Viruses Associated with Human Cancer

Edited by LEO A. PHILLIPS

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Preface

Do viruses cause human cancer? Many eminently distinguished scientists, including some Nobel laureates, are thoroughly convinced that viruses do cause human cancer. There are seven pertinent pieces of scientific evidence supporting a viral etiology for some human cancers:

1. Viral etiology of cancer in animals, including some primates, is a certainty.
2. Epstein-Barr virus (EBV) is associated with two distinct forms of human cancer, Burkitt's lymphoma and nasopharyngeal cancer.
3. Hepatitis B virus is associated with human liver cancer.
4. Herpes simplex virus type 2 (HSV-2) is associated with human cervical cancer.
5. Cytomegalovirus (CMV) is associated with human prostate and colon cancers, and Kaposi's sarcoma.
6. Human T-cell leukemia-lymphoma virus (HTLV) is associated with human leukemia.
7. Retrovirus information has been detected in some human leukemias and breast cancers.

The persistent and intriguing question of whether viruses cause human cancer has been difficult to answer definitively, partially because of the indigenous parasitic nature of viruses in humans, the inhumanity and illegality of proving Koch's postulates in humans with oncogenic viruses, and the multifactorial nature of human cancers.

Persistent efforts to understand how a virus transforms a normal cell into a tumor cell have yet to establish firmly the mechanisms involved. Available data from human tumors thus far have failed to show the presence of complete copies of virus-specific DNA or RNA sequences that are related to currently recognized viral probes.

For a variety of human cancers, particularly those that occur at a young age, hypotheses based solely on the accumulation of damage caused by chemical or physical carcinogens seems unlikely. Continuing search for candidate viruses that may cause various forms of human cancer have been substantiated by the association of EBV, HSV-2, hepatitis B virus, CMV, HTLV, and other retroviruses with human cancer.

This text is concerned with the association of both oncogenic DNA and RNA viruses to human cancer. Roger Monier and Norman P. Salzman begin the text by presenting the historical background of oncogenic DNA viruses. John Hay and Roger J. Watson subsequently discuss in detail the ultrastructural components of herpesviruses, followed by an up-to-date study of the association of herpesviruses and cervical cancer by Laure Aurelian. Joseph S. Pagano and Berch E. Henry II discuss the biochemical aspects of the Epstein-Barr virus and its relation to human malignancy, while Eng-Shang Huang and co-workers present evidence for the association of cytomegaloviruses with human cancer. V. Bhaskara Reddy and Sherman M. Weissman delineate the fine structure of papovaviruses encompassing the DNA sequence of SV40. This is followed by a study of the association of papovaviruses with human cancer by Peter M. Howley. Maurice Green and William S. M. Wold present a comparative study of the gene sequences of adenovirus in normal and malignant human tissues, followed by an up-to-date study of the association of hepatitis B virus and liver cancer by Joseph L. Melnick.

The study of oncogenic RNA viruses unfolds with an historical consideration by Peter Ebbesen, followed by a detailed study of the biochemical and biophysical aspects of retrovirus nucleic acids by Leo A. Phillips and co-workers. Ralph B. Arlinghaus then discusses the translational products of the retrovirus genome. Subsequently, the association of retroviruses with breast cancer is discussed by Ricardo Mesa-Tejada and Sol Spiegelman, and L. Ceccherini Nelli and Robert C. Gallo discuss the relationship between retroviruses and human leukemia. Next, Gurmit S. Aulakh and his associates analyze the implications of murine leukemia virus information present in some human cancers. This is followed by a detailed discussion by Myron Essex of the possible modes of transmission of oncogenic RNA viruses. Finally, Leo A. Phillips and co-workers present some studies on retroviruses and cells at the nucleotide level to establish an experimental basis for a molecular approach to human cancer.

Oncogenicity, which is expressed relatively infrequently under special circumstances, may simply be a reflection of a more general phenomenon that takes place during differentiation. Some steps forward in understanding the nature of cellular function have been taken unexpectedly while exploring the mechanisms controlling viral function. It is hoped that, with time, these pieces of information will fall into place and perhaps will lead to measures for the prevention and control of cancer. This book is a small contribution with that thought in mind.

