

Characterization of primary cultures of adult human epididymis epithelial cells

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Objective: To establish cultures of epithelial cells from all regions of the human epididymis to provide reagents for molecular approaches to functional studies of this epithelium.

Design: Experimental laboratory study.

Setting: University research institute.

Patient(s): Epididymis from seven patients undergoing orchiectomy for suspected testicular cancer without epididymal involvement.

Intervention(s): Human epididymis epithelial cells harvested from adult epididymis tissue.

Main Outcome Measure(s): Establishment of a robust culture protocol for adult human epididymal epithelial cells.

Result(s): Cultures of caput, corpus, and cauda epithelial cells were established from epididymis tissue of seven donors. Cells were passaged up to eight times and maintained differentiation markers. They were also cryopreserved and recovered successfully. Androgen receptor, clusterin, and cysteine-rich secretory protein 1 were expressed in cultured cells, as shown by means of immunofluorescence, Western blot, and quantitative reverse-transcription polymerase chain reaction (qRT-PCR). The distribution of other epididymis markers was also shown by means of qRT-PCR. Cultures developed transepithelial resistance (TER), which was androgen responsive in the caput but androgen insensitive in the corpus and cauda, where unstimulated TER values were much higher.

Conclusion(s): The results demonstrate a robust in vitro culture system for differentiated epithelial cell types in the caput, corpus, and cauda of the human epididymis. These cells will be a valuable resource for molecular analysis of epididymis epithelial function, which has a pivotal role in male fertility. (Fertil Steril® 2015;103:647–54. ©2015 by American Society for Reproductive Medicine.)

Key Words: Epididymis epithelial cell culture: caput, corpus, cauda

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The biologic functions of the epididymis are critical for normal spermatozoa maturation, because it is during their passage through this organ that spermatozoa acquire full motility and fertility. The three anatomic segments of the epididymis, the caput (head), corpus (body), and cauda (tail), have distinct roles: The caput and corpus are crucial for the early and late processes of spermatozoa maturation, and the cauda serves

as a storage site for functionally mature spermatozoa.

The major cell types within the epididymis epithelium are similar along the length of the organ and include principal cells, clear cells, basal cells, and narrow cells, among others (1). However, cells within each region have distinct functions and a unique proteome. Studies of region-specific epididymal proteins show that certain cell types can express quite different subsets of

genes, which contributes to the different physiologic functions of the segments (2–4). The different protein expression signatures along the epididymis are controlled by particular transcription factor networks that coordinate region-specific functions (5, 6). These regulatory mechanisms within the caput, corpus, and cauda of the epididymis establish critical sequential changes in epididymis luminal environment, which are required for normal sperm maturation and thence male fertility.

Although a few critical proteins are well studied in the human epididymis epithelium (7–9), there are few genome-wide data sets that adequately describe the transcriptional profile of these cells (5, 10, 11). Moreover, most relevant data derive from tissue

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segments, rather than isolated epithelial cells, thus complicating the analysis. The epithelial cell layer responds to hormonal and other biologic signals from underlying tissue, blood supply, and the luminal fluid, so it has been argued that these need to be studied together (12). However, many of these stimuli can be mimicked during culture in vitro, and only the pure epithelial cell populations can generate robust data on their differentiated function. Microarray analyses of epididymis tissues of different animal species revealed segment- or region-specific gene expression patterns (13–17), but the function and relevance to epididymis biology of many of these genes is unknown. Primary cultures of epididymis epithelial cells from mice (18) and rats (19–21) generated valuable information on the expression profiles of these cells. However, many genes show different expression patterns in human and rodent epididymis (22, 23), as do several major epididymal secreted proteins (3). In addition, human epididymal tissue has structural and morphologic characteristics that are distinct from other species (24). This species-specific variation necessitates the use of human primary epididymal epithelial (HEE) cells to understand the molecular basis of human epididymis function in health and disease.

