ISOLATION AND CHARACTERIZATION OF A NOVEL HUMAN PROSTATIC STROMAL CELL CULTURE: DuK50

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SUMMARY

A novel human prostatic stromal cell culture, designated DuK50, has been passed in vitro > 12 mo. Tissue cultures were obtained from material harvested within a normal region of a radical prostatectomy specimen. These monolayers exhibited normal fibroblastic characteristics with each cell having a flattened, elongated appearance. Karyotypic analysis revealed a normal, male 46, XY chromosomal content with no numerical or structural abnormalities. DNA analysis using a Cell Analysis Systems Image Analyzer confirmed a euploid DNA content (7.9 pg DNA). Cellular markers for verification of stromal cell type were performed by immunohistochemical techniques. DuK50 stained positive for vimentin and fibronectin. Immunostains for epithelial cytokeratins and prostate-specific antigen were negative, which ruled out contamination with prostatic epithelial cells. Negative immunostaining with desmin monoclonal antibody and light staining with smooth muscle actin alpha is consistent with the staining pattern of myofibroblasts. Response to various androgens, measured by a microculture tetrazolium assay technique, revealed a significant growth stimulation of DuK50. Soft agar invasiveness assays and tumorigenicity studies in nude mice were negative. DuK50 exhibits a rapid doubling time with excellent plating efficiency, thrives in a readily available media supplemented with fetal bovine serum, and passes with routine trypsin protocols. The availability of this prostatic stromal cell culture may facilitate studies on this cell type's role in growth factor modulation, drug and steroid metabolism, and stromal-epithelial interactions in the prostate.

Key words: prostatic; stromal; cell culture.

INTRODUCTION

Prostatic morphogenesis and maintenance is dependent on androgenic steroids. Because the prostate is a heterogeneous gland composed of stromal and epithelial cells, it is important to study each cell type and their relationship. Possible interactions between stromal and epithelial cells in the prostate have been the subject of numerous studies (Cunha, 1976; Lasnitzki and Mizuno, 1979, 1980; Cunha and Chung, 1981; Cunha et al., 1983). An increasing body of evidence now proposes that some hormonal responses may not be invoked in the epithelial cells but rather in the adjacent stromal cells (Chung and Cunha, 1983; Cunha et al., 1983). Prostatic stroma is likely a primary site of testosterone conversion to dihydrotestosterone (Wilkin et al., 1980). High levels of 5 alpha reductase activity are localized in the stroma (Cowan et al., 1977). Furthermore, the differentiation and function of epithelial cells may be regulated by the surrounding stromal cells (Cunha, 1976; Cunha and Chung, 1981; Cunha, 1983).

Benign prostatic hyperplasia (BPH) is considered to be primarily a disease of the stroma. The ratio of stroma to epithelium shifts from 2:1 in normal prostate to 5:1 in BPH (Bartsch et al., 1979). BPH arises most frequently in the transition zone where stromal cells are most dense (McNeal, 1978). Localized stromal proliferation may be the first event in the pathogenesis of BPH (Mostifi, 1970; Pradhan and Chandra, 1975). Thus, a reactivation of embryonic activity in stromal cells may be responsible for the pathological enlargement of the prostate. Concomitant glandular proliferation may be the result of a humoral inducing agent produced by the stroma (McNeal, 1975, 1978). This evidence demonstrates the importance of stromal-epithelial interactions in the development and maintenance of the normal prostate and underscores the potential role of stromal-epithelial interactions in diseases of the prostate. In light of this evidence, we have sought to isolate prostatic stromal cells for use in coculture experiments with primary epithelial cultures already in production by our laboratory. In this report, we describe the isolation and characterization of DuK50, a normal human prostatic stromal cell culture.

MATERIALS AND METHODS

Procurement of specimen for culture. Approximately 1.0 cm³ of prostatic tissue was harvested from a radical prostatectomy specimen removed from a 76-year-old male patient at Duke University Medical Center, Durham, NC. The tissue was placed sterilely in tissue transport medium (TTM): Ham's F12 media, 2 mM glutamine, and 2 uM insulin (Sigma Chemical Co., St. Louis, MO). Adjacent areas of prostatic tissue were snap frozen for routine histologic evaluation by standard hematoxylin and eosin (H&E) staining and normal morphology was confirmed (Luna, 1968).

