Urological Research 5, 159-162 (1977)

Urological Research

RESEARCH METHODS

Tissue Culture in the Investigation of Prostatic Cancer

L. M. Franks

Department of Cellular Pathology, Imperial Cancer Research Fund, London, UK

Tissue culture has much to offer in studies on prostatic cancer though the amount of information it has actually produced is still relatively small.

The main virtue of tissue culture is that it allows the separation of cells from the host and the control of their environment. This is of particular importance in the prostate where, in the intact individual, we have only a very uncertain knowledge of the endocrine environment around the specific epithelial cells. However precise our methods may be for measuring hormones in the blood or urine we can only guess at the concentrations available to the prostate cells. The second virtue of tissue culture is underlined by the last statement - "available to the prostate cells". The prostate is a complex organ with many different cell types. Tissue culture allows the isolation and study of the direct effect of different agents on specific cell types such as epithelium, muscle and stroma. It also allows for the analysis of the products of specific cell types and the effects which are due to the interaction of these different cell types.

There are two main types of tissue culture both of which have been used in the study of prostatic disease. The first is organ culture, a misnomer since only fragments of the organ rather than whole organs are maintained. The basis of the technique is that the tissue is maintained in an organised form with epithelium and stroma in its normal relationship. The second type of culture system is the cell culture in which cells usually of a single type but sometimes mixed, are maintained in a disorganised and often rapidly growing form. Both types of culture system are valuable but to obtain the maximum amount of information the technique which is most appropriate for the particular problem to be solved must be selected. Thus, for questions concerned with physiology of the gland, the organ culture system is likely to be more informative. Questions concerned with the behaviour of individual cells are more likely to be answered by cell culture systems.

The main problem, as far as human tissue is concerned, is that prostatic carcinoma is rarely removed by radical surgery, so that human carcinoma cells are not readily available in any quantity. If cells are to be grown they must be alive and most biopsy methods used to obtain prostatic tissue tend to damage the cells. A second problem is that even if living tumour cells are obtained only a relatively small proportion of tumours can be maintained in culture for any length of time.

A further problem is that it is almost impossible to obtain and grow normal human prostatic epithelium. This particular problem is not confined to the prostate or to human tissue but is a problem which applies to all mammalian cells (6). The usual source of human material is from benign nodular hyperplasia and this is NOT normal prostate.

These difficulties inevitably lead to the use of an animal model system but these too have their own problems as all who have been concerned with the experimental investigation of

prostatic cancer are only too well aware. The prostate in almost all species differs in position and structure from the human prostate and spontaneous prostatic cancer is very rare.

Despite these problems there are many things which have been learnt and many more which can be learnt using tissue culture techniques. Tissue culture can be of value in three areas:

1. The effects of hormones on the normal and abnormal prostate
2. In vitro carcinogenesis
3. The study of stromal-epithelial interaction

1. THE EFFECTS OF HORMONES

Much of this work has been done by Lasnitzki (9) and, in general, it has confirmed the expected effects, i.e. that in mouse or rat prostate, androgens are necessary for normal epithelial maintenance and function, and that oestrogens are inhibitory. This response is altered by age (3) and it has been shown that hormones such as insulin have a remarkable effect on the rodent gland (4). Lasnitzki has also studied the effect of pituitary hormones and the effects of hormone interaction (9). Perhaps the most important part of her work, in collaboration with Beaulieu and his colleagues, has been concerned with the studies on androgen metabolism. Most of this work has been done using the normal rodent prostate and has great potential, particularly if the methods can be applied to human tissues.

There have been a number of reports on the effects of hormones on benign prostatic hyperplasia, e.g. McMahon and Thomas (12), Lasnitzki et al. (10), McRae et al. (13). Riches et al. (15) and some on prostatic cancers e.g. McMahon et al. (11), and Webber (17), but all suffer from problems concerned with sampling and difficulties in assessing the results of treatment. Most have used organ culture systems and results have been assessed by structural alterations. Unfortunately, many of the structural changes in the prostatic epithelium can occur in the absence of hormones and it is difficult to control the effects of injury and regeneration.

Cell culture systems have also been used but there are no cell lines derived from normal tissues. Fraley et al. (1) have reported the establishment of a permanently transferable cell line from a human prostatic adenoma but it seems likely that this line (MA 160) may be the result of HeLa cell contamination. Schroeder et al. have grown cells from a number of prostatic cancers, but these have been maintained for only 2 or 3 months (16). Thus, the general experience is that cells can be grown from most explants of prostatic tissue, benign or malignant, but in almost all studies the cells rapidly die out. The outgrowth that occurs is invariably a mixture of epithelial and mesenchymal cells from all the cell types originally present in the explant.

The conclusions to be drawn are that in this field the future depends on the development of basic techniques.

2. IN VITRO CARCINOGENESIS

A model system in which neoplastic transformation could be studied would be of great value, not only for prostatic cancer but for most other cancer studies. There is no satisfactory model for prostatic carcinoma and almost all the work which has been done in in vitro carcinogenesis, has been concerned with neoplastic transformation by viruses or chemical carcinogens of mesenchymal cells - the so-called fibroblasts which are, in fact, derived from small blood vessels. These cells very rarely produce tumours spontaneously in man or the experimental animal. Recently, it has been possible to transform epithelial cells in vitro, using a technique which is simple in principle but requires some care and dexterity in practice. These transformed cells are from adult mouse salivary gland and produce epithelial tumours when reimplanted into syngeneic hosts (8). After refining the technique it is planned to apply it to other organs.