The editor wishes to thank his distinguished colleagues for their contributions to this timely text and wishes to thank also the professional staff at Marcel Dekker for their assistance and cooperation. It is hoped that this text will serve as a valuable reference book to a multidisciplinary audience.

Leo A. Phillips

Acknowledgments

After the death of Dr. Leo A. Phillips this book was brought to completion with the assistance of his colleagues.

Special thanks go to Dr. Gurmit S. Aulakh who coordinated the final proof-reading and indexing with the publisher and contributors.

My thanks also go to all who helped bring the book to publication, in particular Drs. Mohinder S. Kang and Ramaswamy Narayanan. Without the hard work and diligence of all these friends and colleagues the book could not have been finished.

Mrs. Hattie M. Phillips

Leo A. Phillips (1931-1982)

This book is a small contribution to the living memory of Dr. Leo A. Phillips (Ed.), who died before the book was published. Leo A. Phillips, a graduate from the University of Kansas, was the Head of the Prophylaxis Working Group in the Laboratory of Viral Carcinogenesis at the National Cancer Institute.

In addition to being a member of various professional societies including the New York Academy of Sciences, Leo A. Phillips had organized and participated in various symposia connected with retroviruses. His main research interest involved the biochemistry and biophysics of the nucleic acids in retrovirus; in particular, the nucleotide track composition and molecular organization of the nucleic acids of oncogenic viruses, mammalian and human cells. His research centered on efforts to establish a scientific basis for a molecular approach to cancer prophylaxis and therapy.

Leo A. Phillips was a respected scientist at the National Cancer Institute. To an equal extent, he was a respected humanitarian. His death will be felt by all those who had come to know him both as a professional scientist and as a personal friend.

This book is dedicated to his memory.

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ONCOGENIC RNA VIRUSES

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ONCOGENIC DNA VIRUSES

1 History and Overview of Oncogenic DNA Viruses

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I. Early Studies with Oncogenic Viruses

Two separate areas of medical research were gaining importance at the end of the 19th century. One of them had as a base Koch's remarkable studies on the causes of infectious diseases. Starting with his early studies on anthrax in sheep, Koch had developed procedures for isolating pure cultures of bacteria, and had defined how medical microbiologists should identify the causative agents of a disease. This involved showing that the organism was present in the diseased tissue, and then isolating it in pure culture. On injection of the pure culture into a susceptible host, the same disease must be produced, and the organism again isolated. Using these procedures, it was possible to identify cholera vibrio, typhoid bacillus, and many other pathogenic bacteria.

For several diseases of plants and of animals, it was not possible to culture the causative agents and to examine them under the microscope. The diseases could be transmitted to suitable hosts but only by causative agents that couldn't be seen under the microscope, agents that were so small they could pass through filters which retained all bacteria. These results, which failed to follow Koch's postulates, led to the discovery of viruses.

A second independent field of medical research was tissue transplantation, and one narrow area of research within this larger field was concerned with transplantation of tumors from one animal to another. When a tumor was successfully implanted in a recipient animal, disease was being transmitted from one animal to another, but there was no idea that a pathogenic agent was being transferred. Instead, tumor transplantation was considered to be like a graft of tissue from one host to another, except that in this case the grafted tissue was malignant. Presumably, the recipient didn't change but simply provided an environment in which the tumor could grow.

When a historian looks back, he can always detail the logical antecedents that served as the base for some important event. One can look back on the field of viral oncology and show how it came logically from virology and transplantation studies, and how virology itself was a consequence of the germ theory of disease. However, scientists know that the merging of different concepts produces quantum leaps in our understanding and is a rare event. What is also intriguing is how often these new ideas lay dormant after they are published. It was the studies of

Peyton Rous that brought together virology and transplantation studies, and this remarkable union was to give rise to the field of viral oncology.

Peyton Rous was able to transmit a spindle cell sarcoma from a hen by injecting minced tumor pieces into recipient hens of the same variety. Later, by preparing cell-free extracts from the tumor, he was also able to induce tumors within a few weeks after injection. Even when the extracts were passed through filters that retained bacteria, they could produce tumors.

Rous [1], discussing these results in 1911, stated: "The first tendency will be to regard the self-perpetuating agent active in this sarcoma of the fowl as a minute parasitic organism. Analogy with several infectious diseases of man and the lower animals caused by ultramicroscopic organisms gives support to this view of the findings, and at present, work is being directed to its experimental verification."

