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In Vitro Culture of Human Prostatic Tissue

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Summary. A procedure is described which yields a significant percentage of long-term mixed cell cultures of human prostatic tissue. Attempts were made to suppress the proliferation of stromal fibroblasts and to characterize the cultured cells as those of prostatic origin. The problems associated with establishing epithelial cell lines are discussed.

Key words: Cell culture - Prostate - Collagenase - Epithelial.

Carcinoma of the prostate is the second most common cause of death from cancer in the adult male and accounts for over 18,000 deaths annually. Despite this high incidence, fundamental research concerning prostatic neoplasia has been hindered by the lack of a suitable experimental model. Spontaneous prostatic tumours do occur in laboratory animals but the rarity of such precludes extensive investigation (2). Concerted efforts to induce neoplastic change in vitro, utilizing either chemical carcinogens (4) or an oncogenic virus (13, 17), have so far failed to yield a representative tumour system. Consequently, any investigative model designed to study prostatic oncogenesis necessitates the cultivation of human prostate cells in vitro.

The object of this report is to describe the techniques employed and the results obtained in this laboratory in establishing monolayer tissue cultures from benign and malignant prostate specimens. These preliminary investigations constitute a portion of a long-range research program regarding the in vitro behaviour of human prostatic epithelium as it relates to the viral and hormonal aspects of prostatic malignancy.

MATERIAL AND METHODS

Hyperplastic and malignant prostatic specimens obtained at operation from adult males were collected under sterile conditions, washed with cold phosphate-buffered saline, and minced into 1-2 mm pieces. Approximately ten fragments were then placed into each of a number of 30 ml plastic flasks or 8 oz. plastic cell culture flasks and suspended in Dulbecco's medium (fortified Eagle's MEM) containing 20% heat-inactivated fetal bovine serum, 10% tryptose phosphate broth, 0.075% sodium bicarbonate, 100 U/ml penicillin, and 100 µg/ml streptomycin. Cultures were incubated at 37° C in a moist environment of 5% CO_2 in air. Medium was initially changed at one week, replenished thrice weekly thereafter, and subcultures performed utilizing an equal part mixture of 0.125% trypsin and 0.02% versene buffer. Following confluent growth, cells from two small flasks were pooled and transferred to a single 250 ml plastic bottle in which they were subsequently maintained. Part of the cell culture fragments were suspended in Dulbecco's medium and 20% fetal bovine serum containing 1 mg/1 ml collagenase for 18 hrs at 37° C. The medium was then replaced with fresh Dulbecco medium and 20% fetal calf serum. The limited number of remaining fibroblasts were removed from the culture at a later phase of in vitro growth with brief trypsinization. Cell cultures surviving more than 10 in vitro passages were maintained in Ham's F-10 medium with 20% fetal calf serum.

Smaller specimens, e. g. needle biopsies,

were similarly prepared but suspended in the 16 mm wells of microtest plates, so as to provide an optimal weight: volume ratio for growth. The microplate monolayers were likewise passaged until they attained confluent growth in 8 wells, at which time they were pooled and transferred to a single 30 ml plastic flask.

RESULTS

Cell Culture

The suspension cultures usually remained dormant for up to 3 weeks, following which time a portion of cells shed from the floating explants became adherent to the flask and proliferated to form a confluent monolayer 4-5 weeks after planting (Fig. 1). Adequate primary growth of cells occurred without exception and usually represented a mixed population of connective tissue elements, myoepithelial, and epithelial cells. However, with serial passage, the fibroblasts tended to obliterate the epithelial foci. Of the 89 prostate specimens processed by the explant suspension method, 39% remain viable in vitro after ten or more transfers (Table 1). However, 6% of all culture attempts yielded a significant proportion of epithelioid cells, the remainder assuming a predominantly fibroblastic morphology. Once established in culture, however, the cells have been maintained equally well in either Dulbecco's or Ham's medium, with several cultures from benign and malignant specimens respectively having been carried through up to 30 passages. The cultures discarded were either lost due to contamination or ceased growing early in subculture.

