Primary Culture of Smooth Muscle Cells from Benigin Prostatic Hyperplasia

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Abstract

Primary cultures of smooth muscle cells (SMCs) were derived from the human prostate obtained from four patients with symptomatic benign prostatic hyperplasia (BPH) undergoing open prostatectomy, using collagenase digestion and preferential adhesion techniques. SMCs attached to the plastic substrate by 6 to 10 h, proliferated by 2 to 3 days, and reached confluency by 10 to 14 days with a “hill-and-valley” pattern. Immunocytochemical staining of cultured SMCs using anti human muscle-specific actin monoclonal antibody (HHF35), confirmed that more than 90% primary cultured cells were positive for HHF-35. Transmission electron microscopy of SMCs revealed dense myofibrils (6-8 nm width) showing focal density, and was compatible to smooth muscle cells. This primary culture will be utilized to provide a useful model to investigate the character of smooth muscle cells and their role in the development of BPH.

Key words: smooth muscle cell; benign prostatic hyperplasia; primary culture.

Introduction

Benign prostatic hyperplasia (BPH) describes a hyperplastic process of the stromal and epithelial elements of the prostate. (Franks, 1975) Bartsch et al. reported that the stromal:epithelial ratio in the normal prostate and BPH was 2:1 and 5:1. (Bartsch et al., 1979) The severity of symptomatic BPH is not related to the size of the prostate adenoma. Shapiro et al. determined that the development of symptomatic BPH was dependent upon the stroma:epithelial ratio, and showed that the stroma:epithelial ratio in men with symptomatic BPH was 1.7-fold greater than in those with asymptomatic BPH. (Shapiro et al., 1992a) These findings have suggested that the stroma plays an important role in the development of BPH. The smooth muscle is one of the major components in the stroma. The characterization of smooth muscle cells from BPH is necessary for the study on the regulation of growth and proliferation of prostatic muscular element. So we have developed and characterized the primary cell culture of SMCs derived from the prostate obtained from the patients with BPH. SMCs in primary culture were identified by their pattern of growth, immunocytochemical staining and ultrastructural features.
Materials and Methods

Smooth muscle cell preparation

Prostate specimens were obtained from four patients with symptomatic BPH who underwent open prostatectomy. Histologically, the prostates were fibromuscular type with a small portion of adenomatous hyperplasia. All tissue specimens were removed aseptically and washed several times in calcium and magnesium free Hanks balanced saline (GIBCO). Tissue was cut into 1 to 2 mm² fragments and incubated with Dulbecco's Modified Eagle's medium (DMEN) (Nissui Pharmaceutical Co. Tokyo) containing 0.1% collagenase (type 1 Sigma Chemical Co.) and 5% fetal bovine serum (FBS, GIBCO) overnight (about 10-15 hours). After the incubation the remaining tissue was passed through the glass pipette and the cell-tissue suspension was filtered through a 500 µm stainless steel mesh. After filtration, the dispersed cells were partly purified by plating for 1 to 2 h on 100 mm Falcon plastic Petri dishes (Becton Dickinson Labware.) to allow preferential attachment of contaminant fibroblasts. (Ricciardelli et al., 1989; Yaffe et al., 1968)

The concentration of cells was measured by hemacytometer using trypan blue (0.4%), and the cells then were seeded at a density 1.0×10⁴ viable cells/cm² in Falcon plastic culture dishes and cultured in DMEN medium with 10% FBS, 10 µg/ml penicillin, and 10 µg/ml streptomycin. The cells were incubated at 37°C, 5% CO₂ : 95% humidified mixed gas. Culture medium was exchanged every 2 days. Cell growth was examined with microscopy every day. Upon reaching confluence cells were harvested for passaging with a trypsin (0.05%) (type 1 Sigma Chemical Co.) and EDTA (0.02%) solution, and plated at 10⁴ cells/cm².

Immunocytochemical staining of cultured SMCs

HHF35, a human muscle-specific monoclonal anti-actin antibody (ENZO Diagnostics Inc. NY) was used to identify cultured SMCs. HHF35 recognized the alpha actin of skeletal, cardiac, and smooth muscle. It does not react with fibroblasts, endothelial cells, or macrophages. (Tsukada et al., 1987) When primary culture cells reached confluence, they were harvested as described above, and were cultured either in culture chamber slides (Nunc Inc.) or in poly-L-lysine coated slides (0.1% Sigma Chemical Co.). After 2 to 3 days (log phase) the slides were rinsed with phosphate buffered saline (PBS), pH 7.4, and fixed with methanol at −20°C for 5 min.