The studies that Rous carried out were done in an elegant way, the conclusions were clear, and the merging of two major fields of biomedical research had been achieved. Did investigators in these two fields realize the remarkable opportunities presented to them? There is no evidence from the published literature that they did.

Rous's early work had little impact and it was more than 20 years before Richard Shope published a series of papers based on tumors found in the footpads of wild cottontail rabbits. In one series of experiments, fibromatous tumors were used to prepare cell-free extracts, which on injection into wild and domestic rabbits produced tumors that were self-limiting in growth and usually regressed. These experiments were done with fibroma virus, and the growths it induced were not considered true cancers [2].

Shope followed these studies by examining wartlike tumors carried on the skin of wild cottontail rabbits [3]. These papillomas were also able to be transmitted using cell-free extracts which were rubbed onto scarified skin of wild cottontail or domestic animals. Interestingly, the warts produced in the domestic rabbits could not be successfully passed a second time. This curious failure to transmit the virus led in later years to a series of interesting experiments in which the infectious papilloma virus was found in the nuclei of the differentiated keratinizing cells on the top of the papillomas in the wild cottontail rabbit. Virus could be extracted from the keratinized layers of cells, but not from the actively proliferating cells at the base of the papillomas. In domestic rabbits, in which papillomas develop, only the noninfectious form of the virus is produced [4, 5]. In a number of cases, tumors in domestic rabbits change into squamous cell carcinomas, and similar changes are also seen in wild cottontail rabbits [6].

Humans are also infected with papilloma viruses. Warts and papillomatous tumors are commonly observed in children, and can be transferred from person to person by inoculation.

These early studies were followed by a series of new isolations of oncogenic viruses in rodents and in fowl during the 1940s and 1950s.

The first key studies with polyoma virus involved the attempted cell-free transmission of mouse leukemia using filtrates from leukemic mice which were injected into newborn C3H mice. Contrary to the expected leukemias, mice developed parotid gland tumors. Further studies demonstrated that the original cell-free extracts contained more than one type of virus. The leukemogenic activity was lost by heating, but the extracts still could induce parotid tumors after 30 min at 64°C [7].

Rapid proliferation of the polyoma virus contained in extracts prepared from parotid tumors occurred when cultured mouse embryo cells were inoculated with these extracts, and its biologic potency increased by tissue culture passage. As the virus was passed in these cultures, the cells became pyknotic and detached from the glass. The tissue-culture-passaged virus showed enhanced potency and injection into newborn mice produced tumors in many organs and tissues; the virus was also able to produce tumors in newborn hamsters [8, 9].

Not only virus, but also viral DNA extracted from polyoma-infected mouse embryo cultures could be used to inoculate normal mouse embryo cultures; and it would give rise to polyoma virus, which on injection still produced tumors in suckling hamsters [10].

By the early 1960s the foundations that would be used by molecular biologists to study viral oncongenicity were in place. Growing cells in culture was becoming easier, and media for growing cells had been greatly simplified. In vitro transformation of cultured cells permitted those events to be analyzed and quantified under conditions that the investigator could manipulate. Colonies, either normal or transformed, could be produced from single cells, yielding genetically homogeneous cell populations. Differences between transformed cell lines and the parent lines gave rise to the expectation that the critical events which determined the transformed phenotype would be understood, and perhaps if they were understood, they could be reversed. Viruses that transformed cells of one type would lytically infect other species of cells, and events unique to or shared by cells undergoing transformation or cell lysis could be examined.

II. Diversity of Oncogenic DNA Viruses

Three groups of DNA viruses which are very different in their structure and biological properties have clearly demonstrated oncogenic potential.

Among the papovaviruses, the mouse polyoma virus and the monkey simian virus 40 (SV40) have been studied with the greatest care. The genetic information of these small viruses is contained in a circular double-stranded DNA molecule of approximate molecular weight 3.5×10^6. The complete sequences of both SV40 and polyoma DNAs have been determined [11-13].

The adenovirus group has many representatives. The human adenoviruses

have been classified into three subgroups, A, B, and C. Their oncogenic potential decreases in that order. Members of subgroup C (adenovirus of antigenic type 2 and of antigenic type 5, which are nononcogenic) and of the highly oncogenic group A (adenovirus 12) have been studied in some detail. Their genomes are linear molecules of double-stranded DNA of approximately 23×10^6 daltons, which display short terminal inverted repeats [14]. The sequences of the left-hand ends of the physical maps of adenovirus 5 and adenovirus 12, which are essential in cellular transformation, have been determined [15-17].