Epithelial Selection

In an ettempt to promote epithelial predominance and/or selectively suppress stromal overgrowth, 10% heat inactivated horse serum was

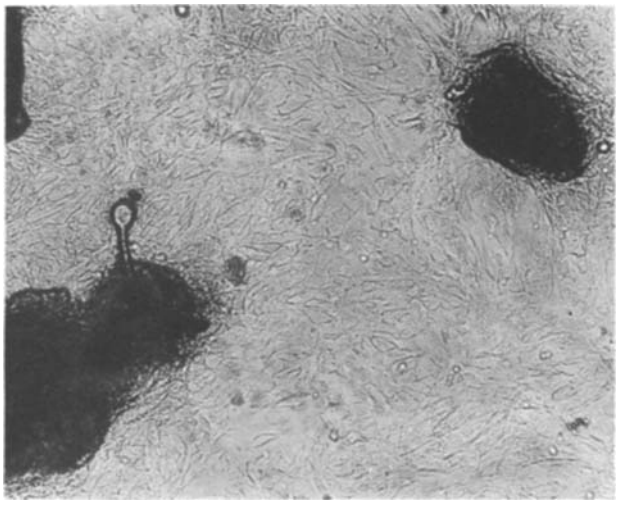

Fig. 1. Primary epithelial monolayer growth from prostatic explants

Table 1. Growth of human prostate in vitro

	BPH[a]	Adenocarcinoma	Total
Specimens processed (explant suspension)	55	34	89
Viable in culture (> 10 passages)	17	18	35
Epithelial morphology (> 10 passages)	3	3	6
Contaminated or lost (0-10 passages)	38	16	54

[a] Benign prostatic hypertrophy

Table 2. Effect of collagenase treatment on epithelioid growth of prostate specimens

Method	Total number of specimens	Cell culture with epithelioid growth		Cell culture with fibroblastoid growth	
		No.	%	No.	%
Collagenase treatment	16	10	62.5	6	37.5
No collagenase added	21	8	38.6	13	61.4

added to the medium in conjunction with 10% fetal bovine serum as recently suggested by Webber (19). However, with passage, the epithelial elements vanished as rapidly as when 20% bovine serum was used alone. In another early passage series, hydrocortisone sodium succinate at concentrations ranging from 0.1-2.0 mg/ml was employed according to the method of Kline et al. (7). Although there was unequivocal disruption of fibroblasts after 6 days in the steroid medium, the residual epithelial foci were nevertheless lost to stromal regrowth in subsequent passage in steroid-free media. The application of collagenase (12) enhanced the establishment of pure epithelioid cell cultures from prostatic tissue (Table 2); 62.5% of collagenase treated tissue fragments yielding pure epithelioid growth compared to 38.6% of nontreated ones. However, the average in vitro lifetime of the epithelioid cell cultures was not extended.

In Vitro Enzyme Analysis

To characterize the cultured cells as those of prostatic origin, coverslip monolayers of random cultures were established and stained for prostate-specific acid phosphatase (3). A three hour substrate incubation revealed scattered perinuclear enzyme activity in those cultures harbouring epithelial cells, supporting evidence for the presence of secretory prostate epithelium. The enzyme was predictably absent however in the purely fibroblastic populations suggesting that only glandular epithelium has the capacity to synthesise tartrate-labile acid phosphatase in vitro.

DISCUSSION

Although the growth of well-defined human prostate cells in culture remains notoriously difficult, technical refinements have permitted maintenance of prostatic tissue in mixed monolayer cultures for limited periods of time (1, 15, 16). However, to date there have been no stable cell lines developed with the possible exception of EB33, an epithelial strain derived from an adenocarcinoma (11). The relative absence of successful in vitro cultures of prostatic epithelium probably reflects not only the inherent difficulty in isolating viable cells from within an abundant stromal mass, but also the unique nutritional and hormonal requirements of the human prostate.

The explant suspension technique employed to establish primary monolayers in this study is somewhat similar to the method of Stonington et al. (18), being adopted from previous work with suspension cultures (9). Presumably, the floating explants become encapsulated with hyperplastic epithelium that produces excresences which are shed and adhere to the substrate, hopefully to form epithelial monolayers (20).

As it is advantageous to isolate the epithelial component in vitro, concerted efforts have been made to significantly reduce the magnitude of fibroblastic overgrowth in mixed culture. Schroeder et al. (16) attempted selective stromal suppression using the nucleotide analogue bromodeoxyuridine. Webber (19) and Puck (14) reported that the use of horse serum during early subculture favours epithelial predominance whereas bovine serum accentuates stromal proliferation. Glucocorticoids have also been demonstrated to possess dose related inhibitory effects on the growth of fibroblasts in vitro (6). However, in our experience, these modalities for promoting epithelial growth have proven ineffective in that, with serial passage, all cultures tended to assume a fibroblastic morphology despite laboratory manipulation.

The value of collagenase in obtaining epithelioid growth from primary cultures is apparent. Almost twice as many epithelioid lines could be obtained with this method. Originally described by Lasfargues and Moore (8) this enzyme gently separates the glandular epithelial components from the fibroblastic stroma allowing the latter to be discarded.

