CHARACTERISTICS OF THE SURFACE CELLS OF THE RAT ANTERIOR PITUITARY GLAND IN CULTURE

Takaki Shimada, Fumio Nakamura and Hiroshi Ishikawa
Department of Anatomy, Jikei University School of Medicine, Nishi-shinbashi, Minatoku, Tokyo 105, Japan

ABSTRACT
In the organ culture of rat anterior pituitary fragments, microvillous cells and ciliated cells rapidly covered the surface of the explant. The cilia showed the 9+2 fibril configuration. Both types of surface cells lacked secretory granules. The rough endoplasmic reticulum and the Golgi apparatus were poorly developed in these cells, while bundles of filaments were frequently observed. The two types of cells formed junctional complexes between adjacent cells. Some surface cells of the explant were immunoreactive against anti-S-100 protein serum. These characteristics resemble those of folliculo-stellate and marginal cells found in the pituitary in situ. Microvillous and ciliated cells covered the glandular cells with their thin cytoplasmic projections. The basement membrane was observed on the basal side of the surface cells. It is suggested that the surface cells originate from inside the explant, and support the glandular cells.

A number of investigators have studied the anterior pituitary of the rat and another mammals by means of various culture techniques. In organ culture, it is usual that the surface of the explant is rapidly surrounded by a unicellular ‘membrane’ which is composed of a certain cell type. Some investigators have paid special attention to the histology and ultrastructure of cultured explants of either whole pituitaries or their fragments (7, 9, 10, 11, 14, 20–26, 29). The origin and exact nature of the cells which form the unicellular membrane is not well clarified; some authors describe them as glandular cells (25), ‘fibroblastic’ cells (6), or non-secreting pituitary elements such as follicular cells or stellate cells (28). The disagreement seems to be caused by the poorly understood behavior of pituitary cells during organ culture. Perhaps, some cells of the anterior pituitary are capable of changing their morphology under culture conditions.

In this study, we attempted to clarify the origin and morphological characteristics of the cells surrounding the surface of the culture explant from the adult male rat anterior pituitary using ultrastructural and immunohistochemical techniques.

MATERIALS AND METHODS
Pituitaries were removed from 50 day-old male Wistar-Imamichi rats. They were placed in a growth medium, and the posterior and intermediate lobes were removed. The anterior pituitary gland was separated into two parts under a dissecting microscope. One part contained the marginal layer, and the another part did not. Each part was then cut into small pieces, placed into 12-well culture plates (Costar) with 1.5 ml of the growth medium per well, and cultured for 1 to 5 weeks. They and whole anterior pituitary glands were cultured in a CO2-incubator (Model WJ-12E, Hirasawa) in a humidified atmosphere of 5% CO2 in air at 37°C.

The growth medium was composed of Ham’s F 10 medium (Flow Laboratories) supplemented with 15% mixed sera (newborn calf serum (Japan Biotest): fetal calf serum (KC Biological): horse serum (Hyclone)=4:2:1),
and 50 U/ml penicillin G and 50 μg/ml streptomycin (Gibco). The medium was changed every 3 or 4 days.

Pituitary explants, whole organs, and explants with or without the marginal layer were fixed after 1, 2, 3, 4 and 5 weeks of culture for microscopic studies.

**Light Microscopy**

Cultured explants were fixed in formol-sublimate (3:7) solution for 6 h at 4°C. After dehydrration in the ethanol gradient, specimens were embedded in Paraplast, cut at 3 μm, and stained with hematoxylin and eosin. Immunostaining was performed according to the avidin-biotin complex (ABC) method of Hsu et al. (12), using an ABC kit from Vector (CA, U.S.A.); the first antiserum against bovine S-100 protein was prepared in our laboratory (27).

**Electron Microscopy**

Cultured explants were fixed in 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 at 4°C...
Fig. 3 Scanning electron microscopic observation of the surface explants with the marginal layer (3a) and of those without the layer (3b) after 5 weeks of culture. Many microvilli and cilia are seen on the surface. More ciliated cells are found in (a) than in (b). $\times 2,000$
Fig. 4 The explant without the marginal layer after 3 weeks of culture. Ciliated cell (CC) and microvillous cells (MC) are seen on the surface of the explant. Junctional complexes (JC) are observed between the neighboring cells. Glandular cells (G) are also seen under the CC and MC. $\times 7,400$. Inset: Cilia with the 9+2 fibril configuration. $\times 74,000$

for 1 h, washed several times with 0.1 M phosphate buffer containing 2% sucrose at 4°C for 1 h, and post-fixed in 1% osmic acid in Millonig's buffer (15).

