types), simian, avian, bovine
Herpesviruses—Epstein-Barr virus associated with Burkitt's lymphoma and nasopharyngeal carcinoma), Marek's disease virus (chicken), Lucké carcinoma virus (frog), herpesvirus 1 and herpesvirus 2 (human), herpesvirus saimiri (squirrel monkey), herpesvirus ateles (spider monkey), herpesvirus sylvilagus (cottontail rabbit)

herpesviruses, the virus has two life styles: (1) *productive infection* of permissive cells, in which up to 200,000 virus particles are produced per cell and the cell is killed; (2) *cell transformation* of nonpermissive cells, in which only several early viral genes function, no virus is produced, and the cell is "immortalized" to divide indefinitely (normal cells from the body when placed in culture have a finite life span).

The second class of DNA tumor viruses are the adenoviruses, comprising about 70 members, of which 31 are of human origin. They provide superb experimental systems for analyzing the molecular biology of the human cell and for analyzing the functions of tumor virus genes in cell transformation. They grow exceptionally well in cultured human cells and at least 12 members can transform rodent cells *in vitro* or produce tumors when inoculated into rodents. Furthermore, only one or at most two viral genes are required to transform a cell, and DNA segments containing "transforming genes" have been identified. The adenoviruses will be discussed in detail later.

Neither papovaviruses nor adenoviruses have been shown to cause cancer naturally in their host species. To demonstrate oncogenicity, the virus must be inoculated into a foreign animal species, or added to their cells in culture. However, the third group of DNA tumor viruses, the herpesviruses, have been shown to cause cancer in their natural host species. Both Marek's disease (chicken) and Lucke adenocarcinoma (frog) are neoplastic diseases