The herpesviruses have the largest amount of genetic information contained in a linear double-stranded molecule of about 100×10^6 daltons. In all herpesviruses, the structure of the genome is complex and involves both repeated and single copy sequences which are distributed in a specific way along the linear DNA molecule for each member of the group. Most of the studies have been done with the human herpes simplex 1 and 2 (HSV-1 and HSV-2) and several lymphotropic herpes viruses (the Marek's disease virus of the chicken or MDV; the herpesvirus saimiri of the squirrel monkey *Saimiri sciureus* or HVS; the human Epstein-Barr virus or EBV).

The small papovaviruses can only code for a limited number of viral-specific polypeptides: Five polypeptides have been described for SV40 and six polypeptides for polyoma. None of these polypeptides participate in the biosynthesis of nucleoside triphosphates or in their polymerization. The papovaviruses are totally dependent on the host cell machinery for their replication and do not induce a shutoff of host-cell functions. The adenoviruses and the herpes viruses encode a number of DNA synthetic enzymes and induce a shutoff of host-cell processes. This indicates that the three groups of viruses use different replication strategies.

None of the above-mentioned viruses are oncogenic in their natural host, with the exception of some strains of MDV which produce lymphomas in genetically susceptible chickens, and EBV, which is probably involved in the etiology of some human Burkitt's lymphomas and nasopharyngeal carcinomas. However, most of them are oncogenic when injected into newborn animals of appropriate species, and they can induce cell transformation in tissue culture. In recent years, most of the experiments on oncogenic DNA viruses have been performed on cells in culture. For this reason, in the rest of this chapter we will critically evaluate the role played by the virus and try to define those viral functions that could control the various steps in cell transformation. Since SV40 and polyoma have received more attention than other oncogenic DNA viruses, our emphasis will be on these two model viruses.

III. Phenotype and Selection of the Transformed Cell

At the present time, there is no accepted definition of the "transformed" cell,

which distinguishes it from the "normal" cell based on a comparison of properties, such as growth control, morphology, cell surface structure, and expression of enzyme activities. One of the difficulties is that no two transformed cell clones are exactly alike and that between "normal" and "fully transformed" cells, many intermediates can be observed.

Fully transformed cells do possess the following properties, which are not displayed by normal primary or secondary cells:

1. Ability to form clones when seeded on a solid surface at low cell densities.
2. Ability to grow continuously, without growth crisis, upon successive prolonged passages in culture (immortality).
3. Low requirement for serum growth factors.
4. Ability to overgrow a continuous layer of cells, a property that enables them to form multilayered colonies (dense foci).
5. Anchorage independence of growth. It has been suggested that a strong correlation exists between anchorage independence of growth and the ability of transformed cells to form tumors when injected into nude mice [18].

A hierarchy seems to exist in the growth properties of various transformed cells, in the sense that cells which display the first three growth properties may not display the latter two properties. However, cells that are able to form dense foci and are anchorage-independent usually display the first three growth properties [19]. Therefore, the selection techniques used to isolate transformed cells are of primary importance with respect to their transformed phenotype. Selection on the basis of anchorage independence certainly selects more stringently for "fully" transformed cells than does selection on the basis of low serum requirements or of colony formation at low cell densities.

All continuous cell lines, such as the mouse 3T3 lines [20] which are very frequently taken as "normal" references in cell transformation experiments, already display the ability to clone on plastic and possess immortality. Therefore, while any of the growth properties listed above can be used to select transformed cells out of a population of virus-infected primary or secondary cells, only the latter three properties can be used when the continuous "normal" cell lines are transformed.

Other properties displayed by transformed cells are morphological changes which are probably associated with changes in the distribution of cytoskeleton elements [21]; membrane structure modification, associated with lectin agglutinability [22,24]; increased rate of hexose transport; increased rate of plasminogen activator production [25-27]; and decreased deposition of and self-coating with fibronectin [28].

Cytoskeleton alterations and increased lectin agglutinability may be causally related and may play an essential role in transformation. The hypothesis of