There have been two attempts at in vitro carcinogenesis in the prostate. Lasnitzki has shown that the application of 20 methylcholanthrene to organ cultures of rodent prostate produces irregular cellular proliferation (9). Heidelberger has extended this work by culturing treated organ cultures and established cell lines from them (7). Some of these produced tumours, but the cells were mesenchymal. In a similar type of experiment Paulson et al. induced a viral transformation of cells from hamster prostate (14). These cells were also tumour producing, but the tumours were also probably mesenchymal.

3. STROMAL-EPITHELIAL INTERACTION

The third area in which tissue culture techniques are of value is in the study of stromal epithelial interactions. Using tissue

culture techniques, it is possible to separate epithelium and stroma, and study the role that each plays in growth and normal function (5). In embryogenesis, the mesenchyme plays an important, perhaps dominant, role in controlling normal growth and differentiation. It has always seemed probable that there is a similar relationship in the adult; in the prostate there is good evidence to suggest that stromal nodular proliferation may initiate epithelial growth in benign nodular hyperplasia, or in the pre-cancerous post-sclerotic hyperplasia.

CONCLUSIONS

Tissue culture has a great potential value in the investigation of prostatic cancer but the technique has not yet been exploited fully. This is due mainly to inherent practical problems, particularly the fact that very few (if any) prostatic tumours can be established in culture as cell lines and the fact that normal human prostatic epithelium is very rarely available for culture. Nevertheless the method can be used to give information on hormone and drug metabolism by the tissue. Further technical developments should allow us eventually to establish cell lines for study from surgically removed tissue but the problem of providing normal control tissue for comparison is likely to remain.

This paper was presented at the International Conference on Prostatic Cancer, Leeds, July 6th-9th, 1976.

REFERENCES

1. Fraley, E.E., Ecker, S., Vincent, M.M.: Spontaneous in vitro neoplastic transformation of adult human prostatic epithelium. Science 170, 540 (1970)
2. Fraley, E.E., Ecker, S.: Tumour production in immune-suppressed hamsters by spontaneously transformed human prostatic epithelium. Journal of Urology 106, 95 (1971)
3. Franks, L.M.: The effects of age on the structure of the mouse prostate and its response to oestrogens and testosterone, in organ culture. British Journal of Cancer 13, 59 (1959)
4. Franks, L.M.: The growth of mouse prostate after transplantation and in vitro culture in natural and chemically defined media: the effects of insulin and normal human serum. Experimental Cell Research 22, 56 (1961)
5. Franks, L.M., Riddle, P.N., Carbonell, A.W., Gey, G.O.: A comparative study of the ultrastructure and lack of growth capacity of adult human prostate epithelium mechanically separated from its stroma. Journal of Pathology 100, 113 (1970)
6. Franks, L.M., Wilson, P.D.: Origin and ultrastructure of cells in vitro. International Review of Cytology 48, 55 (1977)
7. Heidelberger, C.: Chemical oncogenesis in culture. Advances in Cancer Research 18, 317 (1973)
8. Knowles, M.A., Franks, L.M.: Neoplastic transformation of adult epithelial cells in vitro: stages in the progression to malignancy. Cancer Research (Submitted for publication)
9. Lasnitzki, I.: The prostate gland in organ culture. In: Male Accessory Sex Organs. Structure and Function in Mammals. Brandes D. New York, London: Academic Press 1974
10. Lasnitzki, I., Whitaker, R.H., Withycombe, J.F.R.: The effect of steroid hormones on the growth pattern and RNA synthesis in human benign prostatic hyperplasia in organ culture. British Journal of Cancer 32, 168 (1975)
11. McMahon, M.J., Butler, A.V.J., Thomas, G.H.: Morphological responses of prostatic carcinoma to Testosterone in organ culture. British Journal of Cancer 26, 388 (1972)
12. McMahon, M.J., Thomas, G.H.: Morphological changes in benign hyperplasia in culture. British Journal of Cancer 27, 323 (1973)
13. McRae, C.U., Ghanadian, R., Fotherby, K., Chisholm, G.D.: The effect of testosterone on the human prostate in organ culture. British Journal of Urology 45, 156 (1973)
14. Paulson, D.F., Fraley, E.E., Rabson, A.S., Ketcham, A.S.: SV40 transformed hamster prostatic tissue. A model of human prostatic malignancy. Surgery 64, 241 (1968)
15. Riches, A.C., Shipman, P.A., Littlewood, V., Donaldson, I., Thomas, G.H.: Organ culture - a model for defining the hormone dependency of human benign prostatic hyperplasia. In: Human Tumours in Short Term Culture Techniques and Clinical Applications, p. 227. Ed. Dandy P.P. New York: Academic Press 1976
16. Schroeder, F.H., Sato, G., Gittes, R.G.:

Human prostatic adenocarcinoma: growth in monolayer tissue culture. Journal of Urology 106, 734 (1971)
17. Webber, M. M.: Cytopathic effects in primary epithelial cultures derived from the human prostate Investigate Urology 13, 259 (1976)

L. M. Franks, M. D.
Department of Cellular Pathology
Imperial Cancer Research Fund Laboratories
P. O. Box No. 123
Lincoln's Inn Fields
London WC2A 3PX
United Kingdom