Adult HEE cell cultures were established from epididymal fragments, generating small numbers of primary cells (25, 26). However, in-depth studies of epididymis epithelial function by genome-wide approaches currently require more than one million cells per experiment, and many functional studies necessitate cultured cells with a phenotype that is stable over several generations. We previously used cultures of immature HEE cells to map open chromatin genome wide and identified candidate transcriptional networks coordinating gene expression in the epididymis (5). However, because these HEE cells are immature (27) they are unlikely to exhibit all aspects of the differentiated adult human epididymis. To circumvent this limitation, our goal was to test the hypothesis that establishment of equivalent cultures of adult HEE cells would provide suitable reagents to study differentiated functions of the mature HEE. Here we describe a robust method of culturing adult HEE cells on plastic substrates that enables multiple passages before cells lose morphologic and functional differentiation. Cells were cultured separately from the caput, corpus, and cauda regions. Moreover, sufficient numbers of cells retaining specialized epithelial features were generated to enable detailed molecular analysis. These cultures, which are phenotypically characterized here by means of immunocytochemistry, Western blot, and quantitative reverse-transcription polymerase chain reaction (qRT-PCR), will be valuable for the study of regional special functions of the human epididymis.

MATERIALS AND METHODS

Preparation of Primary Cultures

Human epididymis tissue was obtained with Institutional Review Board approval from seven patients (age range 22–36 years) undergoing inguinal radical orchiectomy for a clinical diagnosis of testicular cancer. None of the epididymides had extension of the testicular cancer, and all were freshly placed into normal saline solution until further processing.

Adult HEE cultures were established with the use of methods described previously for immature HEE cells (27) with modification. Epididymal tissue was placed in Hanks balanced salt solution (HBSS), and fat and connective tissue were removed with the use of a pair of Vannas spring scissors and fine forceps under a dissecting microscope. The three different anatomic regions, caput, corpus, and cauda, were separated. Tissues of each segment were cut into 2–3-mm pieces and placed into a 50-mL centrifuge tube with 10-mL digestion buffer containing 150 U/mL collagenase type I, 15 μ g/mL DNase I (both from Worthington) and 0.5 mmol/L Ca^{2+} in HBSS. Samples were incubated for 2 hours at 37°C in a water bath (shaking every 10–15 min) and with gentle pipetting several times with a 5-mL serologic pipette after 1 hour. The digestion buffer was collected after 1 hour and replaced with fresh buffer for the second 1 hour period. The cell suspensions from the first digestion and the entire second digestion mixture were passed through a 360- μ m stainless mesh and then pelleted by centrifugation at 200g for 5 minutes. Primaria flasks (BD Bioscience) coated with type I collagen (1:75; Purecol 5005-B) were used. The adult HEE cells were grown in CMRL 1066 medium (containing 15% fetal calf serum [FCS], 2 mmol/L L-glutamine, 1 μ g/mL hydrocortisone, 0.2 U/mL insulin, and 10^{-10} mol/L cholera toxin). In the 1st week of culture, 100 U/mL penicillin, 100 μ g/mL streptomycin, and 0.25 μ g/mL amphotericin B were added to the culture medium. Subsequently, cells were cultured in media without these additives and maintained in a humid 5% CO_2 incubator at 33°C.

The cells were examined every other day with the use of an inverted phase-contrast microscope (Leica DMIL), and photographs were taken with the use of a digital camera (Leica FCD280). Culture medium was changed every 3 days. The adult HEEs were passaged with trypsin EDTA (0.25% trypsin, 0.53 mmol/L EDTA) before they reached 80% confluence, and the trypsin activity was inhibited with 0.1% soybean trypsin inhibitor.

For cryopreservation, cells were resuspended in fetal bovine serum (FBS) to a concentration of 6×10^6 cells/mL, and mixed with an equal volume of FBS with 10% dimethylsulfoxide. Cryovials containing 1-mL aliquots of cell suspension were then frozen by means of standard protocols and stored in liquid nitrogen. For recovery, the cells were thawed rapidly with the use of standard methods and placed on collagen I-coated tissue culture flasks. Cell viability was >95%.

For experiments on androgen receptor (AR)-mediated pathways, cultures were grown in phenol red-free CMRL 1066 medium containing 15% charcoal stripped FCS with the other supplements described above for at least 3 days before treatment with 200 nmol/L testosterone (Sigma T1500) or 1 nmol/L methyltrienolone (R1881; Perkin Elmer NLP0050) for 12–16 hours.

Immunocytochemistry

Epididymal and epithelial markers reported previously in the literature, including cysteine-rich secretory protein 1 (CRISP1), clusterin, AR, cystic fibrosis transmembrane

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