THE TRANSFORMED CELL

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Preface

This book was conceived as a collection of articles which describe and evaluate many of the differences that may exist between normal and cancer cells. Our intent was to collate in one source the comparative data on purported differences that have surfaced in recent years through a diversity of experimental strategies and investigative techniques. The contributors were asked to summarize the findings in their particular area of expertise and to evaluate critically the data in light of their contribution to our understanding of cancer cell biology. This approach will bring the reader up-to-date on the current theories concerning the differences between transformed cells and their normal counterparts.

Chapter 1 includes a brief description of terminology and basic concepts that appear throughout the book and is intended specifically for those readers whose area of interest may not be directly related to cancer cell biology or oncology. Chapters 2 and 3 should serve as useful background material because they explore the evidence for and against the possible correlation of *in vivo* tumorigenicity to *in vitro* changes in the cytoskeletal system, anchorage-dependent growth, plasminogen activator production, agglutinability by lectins, and cell surface and plasma membrane properties. The next two chapters compare the general aspects of regulation of cell proliferation and the relationships between ion movement and energy metabolism in normal and transformed cells. The important topic of transformation of normal cells by infection with new genetic material from tumor viruses is then reviewed in a separate chapter.

The last six chapters deal with selected cellular properties which have been purported to differ between the normal and transformed cell. Cyclic nucleotides, polyamine metabolism, mobility of cellular water, cell viscosity, intracellular pH, and element concentration are discussed. At least three of these six chapters discuss properties which have received little attention in the recent past and therefore contain much new information and present several new approaches to the study of cancer cell biology.

As the differences between normal and cancer cells are critically explored and established they will serve as points of selective therapeutic attack. This book should stimulate new modes of evaluating normal and cancer cells and should suggest to the reader new and unique, but rational, approaches to the study of cancer cell biology and cancer therapy.

It is clear that deficiencies in our basic knowledge of cells remains a major obstacle to the development of effective cancer therapy. We are convinced that the relentless pursuit of how the properties of cancerous cells compares to that of noncancerous cells can lead to fundamental advances in our knowledge of cells and to the control and eventual cure of cancer.

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The Transformed Cell: Some Introductory Comments

IVAN L. CAMERON AND THOMAS B. POOL

I. ELEMENTARY BACKGROUND, TERMS, AND CONCEPTS

A cancer cell can be defined as a cell that has lost control of growth and of position; thus, the cancer cell is not under the same restraints or controls as normal cells. Various forms of differentiated tissue cell types can give rise to cancer cells through the process of cell transformation. In most cases, the transformed cell retains some of the characteristics of its tissue cell type of origin and this forms the basis for the identification and the classification of the tumor cells. The various forms of cancer are often very different; therefore, cancer may be said to include a variety of diseases.

Let us briefly review some properties of tumor cells *in vivo* then examine some properties of transformed cells *in vitro* [for general works on this topic, see (5,9,11)].

Many normal cell populations in the body demonstrate active cell proliferation which is carefully controlled so that cell birth is balanced by cell death and loss. The tumor cell escapes this careful balance so that a tumor mass or neoplasm results. Some tumor cell types proliferate rapidly and

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others slowly, but in each case the birth rate exceeds the cell death or cell loss rate. Thus, a common property of tumor cells is the loss of growth control.

Loss of growth control alone will, in many cases, result in the production of a benign tumor which can be removed by surgery, but tumor cells may also demonstrate another property called malignancy. This property is characterized by alterations in the tumor cell's ability to stay with its fellow tumor cells. Instead it disassociates itself from its fellow tumor cells or site of substrate attachment and migrates away. Together, these qualities give the tumor cell the ability to invade normal tissues and sometimes to destroy the invaded normal tissue. Tumor cells may eventually metastisize by being able to enter and survive in the circulatory system as well as to arrest in capillaries, where the tumor cell is capable of further invasion at distant sites from its origin.

Finally, tumor cells *in vivo* are sometimes antigenic. In those cases where the tumor cell is antigenic to the host, it can have the ability to evade or block the immune surveillance system of the host (5).

When pieces of normal tissue are dissociated and placed in appropriate tissue culture conditions, cells grow out as primary cells which can be subcultured as secondary cells. Such cells can only be subcultured for a finite number of times, depending on the species of origin and/or on the age of the animal of origin, before the cultured cells fail to proliferate. On occasion, a few of the progeny of the secondary cell culture acquire the property of infinite proliferation. Such cells form a cell line. When pieces of tumor are cultured, in the same way as normal tissue, the tumor cells demonstrate infinite cell proliferation characteristics. Thus, normal and tumor cells have different properties *in vitro*.

Briefly, the transformed or tumor cells are said to demonstrate the following properties when grown *in vitro:* form more than one layer of cells in culture, loss of contact inhibition of growth so that high cell densities are obtained, lower requirements for serum growth factors, loss of anchorage dependence for growth, infinite proliferation, a poor ability to spread or flatten on the substrate, reduced contact inhibition of movement, show alteration in the cell surface such as changes in antigenic properties and production of proteolytic enzymes.

It is the hope of those who study the properties of normal and tumor cells *in vitro* that their findings will correlate with the properties of tumor cells *in vivo*. It is easy to see that the loss of growth control properties are common to tumor cells *in vitro* and *in vivo* but it is somewhat more difficult to establish how the *in vitro* properties of tumor cells relate to invasion and metastasis *in vivo*. It is known that many transformed cells *in vitro* do form

tumors when placed into a suitable host but often the tumor cells lack the ability to, or simply do not invade or metastasize in the host.

