HUMAN SMOOTH MUSCLE CELL CULTURES OF THE STOMACH
MORPHOLOGIC AND BIOCHEMICAL STUDIES

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SUMMARY

Smooth muscle cell cultures were prepared from stomach explants obtained surgically from 10 patients with duodenal ulcer. The cultured cells grew in either overlapping layers in "hills and valleys" or in parallel arrays. The ultrastructure studies showed plasmalemmal vesicles, bundles of myofilaments associated with dense bodies, and gap junctions. The synthesis of contractile proteins illustrated the preponderance of actin on myosin and tropomyosin. The synthesis of contractile proteins in stomach smooth muscle cell cultures is significantly higher than in skin fibroblast cultures, i.e. 20 X higher for myosin, 10 X higher for actin, and 30 X higher for tropomyosin.

Key words: Smooth muscle cell cultures; contractile proteins.

INTRODUCTION

Smooth muscle cell cultures of the stomach have not yet been reported. The tissue culture experience with cultured smooth muscle cells in humans has been limited to uterus (1) and vessels (2). This report describes the establishment and characterization of normal human smooth muscle cell cultures of the stomach based on surgical samples.

MATERIALS AND METHODS

Human stomach samples were obtained from 10 patients who had surgery for duodenal ulcer.

Cell lines and culture conditions. The piece of tissue was immediately placed in a sterile specimen vial containing complete growth Medium 199 (GIBCO, Grand Island, NY) with 20% fetal bovine serum. The specimen was not stored in the refrigerator for longer than 18 to 24 h. The primary culture was set up whenever possible in the same day the surgery was performed. The piece of tissue (stomach) was transferred to a sterile petri dish, washed several times with complete growth medium, after which a careful dissection was done by stripping off the serosa, mucosa, fat, blood vessels, and connective tissue. Before mincing, the muscle sample was checked under the microscope to ensure that it was well cleaned. The muscle was minced with fine scissors into small fragments, each measuring approximately 1 mm² and explanted on noncoated 35-mm diam. dishes. A very small amount of medium was added, just enough to cover the fragments and to prevent them from drying. The culture dishes were left undisturbed for 2 d. After this period, when the fragments were well attached to the substratum, fresh culture medium was added. The culture medium was thereafter changed twice weekly. The cells were cultured at 37°C in a humidified incubator with 95% atmospheric air: 5% CO₂.

In our study the best results were obtained by using the explant procedure, where the outgrowth was noticed after 7 to 10 d. Other procedures to set up the primary culture, such as soaking the tissue samples for 1 h in 0.1% to 0.3% collagenase and then treating the fragments with proteolytic enzymes (trypsin 0.25% or 0.1% collagenase or both) (3-5) were not as successful as the explant procedure. The smooth muscle cells in the primary cultures were reaching confluency by 3 to 4 wk when they were subcultured. The muscle cells were dissociated in a mixture of 0.05% trypsin and 0.02% EDTA. The culture medium was removed, the plates rinsed with phosphate buffered saline (PBS), a small amount of trypsin and EDTA were added. The plates were kept in the incubator for 5 min at 37°C. After this period the trypsin and EDTA were removed and the plates were kept in the incubator for 5 more min. This interval and treatment were enough to get the cells rounded up. At the end of the 10 min, fresh medium was added and the surface of the petri dish flushed gently to detach the cells. The cell suspension was transferred to a centrifuge tube and spun down at 800 rpm for 10 min. The resulting pellet was resuspended in complete culture medium and the cell suspension was counted in a hemocytometer. The plating was done on noncoated 35-mm diam. dishes at a cellular density of 1 X 10⁶ cells/dish with 2 ml of medium/dish. Usually the primary smooth muscle cell cultures contained also some epithelial cells. However, these cells were not more present in the next passages. Most likely the growth
FIG. 1. Phase contrast micrograph of stomach cells which appear ribbon or spindle shaped, with tapered ends with an oval or sausage-shaped nucleus, containing two or more nucleoli. The cytoplasm is phase dense and homogenous and contains few visible inclusions. The cells are oriented along an axis in parallel. First passage. ×714.

FIG. 2. Phase contrast micrograph of stomach cells. After reaching confluency the cells pile up and form "hills and valleys" (arrows). Between the "hills" there are areas containing no cells or 1 to 3 layers. First passage. ×357.