Immunocytochemical staining was performed as a modified avidin biotin complex (ABC) method and was done using SAB-PO Kit (Nichi Co. Japan). (Hsu et al., 1981) HHF35 was diluted 1:200 in 1% bovine serum albumin (BSA)/0.05 M PBS (pH 7.6) and cells were incubated in the solution for 1 h at 24°C. The biotinylated bridge serum was applied for 30 min at 24°C, then the cells were treated by ABC for the same time. After incubated with 0.02% 3,3-diaminobenzidine tetrahydrochloride (10 min), (Hsu et al., 1982) the slides were then washed in water, counterstained with hematoxylin. Stained Cells were examined by light microscopy.

Transmission electron microscopy

Cultured cell were examined by electron microscopy. The cells were washed twice with
0.05 M PBS (pH 7.6) and harvested with EDTA (0.02%) and trypsin (0.05%). After centrifugation the pellets of cultured cells were fixed for 1 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.6) at 24°C, and were postfixed in 1% osmium tetroxide for 1 h, were dehydrated through a graded alcohol series followed by propylene oxide and were embedded in Quetol 812 (Nisshin EM Co. Ltd Tokyo). Sections were cut in 70 nm thickness, were stained with uranyl acetate and lead citrate, and were observed with JEM 100CX electron microscope.

Results

Cytology and growth behavior of cultured SMCs

SMCs were isolated from prostate tissue seeded at plastic dishes. The cells flattened and attached to the plastic substrate by 8 to 10 h and showed a ribbon or spindle shape within 24 h. After 2 to 3 days, the cultured cells began to proliferate. After 7 to 10 days, the cultured cells became confluent and the cells started to grow in multiple layers with a “hill-and-valley” pattern (Fig. 1).

In primary culture, the epithelial cells present had small clumps or single appearances, single epithelial cells became vacuolized and did not proliferate. Small clump epithelial cells had a slow proliferation rate in DMEM medium with 10% FBS. Epithelial cells were only observed before the first subculture.

Immunocytochemical staining of cultured SMCs

The cells were stained with HHF35 antibodies in an “actin-like” pattern decorating stress fibers running the entire length of each cell (Fig. 2). We confirmed that more than 90% of primary culture cells had smooth muscle cells.

Fig. 1. Prostate smooth muscle cells are showing characteristic “hill and valley” morphology pattern. ×100
Fig. 2. Immunocytochemical staining of cultured smooth muscle cells with the muscle-specific antibody HHF35 Fig. 2. ×200

Fig. 3. Transmission electron micrograph of smooth muscle cells in culture shown myofilament (arrow head) bundles dense bodies (arrow).

Ultrastructure of cultured smooth muscle cells

Under transmission electron microscope observation, SMCs exhibited a lot of fine fiber of 6-8 nm, in which the area of focal density or fuliform density were scattered. The other organelle were scanty. The dense patch was recognized on the cell membrane. The caveocae were demonstrated near the cell membrane (Fig. 3).
Discussion

The present study was to develop SMCs isolated from BPH tissue. A highly pure primary culture of SMCs was established. By immunocytochemical staining more than 90% of cells were identified as smooth muscle cells. The cytologic features and growth patterns of the cultured cells were similar to the characteristics of cultured visceral and vascular smooth muscle cell. (Chamley-Campbell et al., 1979; Devore-Carter et al., 1988; Ionasescu et al., 1985; Palmberg et al., 1986; Ricciardelli et al., 1989; Ross et al., 1980) Immunocytochemical studies revealed that cultured cells were stained positive with muscle–actin–specific antibody HHF35. In the primary culture, the SMCs were recognized with HHF 35 during their proliferative state. Cultured SMCs possessed ultrastructural feature of smooth muscle cell, such as myofilaments with focal density. (Palmberg et al., 1986; Ricciardelli et al., 1989)

The human prostate is comprised primarily of glandular epithelium, smooth muscle and connective tissue. Shapiro et al. (Shapiro et al., 1992b) determined that the area density of smooth muscle in the prostate of BPH range from 20% to 40%. According their reports, BPH contained a big proportion of smooth muscle, although BPH tissue contained great number of fibrous tissue. (Shapiro et al., 1992b) A high yield of SMCs could be obtained utilizing enzyme digest and preferential adhesion techniques. In our specimen, the clinical histological diagnosis showed all specimens were mainly fibromuscular hyperplasia. It indicated that the highly pure primary culture of SMCs could be successfully established.

Recently, cell culture techniques have become an important experimental tool in the study of SMCs. Although there are most knowledges of both vascular and visceral smooth muscle in cell and tissue culture, (Carter et al., 1988; Ionasecu et al., 1985; Palmberg et al., 1986; Ross et al., 1980) the studies on cultured prostate smooth muscle cells are very few. These primary culture of SMCs from human prostate may be useful for the studies on ther regulation of growth and proliferation of prostatic SMCs during hyperplastic developmont. This may help to provide insights into the pathogenesis and therapy of BPH.

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