The difficulty in determining the origin of cultured cells is not unique to the prostate but ubiquitous among tissue culture systems. The demonstration of vestigial amounts of acid phosphatase may lend support to the presence of functioning prostatic epithelium in vitro, but histochemical staining remains notoriously nonspecific. Sophisticated immunoassays for prostatic acid phosphatase (5) and tissue antigens (10) have recently emerged and should prove to be much more reliable in characterizing prostatic cells in culture.

The development of cell cultures is of paramount importance to any study concerning the behaviour of human prostatic epithelium as it relates to the immunological, hormonal, and viral aspects of prostatic malignancy. The significance of this research lies in the fact that a procedure has been devised which has yielded relatively long term cultures of mixed cell morphology from both benign and malignant human prostatic tissues. Following the establishment of stable prostatic cell lines, our intent is to elucidate the possible aetiological role of viruses, specifically herpes and cytomegalovirus, in the development of human prostatic neoplasia. Immunological studies to detect intact virus or their respective precursors harboured in tumour-derived prostatic cells are currently in progress.

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REFERENCES

1. Brehmer, B., Marquardt, H., Madsen, P.O.: Growth and hormonal response of cells derived from carcinoma and hyperplasia of the prostate in monolayer cell culture. Journal of Urology 108, 890 (1972)
2. Brendler, H.: Experimental prostatic cancer: background of the problem. National Cancer Institute Monographs 12, 343 (1963)
3. Burstone, M.S.: Histochemical demonstration of acid phosphatase with naphthol-AS-phosphate. Journal of the National Cancer Institute 21, 523 (1958)
4. Chen, T.T., Heidelberger, C.: In vitro transformation of cells derived from mouse prostate in the presence of 3 methylcholanthrene. Journal of the National Cancer Institute 42, 915 (1969)
5. Cooper, J.E., Foti, A.: A radioimmunoassay for prostatic acid phosphatase. Investigative Urology 12, 98 (1974)
6. Kaufman, N., Mason, E.J., Kinney, T.D.: The effect of steroids on fibroblast migration in vitro. American Journal of Pathology 29, 761 (1953)
7. Kline, I., Leighton, J., Belkin, M., Orr, H.: Some observations on the response of four established human cell strains to hydrocortisone in tissue culture. Cancer Research 17, 780 (1957)
8. Lasfargues, E.Y., Morre, D.H.: A method for the continuous cultivation of mammary epithelium. In Vitro 7, 21 (1971)
9. Maitland, H.B., Maitland, M.C.: Cultivation of vaccinia virus without tissue culture. Lancet 215, 596 (1928)
0. Moncure, C.W., Johnston, C.L., Koontz, W.W., Smith, M.J.V.: Investigation of specific antigens in prostatic cancer. Cancer Chemotherapy Reports 59, 105 (1975)
11. Okada, K., Schroeder, F.H.: Human prostatic carcinoma in cell culture: preliminary report on the development and characterization of an epithelial cell line (EB33). Urological Research 2, 111 (1974)
12. Owens, R.B., Smith, H.S., Hackett, A.J.: Epithelial cell cultures from normal glandular tissue to mice. Journal of the National Cancer Institute 53, 261 (1974)
13. Paulson, D.F., Fraley, E.E., Rabson, A.S.: SV40 - transformed hamster prostatic malignancy. Surgery 64, 241 (1968)
14. Puck, T.T., Cierciura, S.J., Fisher, H.W.: Clonal growth in vitro of human cells with fibroblastic morphology. Journal of Experimental Medicine 106, 145 (1957)
15. Schrodt, G.R., Foreman, C.D.: In vitro maintenance of human hyperplastic prostate tissue. Investigative Urology 9, 85 (1971)
16. Schroeder, F.H., Sato, G., Gittes, R.F.: Human prostatic adenocarcinoma: growth in monolayer tissue culture. Journal of Urology 106, 734 (1971)
17. Shah, K.V., Pond, H.S.: Attempts to produce urinary bladder cancer in dogs by inoculation of SV40 into the urothelium. Investigative Urology 10, 141 (1972)
18. Stonington, O.G., Hemingsen, H.: Culture of cells as monolayer derived from epithelium of human prostate. Journal of Urology 106, 393 (1971)
19. Webber, M.M.: Effects of serum on the growth of prostatic cells in vitro. Journal of Urology 112, 798 (1974)
20. Webber, M.M., Stonington, O.G., Poche, P.A.: Epithelial outgrowth from suspension cultures of human prostatic tissue. In Vitro 10, 196 (1974)

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