Virus-transformed Cell Membranes

Edited by

CLAUDE NICOLAU

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Institut für Strahlenchemie
im Max-Planck-Institut
für Kohlenforschung
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Introduction

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Research on membranes has expanded rapidly in the past few years. Cell membranes, although highly complex supramolecular structures, can now be studied for the physical and chemical properties of their individual components as well as for their properties when these various components are organized into the membrane structure. Model membranes have been used for many studies, but for a more thorough understanding of naturally existing biomembranes there are great difficulties still to be overcome.

Cell membranes have multiple biochemical, physiological and immunological functions. In particular, it is clear that a number of vital roles are played by the plasma membrane as the cell's limiting boundary and point of contact and interaction with its environment. The principal biochemical and physiological functions of the plasma membrane include enzymatic and enzyme-regulation functions, transport activities, immunogenic activity and regulatory roles for many activities of the cell. In interactions with other cells, the membrane has important functions in cell contact and recognition and in cell-to-cell fusion.

Because oncogenic transformation of cells is accompanied by alterations in both structure and function of cell plasma membranes, efforts to define and elucidate these alterations and to understand how they are initiated—possibly the basic lesions of cancers—have assumed great importance in modern cancer research and particularly in studies of virus-transformed cells.

It is anticipated that the information obtained from the studies reviewed in this book—and from the areas of research toward which they point—will (i) identify the surface property or properties associated with loss of growth control, (ii) define the surface glycoconjugate alterations that are associated with high oncogenic potential and (iii) define surface membrane changes common to cells transformed by viral, chemical or physical agents. Hopefully, these reviews pinpoint aspects of the malignant cell surface which may serve

as the focal point for an approach to cancer prevention and therapy. The contributors to this volume are themselves actively engaged at the forefront of the fields of research that they discuss.

Much of the work on surface proteins of virus-transformed cells has been designed to elucidate the mechanism of malignant transformation and to assess the role of surface protein alterations in this process. Other studies have been concerned with the development of antigenic and enzymatic reagents for diagnostic, prophylactic or therapeutic applications in malignancies. In Chapter 1, Vaheri presents the information that has been collected concerning surface protein changes in virus-transformed cells. He evaluates some of the important new technologies that have contributed to progress in this area of research. These include polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate for identifying polypeptides by their apparent molecular weights, application of virus mutants temperature sensitive for transformation and of immunochemical and surface labelling methods, and several tumour virus–cell systems available for use as transformation models. In characterization of surface proteins, rather than presenting a complete collection of crude data the author assesses the relevance of the findings, correlates them, presents conflicting evidence and proposes areas where significant new information would seem to be technically within reach. In discussing alterations in surface proteins, particular emphasis is placed upon interactions between these proteins and other cell surface components. Interactions with extracellular components such as plasma proteins, enzymes and their inhibitors are also dealt with. Stress is also placed on the functional roles that the altered surface proteins may have in normal cell physiology and in the mechanism of viral transformation. Working hypotheses and prospects arising from the data are presented, but Vaheri explicitly avoids construction of unifying theories, feeling that some theories, speculations and general models have led to highly reiterated work that has done little to advance the field.

The outermost boundary of the cell surface consists of the oligosaccharide moieties of both lipids and proteins which are thought to be involved in cell-to-cell recognition and adhesion. For example, SV40-transformed cells specifically adhere to Sephadex beads derivatized with D-galactose, but not to beads derivatized with D-glucose or *N*-acetyl-D-glucosamine. Surface carbohydrate involvement has also been seen in platelet aggregation and in aggregation of mouse teratoma cells. In view of the alteration of cell surface interactions in the transformed state, carbohydrates are a logical focal point in the study of transformation. Whereas membrane glycoproteins of on-

cogenically transformed cells are included in the first chapter, Chapter 2 by the Steiners deals mainly with the status of glycolipids in malignant cells and with the activity of the membrane-bound enzymes involved in glycolipid biosynthesis. They also discuss the changes in the organization of these components in the cell surface.

