NORMAL AND BENIGN HUMAN PROSTATIC EPITHELIUM IN CULTURE

I. Isolation

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SUMMARY

Isolation of normal human glandular epithelia and their growth and maintenance in vitro have been major problems. The primary objective of studies presented here was to isolate postpubertal, normal human, viable prostatic epithelium for in vitro cultivation. The long-term objective of these investigations was to develop an in vitro human cell model system for studies on prostatic carcinogenesis. A method for isolation of viable, normal and benign human prostatic epithelium, using collagenase for tissue dissociation, is described. Intact acini were isolated, which, on plating gave rise to vigorously growing monolayer cultures of epithelial cells. The purity of epithelial cultures partly depended upon the source of tissue. Specimens of normal prostate and those of benign tissue derived from open prostatectomies provided primarily pure epithelial cultures with occasional fibroblast colonies in some cultures, which could be removed. Cultures from some specimens of transurethral resection of the prostate (TURP) contained many fibroblast colonies due to incomplete separation of acini from the stroma. This resulted from incomplete digestion of denatured tissue caused by electrocauterization during surgery. Cultures established in this manner are being used to study the effects of hormones, vitamins and other growth regulators in order to establish growth requirements of these cells in vitro, which would facilitate their long-term maintenance.

Key words: human; prostate; epithelium; culture; collagenase; model system.

INTRODUCTION

Very few studies have been made on the isolation, growth and maintenance of normal, human prostatic epithelium in vitro. The major objective of studies being conducted by this investigator is to develop an in vitro cell model system using postpubertal, normal human, prostatic epithelium, which could be used to study early steps in prostatic carcinogenesis. In order to develop such a model system, it is necessary to isolate normal epithelium, to establish its nutrient requirements in vitro and to establish its prostatic epithelial origin. This paper deals with studies on the isolation of prostatic epithelium.

Current information suggests that 80% of all human cancers may be caused by environmental carcinogens. Considerable emphasis is therefore being placed on the identification of these carcinogens and on the methods of interfering with the process of malignant transformation. Nearly 85% of all human tumors arise from epithelial cells. It is therefore important to identify and determine the mechanism of carcinogen-target cell interaction in organs that show a high incidence of cancer. Therefore, it is important to develop in vitro model systems of human epithelial cells. This requires isolation of viable, normal epithelial cells as described in this paper.
The incidence of benign tumors of the prostate may be as high as 80% in men over the age of 40 (1). At least 30% of all men over 50 years may have histological carcinoma and this figure increases to 50% after the age of 70 (2). In view of this, it is important to understand the mechanism of carcinogenesis in the prostate.

Lechner et al. (3) have reported successful cultivation of neonatal prostatic epithelium. These cultures were initiated from cells spilled after mincing the tissue. No work to date has been reported on the successful isolation, cultivation and maintenance of normal, postpubertal human prostatic epithelium. The significance of using postpubertal prostate must be emphasized. Since prostatic carcinomas arise from adult, androgen-responsive epithelium, it is important to use such epithelium for developing an in vitro model system.

It has generally been difficult to grow and maintain normal human epithelial cells in vitro. This is paradoxical when one realizes that the majority of human cancers are of epithelial origin. Various methods for epithelial cell isolation and culture have been used, e.g. nonenzymatic isolation by scraping of bovine pancreatic duct (4); enzymatic digestion of minced pancreatic tissue (5-7); enzymatic digestion of minced pancreatic tissue (8) and human endometrium (9); and collection of normal epithelial cells from human milk (10). Waymouth (11) has reviewed various methods for tissue digestion and subculturing.

Physiological breakdown of collagen in many mammals and amphibians is accomplished by the action of specific collagenases, produced in very small amounts as needed. These are apparently not stored in vivo (12,13). Collagenase activity has been detected in normal and diseased human skin, in the edges of healing wounds, in growing bone, in involuting uterus, in inflamed human tissues, e.g. rheumatoid arthritis, and in regenerating newt limbs (12). On the basis of these observations it is logical to conclude that the same enzyme might be used for isolation of cells from tissues after digestion with collagenase.

Collagenases by definition are enzymes capable of dissolving fibrous collagen by peptide bond cleavage under physiological conditions of pH and temperature. The specific substrate, collagen, comprises 33% of the total protein in mammalian organisms (13). Collagenase is nontoxic at a neutral pH, is active within a pH range of 6.5 to 7.8 and requires Ca\(^{2+}\) ions for its activity and stability. It can therefore be dissolved in complete tissue culture media. Since it has its specific action on collagen, it is active even in media containing 5 to 10% serum. Thus, minced tissue can be placed in a complete culture medium enriched with serum and still be exposed to the dissociating activity of collagenase. This results in improved cell viability as compared to serum-free dissociating media containing proteolytic enzymes (14).

Lasfargues (14-17) pioneered the use of collagenase for digestion of tissue for cell dispersal in the preparation of primary cultures of mouse mammary epithelium. Since prostate has a histological composition similar to that of the mammary glands, I have used collagenase for the isolation of prostatic epithelium.

Collagenase has been used in recent years for dissociation of animal tissues and for isolation of epithelial cells of various types, e.g. aortic endothelium (18), liver (19-22), pancreatic epithelium (23) and mammary epithelium (15,24-26). In the majority of these cultures some fibroblasts did get carried over into the cultures.

Cultures of animal prostatic epithelium to date have primarily been established from explant cultures (27-29). Human prostatic epithelium has also primarily been grown in vitro using explant cultures (5-7, 30-38). Enzymatic digestion of prostatic tissue using trypsin and pronase has also been tried (31,33,39). Stone, Stone, and Paulson (40) used collagenase for dispersal of benign prostatic tissue.

One of the major problems in culturing human epithelial cells has been the contamination with fibroblasts. I first initiated investigations in 1974 on the usefulness of collagenase in establishing pure monolayer cultures of prostatic epithelium. There were two major reasons for this: (a) to inhibit the growth of fibroblasts in mixed cultures initiated from explants and (b) to isolate prostatic acini from prostatic tissue, which could then be used to initiate pure cultures of prostatic epithelium. Such a method would also facilitate setting up of a large number of cultures in a fraction of the time (2-3 days) spent on cutting explants for 200-300 cultures. In order to eliminate fibroblasts from mixed cultures, collagenase was tested for its cytotoxicity to fibroblasts, especially with the knowledge that it did not have deleterious effects on epithelial cells and did not damage their cell membranes. Work was begun on both normal and benign human prostatic tissue (41).

Separation of epithelial cells from the stromal elements, which form a major part of prostatic tis-