Paper

Oncology 1977;34:100-101

Model Systems for the Study of Prostatic Cancer

D.J.

Merchant

Eastern Virginia Medical School, Norfolk, Virginia

Key Words

Prostatic adenocarcinoma
Model systems
Cell cultures
Organ cultures
Animal models

Abstract

Application of suitable in vivo and in vitro model systems are necessary to further investigations in prostatic adenocarcinoma. Model systems are defined, and discussed in terms of their potential application to the study of the neoplastic process.

Request reprints from: D. J. Merchant, Ph D., Department of Microbiology and Immunology, Eastern Virginia Medical School, P.O. Box 1980, Norfolk, VA 23501 (USA)

Man is the only species in which adenocarcinoma has been demonstrated to occur spontaneously, with significant frequency, though nodular hyperplasia may occur in approximately 80 percent of senescent dogs (8–10 years old) [2]. Nevertheless, basic information concerning many aspects of prostatic cancer cannot be obtained solely by study of the natural history of the disease in man and continued progress in our understanding and control of prostatic cancer depends significantly, on the ability to develop appropriate model systems. It is to be expected that critical information could be obtained and significant progress made possible in each of the following areas by application of suitable model systems.

Etiology

Mechanisms of carcinogenesis

Tumor progression

Metastasis

Pathogenesis

Hormone metabolism

Tumor genetics

Tumor immunity

Chemotherapy

Hormone therapy

Radiation therapy While spontaneous adenocarcinoma does not occur with sufficient frequency in most species to serve as a model system, the disease has been noted in aged animals, particularly in rats. Transplantable tumors have been developed from such material and offer considerable promise as models. The two systems which have been studied most extensively are those developed by Dunning in the Fischer rat [3] and by Pollard in germ free Wistar rats [6].

The recent report by Shain [9] of the high frequency of spontaneous adenocarcinoma in the ventral prostate of aging AXC rats suggests an additional animal model though confirmation is required.

In vivo models are likely to be useful particularly in the study of metastasis, progression, pathogenesis, tumor genetics, tumor immunity and therapy. Several important questions must be answered, however, before we can have confidence in the fidelity of such models. It is well-known that there are important differences in embryogenesis of the prostate between man and other species [1, 7]. This results in differences in biochemistry and physiology of the prostate. Sufficient information is not available at this time to make a judgement concerning the alternatives that adenocarcinoma of the ventral prostate of the rat and adenocarcinoma of the posterior prostate of man represent parallel or divergent biological systems. Certainly, a similarity in histologic pattern between the animal tumors and human carcinoma is not sufficient and it is important that several biochemical markers be developed before a significant relationship can be established. Moreover, the natural history of the experimental disease in animals as well as its sensitivity to therapeutic modalities should bear a recognizable relationship to human prostatic cancer.

An important variable which must be controlled in transplantable tumor models is mutation and selection. There is danger of considerable biologic drift with continued passage and an even greater danger of variability between substrains when maintained in differing animal colonies. Any potential model should be tested early for its capacity to be frozen down and recovered by subsequent animal passage. When such a procedure has been demonstrated to be practical, a large batch should be frozen for future reference.

With the availability of "athymic" nude mice, it has been possible to develop transplantable models of certain human tumors. While such a system bypasses some of the problems related to variability of the prostate between species, there is uncertainty as to the extent of suppression of thymic lymphocytes and results are not always as predictable as desired. Moreover, hormonal and other biochemical effects of host metabolism may be difficult to control and interpret. Nevertheless, this system is under active investigation using prostatic tumors and should be pursued as an additional model [5,8]. Development of in vitro model systems using human prostatic tissue offers another approach and avoids the question of differences between human and animal prostates. In vitro systems can take any one of three basic forms, each having specific advantages and disadvantages.

Organ cultures, which represent a form of tissue survival in vitro, can be maintained for limited periods of time, usually a few days to a maximum of 1–2 weeks. Small fragments of tissue are provided with nutrients and conditions for maintenance and differentiation but outgrowth of cells is discouraged. Evaluation of biochemical changes can be made by utilizing sensitive techniques such as radioassay, or histologic changes can be noted by sacrifice of fragments for sectioning and staining at suitable intervals. The utility of the system is limited by the length of time over which cultures can be maintained, the

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sensitivity of evaluation techniques and the need for multiple fragments of similar architecture and physiology for serial analysis.

Organ cultures may provide important information concerning hormone responsiveness, response to chemo therapeutic agents, tumor progression and other questions. The small amount of tissue available from needle biopsies and TUR specimens limits the broad application of organ culture methods. Likewise, the short time intervals involved restricts the information which can be obtained regarding such questions as mechanisms of carcinogenesis which are believed to

involve rather long time intervals under natural conditions *in vivo*. In contrast to organ cultures, cell culture systems depend upon stimulation of cell proliferation. Two basic types of cell cultures can be exploited, viz: primary cell cultures and continuously propagated cell strains or cell lines. Either type of culture can be initiated as outgrowth from tissue fragments (explants) or by culture of cells dispersed from the tissue by enzymatic digestion or disaggregation with chelating agents. The latter, however, appear to be relatively ineffective for prostate. By definition, primary cell cultures are those involving minimal cell proliferation and which usually involve the original outgrowth and at most two or three subsequent subcultures. Cell strains are defined as cell populations which can be sub-cultured over a finite number of generations but which subsequently stop dividing and eventually die unless they are transformed. Cell lines can be subcultured for indefinite periods and in many instances this appears to be unlimited. Cell lines usually are aneuploid.

Each of these cell culture systems appears to have advantages and disadvantages as a model system.

Primary cell cultures usually contain a mixture of cell types and therefore are more representative of the original tissue. Even cells which have limited capacity for replication may be represented. Such cultures may be particularly useful in the study of tumor progression, hormone metabolism and such aspects of etiology as activation and amplification. On the other hand pure cultures of a single cell type which can be expanded and be available as reproducible systems have numerous uses. One application of particular current interest is the isolation and identification of tumor specific antigens.

The use of any cell culture system as an *in vitro* model, however, has many obstacles which must be overcome if the data obtained is to have meaning. Many of these problems have been dealt with in detail previously [4] but a brief discussion is in order here.

With few exceptions the amount of tissue available from prostate cancer is quite limited. The variability in architecture which usually characterizes such tumors makes it very difficult to achieve reproducibility between tissue samples from the same clinical specimen. The problems are significantly magnified when cultures are initiated from different clinical specimens due largely to biological variation between patients.

While the isolation and characterization of “pure” cell strains or cell lines provides a relatively uniform and reproducible system it introduces new questions. Obviously if the culture consists of a single cell type it represents a population selection. What has been selected? Does it have any relationship to the tumor cell (acinar epithelium)? As it is maintained in culture, is there mutation and selection or does “dedifferentiation” occur? Unfortunately, information concerning the tumor cell *in vivo* is minimal and genetic markers of adequate stability are not available yet in sufficient numbers to answer these questions.

Another troublesome question which cannot be answered with our present knowledge is: to what extent can any cell line or cell strain (or even several such lines or strains) be considered to be representative of human prostatic cancer in the broad sense. Under the best of circumstances it is likely that a cell line or strain would be representative of a particular tumor. The known biological variability from one tumor to another suggests that analogy beyond that point is tenuous at best. It is possible that a single cell line or strain might represent only one element in a given tumor.

In summary, the need for model systems is acute and there appear to be strong arguments and potential applications for each of the models discussed. However, the questions raised are real

and it would be unwise to apply any model without being aware of the problems and attempting to overcome or answer them.

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