Physical studies of the cell membrane are reviewed by Nicolau, Hildenbrand and Johnson (Chapter 3). Investigations in this area have produced much important information concerning membrane structure and architecture. Studies made with naturally existing biomembranes have confirmed the bilayer arrangement of extended fractions of the phospholipids and have further demonstrated that the cell membrane is a truly dynamic structure. The non-random nature of the lipid pattern in the organization of the membrane indicates that enzyme complexes may be incorporated into regions of rigid lipids, whereas the rest of the large protein–lipid complex may move with relative freedom in a fluid lipid bilayer.

Measurement of lateral mobility of lipids in animal cell membranes has been made possible by the use of fluorescence, spin label, and nuclear magnetic resonance techniques; behaviour of these lipids with respect to temperature may also be observed by means of these technologies. Mobility measurements with phospholipids have indicated the very complex dynamics of these molecules within the membrane. In many instances chemical studies seem to indicate that lipid composition is similar for normal and transformed cell membranes. However, the alterations observed in the lipid dynamics of these cells suggest that differences occur in the organization of lipids in the cell membranes after transformation. The authors offer a word of caution: no valid conclusion can be drawn solely upon the basis of physical measurements of the extremely complex system that is the cell membrane. Physical comparison of normal and transformed cell membranes has suffered from shortcomings arising in part from oversimplification of interpretations and in part from accepting artifacts as evidence. These authors emphasize that physical studies must always be coupled with detailed biochemical and analytical work in order to provide meaningful answers; otherwise, data based on artifacts may be confused with truths.

Perdue's chapter (Chapter 4) on transport across animal cell membranes includes the following topics: (i) transport of purines, pyrimidines and nucleosides; (ii) transport of amino acids, including stimulation of this transport by hormones, plant mitogens and serum; (iii) transport of hexose; (iv) transport of monovalent cations; (v) transport of anions; (vi) changes in the rate of nutrient and ion transport as a function of the cell cycle; (vii) the

involvement of sulphydryl groups in transport; and (viii) hypotheses concerning the activation and loss of transport control in transformed cells.

Noonan's review (Chapter 5) deals predominantly with the evidence relating lectin-initiated cell agglutination to the transformed cell phenotype. It concentrates on (i) the agglutinability, by lectins, of cells transformed by DNA and RNA tumour viruses, and the comparison of these with spontaneously or chemically transformed cells; (ii) the use of plant lectins to derive cell variants having altered surface properties; (iii) an outline of what is known concerning the molecular basis of lectin-initiated cell agglutination; and finally (iv) a brief discussion of work which the author and his colleagues have carried out on a series of cell variants that seems to hold promise for further clarifying the molecular basis of lectin-induced cell-to-cell agglutination.

The considerable body of evidence now available suggests that lectin-initiated cell agglutination is a good marker of the transformed state. This, however, is not infallible. The question of whether true *in vivo* neoplastic cells are more agglutinable than their normal counterparts is still open to discussion. Much further work in this difficult area is needed before conclusions can be drawn concerning any relation of agglutinability to *in vivo* tumourigenicity. To date, the evidence continues to support the early suggestion that viral infection and subsequent transformation 'switches on' a host gene which in turn is responsible for modifying the surface conformation from the non-agglutinable to the agglutinable state.

The data available suggest that lectin-mediated cell killing can be used to derive cell variants, many of which may contain modified glycosyltransferases. Since glycosyltransferases have been implicated in the phenomenon of contact inhibition of growth, these variants should prove important in testing how agglutinability relates to tumourigenicity. Another important contribution of lectin probes (particularly concanavalin A) rests upon their utility as excellent tools for studying the mechanism by which a cell controls the relative mobility of its surface glycoproteins.

