GLAND FORMATION FROM HUMAN ENDOMETRIAL EPITHELIAL CELLS IN VITRO

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(Received 7 March 1988; accepted 24 June 1988)

SUMMARY

We have developed methods for the culture of human endometrial glandular epithelia in vitro. The culture medium is serum-free and is used in combination with Matrigel, an extracellular matrix material applied as a coating on cell culture plates. Cell growth begins as a monolayer, but the cells subsequently form glandular or organoid structures. The glands are composed of polar columnar cells facing a central lumen, which is enclosed by the apical surfaces of cells displaying numerous microvilli and sealed by tight junction complexes. The ability to study in vitro the complex process of glandular morphogenesis represents an important new tool in cell biology which may be used to investigate growth regulation, hormone production and dependency, and cellular recognition and interactions. Ultimately, these characteristics may be applied to study the alterations of glandular epithelia associated with neoplasia.

Key words: endometrium; epithelial cells; organogenesis.

INTRODUCTION

The lack of suitable cell culture systems has inhibited research on epithelial cells ranging from the study of differentiation to carcinogenesis. To overcome this problem, we have developed a system for the cultivation in vitro of glandular epithelium from human endometrium.

The human endometrium is comprised of glands lined by columnar epithelia which are surrounded by nonepithelial, hormone-responsive stromal cells. Monolayer cultures of both cell types have been established in tissue culture (1,2,5-7,10,15,18), and the cells have been characterized with respect to histochemistry (16) and structural elements (12). Stromal cells typically propagate well in culture (15-18). However, most previous cultures of glandular epithelial cells have been short-lived, refractory to subculture, and the cells were flat and squamoid rather than columnar as found in vivo (15-18). The endometrial glandular epithelium has also been cultured as free-floating vesicles which reorient to form organoid structures in collagen gels (6). Our method allows long-term culture and passage of the endometrial epithelial cells. Furthermore, the epithelial cells are columnar, and the cellular interactions lead to the formation of glandular structures which closely approximate those formed in vivo.

MATERIALS AND METHODS

RPMI 1640 (#430-1800) L-glutamine (#810-1051), and nonessential amino acids (#320-1140) were from GIBCO Laboratories, Grand Island, NY; 17-beta-estradiol (E-8875), hydrocortisone (H-4001), prostaglandin F2α (P-7652), putrescine (P-7505), glutathione (reduced) (G-4251), and dl-alpha-tocopherol (T-3251) were from Sigma Chemical Co., St. Louis, MO; ITS+ [insulin, transferrin, selenium, bovine serum albumin (BSA), linoleic acid] (#40352), Matrigel (#40234) and dispase (#40235) were from Collaborative Research Inc., Bedford MA; and the 35 × 10-mm tissue culture dishes (#3001) were from Falcon, Oxnard, CA. RL95-2 cells are from the American Type Culture Collection, Rockville, MD.

Growth medium was composed of RPMI 1640 supplemented with 10% conditioned medium from RL95-2 human endometrial carcinoma cells (19), 4 mM glutamine, 100 nM hydrocortisone, 10 nM estrogen, 28 nM prostaglandin F2α, 100 μM putrescine, 40 μM glutathione, 23 nM tocopherol, and ITS+. Incubation was at 37°C in a NAPCO model 3100 three-gas incubator in an atmosphere of 10% CO2:13% O2:77% N2.

Conditioned medium was prepared from RL95-2 cells by passaging a confluent culture at a 1:8 split ratio in RPMI 1640 medium containing 1% fetal bovine serum. After 24 h the medium was changed to RPMI 1640 supplemented with ITS+. This latter serum-free, conditioned medium was collected after 72 h, filter
sterilized with a 0.22-μm filter to exclude microorganisms and RL95-2 cells, and stored at -20°C until use.

Hydrocortisone, prostaglandin E₂, and estrogen were added to the media to create a better approximation of in vivo proliferative conditions. Glutathione and tocopherol were added to lower the oxidative potential of medium. Putrescine, insulin, transferrin, selenium, and BSA were standard additives to serum-free media. Conditioned medium from RL-95 cells was added to enhance growth of the glands by virtue of growth factors produced by transformed cells. The only absolute requirement noted for growth was insulin.

Endometrial tissue was obtained with consent from patients undergoing hysterectomies at North Carolina Memorial Hospital. Specimens obtained in the proliferative phase of the cell cycle from young donors (less than 50 yr old) proliferated best in culture. The data presented have been reproduced with more than 10 specimens. After pathological examination, specimens of normal endometrial tissue were transported to the tissue culture laboratory, where, before culture, the glands were separated from the surrounding stromal cells by collagenase treatment followed by differential centrifugation in a modification of published methods (13,16). The tissue was minced and then treated with 2 ml of a solution of 2% collagenase for 3 to 4 h. The tissue was further dissociated by repetitive pipetting and diluted with 10 ml of growth medium. This mixture was centrifuged at 400 rpm for 2 min. The supernatant contained the stromal cells, and the pellet contained the gland fragments. The