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IMMUNOCYTOCHEMICAL CHARACTERISATION OF CULTURES OF HUMAN BLADDER DETRUSOR AND MUCOSA CELLS

Hypothesis / aims of study

Recent studies have suggested an important role of the urothelium and lamina propria in the regulation of bladder function. Urothelial cells have been shown to have sensory properties, whereas suburothelial myofibroblasts may act as a mediator between urothelial cells and subepithelial nerves and the detrusor. Previous attempts to understand the pharmacology of the human bladder have relied heavily on isolated smooth muscle studies. Such results yielded information mainly about the detrusor and were difficult to conduct in biopsy samples. In recent years, several groups have established urothelial cell cultures from animals or human cancer bladders. Detrusor cultures are much less common. The primary aim of this study was to prepare cultures of urothelium and detrusor muscle from human bladder biopsies. The second aim was to characterise urothelial and myofibroblasts populations present in the mucosa. The long term aim is to investigate ATP release in response to a variety of agonists in the different cultures characterised.

Study design, materials and methods

Preparation of cultures: Human bladder biopsies were obtained from control patients undergoing cystoscopy following a previous history of superficial bladder cancer. Tissue was collected into sterile saline, rinsed in growth medium then dissected to remove connective tissue. Bladder smooth muscle and mucosa were trypsin digested (0.25% Trypsin, 1 mM EDTA) for approximately 20 min at 37^0C in 95% air, 5%CO_2. After digestion, dissociated cells were passed through a 100µm nylon cell strainer (to remove undigested tissue and connective tissue) then centrifuged at 1,500 rpm for 5 min. Cells were plated in minimum volume of growth medium (RPMI supplemented with 10% FBS, 2 mM glutamine, 20 ng/mL EGF, 25 mM Hepes buffer, 100 U/mL penicillin, 100 µg/mL streptomycin and 0.25 µg/mL fungazone) to permit cell attachment, then maintained in medium until confluence was reached (approximately 10 to 14 days).

Primary cultures (P0) of bladder mucosa cultures appeared to contain multiple cell types consistent with urothelial cells, umbrella cells, fibroblasts and myofibroblasts. Primary cultures of detrusor appeared to contain smooth muscle cells and fibroblasts. To further purify the individual cell types, cultures were treated with MACS Anti-Fibroblast Microbeads (MACS, Miltenyi Biotec) at the first passage. Fibroblasts and myofibroblasts are bound by the microbeads while the urothelial cells remained unbound. Following MACS bead treatment, two preparations were obtained: (a) a urothelial cell enriched preparation and (b) a fibroblast enriched preparation.

Characterisation of cultured cells: Immunocytochemistry was performed to investigate the characteristics of the individual cell populations using the Dakocytomation envision system, the antibodies as described below (see Table 1) and Fast Red as chromagen. Characterisation of cell culture groups is based on their staining with selected markers. Staining was ranked thus: +++, majority of cells in the culture positive; ++ some cells positive; +, small minority of cells positive; ND, staining not seen.

ATP release studies: Preliminary ATP release studies have been conducted upon cultured bladder mucosa (P2 to P3). Cultured cells were equilibrated by washing with oxygenated Krebs for 15 minutes before ATP release was stimulated with agonists, which included the vanilloid receptor agonist, capsaicin (10 µM) and low pH (5.3). ATP release was quantified using the Sigma Bioluminesence assay kit and a Turner 20/20 luminometer and was normalised to protein content of the wells. Protein content was determined by the Lowry method.

Results

To date biopsies have been collected from 16 patients, with mucosal cell cultures successfully set up from 12 of these patients. Biopsy sizes ranged from 1.5 x 2 mm up to 3.5 x 4 mm. The

biopsies seldom contained more than 50% muscle. Of the biopsies collected, only 8 contained detrusor muscle, and these were successfully cultured.

Mucosal cell cultures: These cells have a generalized "webbed cobblestone" appearance with positive staining for cytokeratins (A0575/ AE1/AE3). At passage 0 some large, smooth contour cells were seen that stained positively for cytokeratin CK20 (a marker for umbrella cells). In subsequent passages (1 to 4), CK20 staining was no longer visible.

Once confluent, the cells were passaged and treated with MAC microbeads. This treatment yielded two separate cell groups. The first group was enriched for urothelial cells (MAC -ve fraction, depleted of fibroblasts) and demonstrated intense staining for cytokeratin markers with some faint staining for 5B5 and α-SMA (Table 1). This staining pattern is indicative of urothelial cell cultures that contain some myofibroblasts/ myoepithelial cells. The second group was the MAC +ve fraction which was enriched for fibroblasts: this demonstrated consistent staining for 5B5 and some staining for α-SMA and cytokeratins (Table 1). These results suggest that the second group contains not only fibroblasts (5B5 +ve, α-SMA -ve) but also myoepithelial/ fibroblast cells (5B5 +ve, α-SMA +ve).

Detrusor muscle cultures: These cells have a tapered stellate appearance with central nuclei. Detrusor cell cultures demonstrated staining consistent with smooth muscle, ie strong reactivity for α-smooth muscle actin (Table 1). Following MAC microbead treatment, detrusor fibroblasts were isolated, which demonstrated strong staining for 5B5 and minimal staining for α-SMA (Table 1).

Table 1. *Summary of immunocytochemical characterisation of cultured human bladder cells*

	AE1/AE3	A0575	α-SMA	5B5
Cells labelled	Urothelial cells	Urothelial cells	Smooth muscle, myofibroblasts/ myoepithelial	Fibroblasts/ myoepithelial
Mucosa: Urothelial enriched	+++	+++	+	+
Mucosa: Fibroblast enriched	+	+	++	+++
Detrusor	ND	ND	+++	+
Detrusor fibroblasts	ND	ND	+	+++

+++ Staining in the majority of cells; ++ staining some cells; + Staining in few cells; ND, no staining detected.

ATP release studies: Preliminary functional studies using bladder mucosal cells at passage 2 to 4 have demonstrated that ATP is released in response to vanilloid receptor agonists capsaicin and acid (pH 5.3).

Interpretation of results

We have established two subtypes of mucosal culture from biopsies of human bladder. The first culture is enriched for urothelial cells and stains positively for cytokeratins. The second group is enriched for myofibroblast type cells and stains positively for α-SMA and 5B5. We have also established detrusor cultures from these biopsies.

Concluding message

To our knowledge this is the first report of the culture and characterisation of both urothelium and detrusor taken from the same cold cup biopsies in the human. This now opens the prospect of cell culture studies in biopsy samples from disease states that are otherwise difficult to study, such as idiopathic detrusor overactivity.