A number of changes in cell membrane antigenicity are specific for the transforming virus, i.e. cells transformed by a given virus express the same or very similar membrane antigens whatever the cell species or the site from which the cell was derived. Because of the unique and consistent virus-specificity of certain of these membrane alterations, detection of the alterations can serve as a clue to suggest which virus has transformed the cell. Lausch and Rapp (Chapter 6) review recent advances that have been made regarding expression of virus-associated antigens on the surface of cells transformed by

human herpesviruses. Study of virus-specific changes in the cell membrane could prove useful for epidemiological surveys to determine the possible association of these agents with human cancers. The authors discuss the relationship of herpesvirus-associated membrane antigens to antigens found on virus particles and on the surface of cells that are productively infected by these agents rather than transformed. This sharing of antigens by transformed cells, infected cells and virion envelopes indicates that the antigens are coded for by the genome of the specific herpesvirus involved.

It is likely that the number of membrane antigens appearing on the surface of the transformed cell will be found to be substantial; in addition to those antigens which may be virus-coded, others probably will be found to represent previously repressed host cell gene products. It also is possible that some new membrane structures represent virus gene products modified by the host cell, or host cell gene products modified by the virus.

Cross-reaction of virus-associated membrane antigens has not been observed among cells transformed by the various members of the herpesvirus family discussed here (herpes simplex virus, cytomegalovirus and EB herpesvirus). Whether the antigens are essential to the transformation process, or whether they are necessary for maintenance of the transformed state, is not known. The need to define the membrane antigens in more precise immunological and biochemical terms is emphasized. For this to be achieved, satisfactory methods are needed to permit isolation of the antigens from the membrane in biologically active form. When purified antigens and their corresponding antibodies become available, it should be possible to look more closely at the role of the antigens in virus-induced carcinogenesis and in tumour cell escape from the host immune response.

Contents

2. Glycolipids in Virus-transformed Cells

SHELDON STEINER and MARION R. STEINER

3. Physical Studies of Transformed Cell Membranes

CLAUDE NICOLAU, KNUT HILDENBRAND and SHEENA M. JOHNSON

4. Transport Across Serum-stimulated and Virus-transformed Cell Membranes

JAMES F. PERDUE

5. Lectins as Probes of the Transformed Cell Surface

KENNETH D. NOONAN

6. Surface Antigens on Human Herpesvirus-infected and -transformed Cells

ROBERT N. LAUSCH and FRED RAPP

Chapter 1

Surface Proteins of Virus-transformed Cells

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Many people have spent the best part of their scientific lives cataloguing the structural and physiological modifications that distinguish tumor cells and transformed cells from their normal counterparts, and it is cruel but fair to say that none of this work has produced anything that can help to explain why some cells form tumors and some do not.

(Sambrook, 1972, p. 155)

I. Introduction

The study of surface proteins in virus-transformed cells appears to have two major motivations. Much of the work is designed to elucidate the mechanism of malignant transformation and to assess the role of surface protein alterations in this process. Some of the work, more directly aimed, has the object of development of antigenic, enzymatic or other reagents for diagnostic, prophylactic or therapeutic applications in malignancies. The two approaches still move rather separately and both unequivocal 'transforming proteins' and tumour antigens remain to be isolated. Not uncommonly though, projects are undertaken to isolate the two together as a single entity. Studies on the surface protein antigens are reviewed elsewhere (Lausch and Rapp, Chapter 6 of this volume) and are treated here only when molecular data exist. The object of this chapter is to discuss the information that has been collected on surface protein changes in viral transformation. Background information for the subject has already been presented in a well digested form (Tooze, 1973).

Progress in the study of surface proteins in virus-transformed cells has been mainly paced by improvements in technology. Introduction of polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulphate (SDS) started a new era in membrane protein biochemistry around 1970 by making it possible, easily and accurately, to identify polypeptides by their apparent mol. wts. Application of virus mutants temperature sensitive for transformation and of immunochemical and surface labelling methods are among more recent major contributions to virus–cell technology. Several tumour virus–cell systems are available today and many of them are used, not to study properties of tumour viruses, but frequently rather indiscriminately as general models to study phenotypic changes in tumour cells. The viral transformation models will be presented here not from a technical point of view but in an attempt to evaluate their advantages and limitations. The technology available for the study of surface proteins will be introduced with a similar treatment. In characterization of the surface