Because of the ability to obtain masses of relatively homogeneous cell populations many workers have chosen to work with transformants of normal cells *in vitro*. Favorite "normal" cell lines for study include the mouse 3T3 cell, which has the morphology of a fibroblast in culture and derives from an endothelial cell, and the baby Syrian hamster kidney cell (BHK 21), which is a fibroblast. Cultures of chick embryo cells, which can be transformed by Rous sarcoma virus (RSV), is another favorite for study (Chapter 6). Although the rodent 3T3 and BHK cells are not really normal (i.e., they demonstrate infinite cell proliferation and other tumor cell properties), they serve as a source of cells which can be made to acquire further properties of tumor cells by infection with a cancer-causing virus or by application of chemical carcinogen or radiation.

II. COMMENTS ON TUMORIGENICITY AND CELL TRANSFORMATION

The second chapter of this book discusses the criteria that characterize and therefore define what constitutes a transformed cell. The most reliable criteria for determining if a cell in culture is transformed is to demonstrate its ability to form a tumor when transplanted into either a syngeneic host or a host with a depressed immune system, such as the athymic nude mouse. Even though the demonstration of cell tumorigenicity in a suitable host is considered to be the best test of cell transformation, the tumorigenicity test is still subject to some criticism. For example, in practice a large number of the cells to be tested are usually inoculated into the host and if a tumor develops from this population of cells then one can really only conclude that at least one of the inoculated cells was transformed to the point of being tumorigenic. On the other hand, that no tumor develops after inoculation cells may not be due to the lack of transformed or tumorigenic cells in the inoculum but may be due to the fact that the host is still capable of mounting a successful immune response or that the site or the method of inoculation was not adequate to allow growth of the transformed cell(s) (3,6). It is even possible to get tumor growth, using cells which are normally nontumorigenic, when carried out under special conditions (1). In any event, it is also wise to explant a piece of the growing tumor into culture and to compare the properties of the explanted cells with the properties of the parent cells which have been maintained in culture.

Most of the chapters of this book critically analyze purported differences which exist between normal and transformed cells. In general, these differences include changes in: the cell surface (Chapter 3), cyclic nucleotides (Chapter 7), polyamine metabolism (Chapters 4 and 8), the state of water (Chapter 9), viscosity (Chapter 10), pH (Chapter 11), element content (Chapter 12), growth regulation (Chapter 4), development of an infinite life span and the loss of contact inhibition (5,9,11), (see also Chapter 2), anchorage dependence (except in the case of lymphohemopoietic cells), the cytoskeleton (Chapter 2), as well as changes in energy metabolism and membrane transport (Chapter 5).

Presumably, a fully transformed cell should demonstrate differences from normal cells in most, if not all, of these properties and would be highly tumorigenic upon transplantation. Smets has presented data to support the concept that cells of infinite life span can acquire some of these properties that are characteristic of transformed cells independently from one another (9).

That the properties characteristic of transformed cells can be acquired independently implies that cell transformation is not a one-step event. Although there continues to be some debate as to whether cell transformation can be accounted for by a one-step mechanism (7) the data in Table I give adequate evidence to illustrate that cell transformation properties can be acquired independently. Determination of the number of steps or events involved in the cell transformation process can be influenced by the choice of the cell type being transformed to a tumorigenic state. For

TABLE I

Examples of the Independent Acquisition of Properties of Transformed Cells[a]

Cell type	Property	Tumorigenicity in nude mice	Reference
3T3	Infinite proliferation	Nontumorigenic	
SV3T3	Loss of contact inhibition	Low tumorigenicity	10
Chemically transformed embryo cells	Grows in soft agar (anchorage independence)	Nontumorigenic	2
Polyoma virus transformed	Loss of contact inhibition, grows in soft agar, specific changes in membrane glycopeptides	Highly tumorigenic (transplantable)	4

[a] Adapted from Smets (9).

example, chemical transformation of a normal cell with a finite life span should involve multiple steps to become fully tumorigenic, whereas the transformation of a 3T3 cell with an infinite life span to a cell which demonstrates only loss of contact inhibition would involve fewer steps.

In general, rodent cells transform easily either spontaneously or when treated with carcinogens or oncogenic viruses, whereas human cells do not readly transform (8) (see also Chapter 6). In fact, Ponten (8) considers cells from the mouse to be genetically unstable and therefore a poor model to study the multistep process of cell transformation.

The tumor cell *in vivo* apparently represents the last of several steps which led to its escape from the growth control imposed on the normal cells of origin of the tumor cell. Obviously, a transformed culture cell does not encounter as many growth restraints as a tumor cell growing *in vivo*.

The study of cell transformation *in vitro* offers an excellent opportunity to reveal molecular mechanisms of cell transformation. However, transformed tissue culture cells when transplanted into a host are usually not suitable for use as models to study tissue invasion and metastasis which are properties correlated with malignancy. Nor are transformed tissue culture cells considered suitable models for study of the interactions of the host's immune system with tumor cells.

Hopefully, the chapters in this volume will help to elucidate those mechanisms operating in transformed cells as well as those mechanisms involved in the cell transformation process. Only by elucidating these mechanisms can we hope to bridge the gap between *in vitro* studies and an understanding of tumor pathogenesis and tumor growth *in vivo*.

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