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Digestion and initiation of primary culture. Prostatic tissue was placed in a 100-mm sterile tissue culture dish with 5 ml TTM, and lacerated using opposing scalpels under sterile conditions. Single cells and minced tissue were collected with a small bore pipette and transferred to a 50-ml conical tube; TTM was added to a final volume of 10 ml. Digestion was initiated with the addition of 5 ml of collagenase type I (500 units per ml) and 5 ml of hyaluronidase (680 units per ml) (Sigma). Tubes were placed on a shaker at 37 °C for 18 h. Digestion medium was removed by centrifugation at 600 g for 10 min, and the pellet was resuspended in 5 ml of RPMI 1640 (Sigma) plus 10% fetal calf serum (FCS, GIBCO, Grand Island, NY). Cellular material was then separated through a 95-μm filter. Retained cellular material was rinsed from the filter and plated in appropriately labeled culture dishes. The dishes were placed in a water-jacketed incubator at 5% CO₂, 37 °C (Queue Inc., Asheville, NC).

Serial passage. Cells were passed 1:5 weekly with 0.25% trypsin-7 mM ethylene-diaminetetraacetic acid (EDTA) (GIBCO) on reaching confluence. Doubling time determination. Cells were plated at 2 × 10⁵ cells/dish, for 3 d in a 100-mm tissue culture dish with 10 ml of growth media. Doubling time calculations were performed by dividing 96 h by the log base 2 of the cell count at Time 96 h minus the log base 2 of the cell count at Time 0 (Jakoby and Pastan, 1979).

Mycoplasma testing and treatment. Mycoplasma testing was performed by the Duke University Clinical Microbiology Laboratory. An aliquot of antibiotic-free tissue culture medium was used to inoculate FFA broth and agar with inhibitors, 10B broth with inhibitors, and AB selective agar (Remel Co., Lenexa, KN). Standard methods for isolation for Mycoplasma pneumoniae, M. hominis, and Ureaplasma urealyticum were used (Bailey and Scott's diagnostic microbiology, 1990). DuK50 tested positive for mycoplasma and was treated with BM-cyclin (Boehringer Mannheim, Indianapolis, IN) according to the manufacturer's directions using BM-cyclin 1 and 2 in an alternating pattern. BM-cyclin was recently shown to be an effective means of permanently eliminating several species of mycoplasma (Drexler et al., 1994). DuK50 has been isolated in a separate incubator since its initiation.

Immunohistological characterization. DuK50 cells were grown on sterile glass slides, 5 × 10⁵ cells/slide, for 3 d in a 100-mm tissue culture dish with 10 ml of growth media. Slides were removed with tweezers, rinsed gently three times with PBS-A (137 mM NaCl, 3 mM KCl, 1 mM KH₂PO₄, and 152 mM Na₂HPO₄), and air dried overnight. Slides were immunostained the following day by the protocol of Hsu et al. (1981) and Kerns et al. (1990) with few modifications. Briefly, the slides were first fixed in acetone for 10 min, then air dried for 20 min, and incubated 15 min with 5% normal horse serum (GIBCO) to block nonspecific Fc receptor sites. The appropriate primary antibody was applied to the slides and allowed to incubate overnight at 4 °C in a humidified chamber. The monoclonal antibody, AE1/AE3 (Boehringer Mannheim), diluted 1:500 in antibody diluting buffer (ADB, Bio-Meda Corp., Foster City, CA) was used to stain for high and low molecular weight epithelial cytokeratins. The monoclonal antibody, PSA (prediluted from Biogenex Laboratories, San Ramon, CA), was used to stain for prostate specific antigen (PSA). Monoclonal antibodies to vimentin, filaminectin, desmin, and smooth muscle actin alpha were also obtained from Biogenex in a prediluted form. IgG1 (Coulter Immunology, Hialeah, FL), used as a negative control, was diluted 1:200 in ADB. The slides were washed three times in phosphate-buffered saline (PBS, GIBCO), pH 7.4, for 5 min each wash (standard wash protocol). Following primary antibody incubation, biotinylated secondary antibody horse anti-mouse IgG (Vector Laboratories, Burlingame, CA) was diluted 1:100 in ADB and applied for 30 min, followed by washing in PBS. Slides were then incubated with premixed Elite reagent avidin-biotin complex (ABC) (Vector) for 30 min. Slides were then washed in PBS and counterstained with methyl green (Sigma) in sodium acetate buffer (pH 5.2), dehydrated in in-