Scanning electron microscopy After dehydration in the ethanol gradient, specimens were critical-point dried in liquid CO$_2$, mounted on metal stubs and coated with carbon using evaporative coating. They were then coated with a gold-palladium alloy in a sputter coater, and examined with a Hitachi S-700 field emission electron microscope.

Transmission electron microscopy After dehydration in the ethanol gradient, specimens were embedded in Epon-Araldite resin. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined with a JEOL 100B electron microscope.

RESULTS

After 1 week of culture, some movements of cilia were observed in explants without the marginal layer by phase contrast microscopy. In whole anterior pituitaries in culture, some cilia were also seen on the lateral surface of the explants where the marginal layer was not found (Fig. 1, a and b). Necrosis was usually seen in the center of the explant, but the peripheral part had a healthy appearance. As the time of culture was increased, cillum movements were more frequently observed. However central necrosis spread widely. Some surface cells, ciliated and microvillous cells, in explants with or without the marginal layer were immunostained with anti-S-100 protein serum. Immunoreactive cells did not show the same staining intensity in both explants (Fig. 2, a and b).

The scanning electron micrographs of explants with or without the marginal layer revealed that the surface cells had many microvilli and/or cilia. Ciliated cells were more frequently seen in explants with the marginal layer than those without the marginal layer (Fig. 3, a and b).

In the transmission electron microscopic studies, the surface of the explants was divid-
ed into two categories. One was composed of ciliated cells which also had microvilli, and the other was contained microvillous cells that were not ciliated.

Ciliated cells were observed in explants with or without the marginal layer. All of the cells had microvilli, and some had cilia. In these cilia, the "9+2" fibril configuration was seen. Glandular cells were located below the surface layer of cells, and never composed of the surface cell layer in the explant (Fig. 4). The ciliated cells contained numerous mitochondria, free ribosomes, and polysomes. The rough endoplasmic reticulum was poorly developed and some images of the Golgi complex were observed. Junctional complexes were usually seen between adjacent cells at the apical portions of the cells (Fig. 4).

Most of the microvillous cells possessed oval nuclei, but some had nuclei of irregular shape. The cytoplasm contained abundant free ribosomes. The rough endoplasmic reticulum and the Golgi apparatus were poorly developed. Junctional complexes were also seen between neighboring cells. Both microvillous and ciliated cells lacked secretory granules. Lipid droplets were frequently observed in the granular and agranular cells in the explant (Figs. 4 and 5). After 1 or 2 weeks of culture, bundles of filaments were seen in the microvillous cells (Fig. 5).

Basement membrane was observed on the basal side of the microvillous and ciliated cells, which coated the surface of the explant (Figs. 5 and 6). However, the basement membrane was not always seen under the cells. When the microvillous cells extended cytoplasmic projections and surrounded glandular cells, the basement membrane was found under cytoplasmic projections and glandular cells, but not between projections and glandular cells (Fig. 6). After at least 2 weeks of culture, glandular cells were also seen near the surface of the explant, but they never faced the growth medium. Thin cytoplasmic projections of microvillous cells always covered glandular cells.

Connective tissue was conspicuous inside
Fig. 6. Microvillous cells on the surface of an explant without the marginal layer after 3 weeks of culture. The cells extend cytoplasmic projections (CP), and surround glandular cells (G). The basement membrane (BM) exists under the cells and connective tissue (CT) is located in the intercellular space. ×17,000.

the explants, *i.e.* in the necrotic region and in the intercellular spaces (Figs. 5, 6 and 7). Inside the explant, agranular cells formed follicles and their morphological characteristics were similar to those in follicular cells. They extended cytoplasmic projections which surrounded adjacent glandular cells. The follicular cells and glandular cells constituted follicular structures which were surrounded by the basement membrane.

After 1 week of culture, fibroblasts were conspicuous under the microvillous cells. They were more conspicuous in the explants without the marginal layer than in those with
the marginal layer. Further inside, glandular cells still survived (Fig. 7).

DISCUSSION
Generally, in organ culture of the pituitary, the surface of the explant is rapidly surrounded by a certain type of cell. In our ultrastructural observations, microvillous and ciliated cells also appeared on the surface of the explants even when these did not contain the marginal layer of the anterior lobe.

Ciliated cells exist in various regions of the pituitary gland (3, 13, 19, 31). In the anterior
lobe of rats, the follicular and marginal cells possess microvilli and cilia with the 9+2 fibril configuration. The ultrastructural similarities are recognized between follicular cells and marginal cells of the pars distalis (31). In organ culture, microvillous cells and ciliated cells were agranular cells, and contained numerous free ribosomes, but the rough endoplasmic reticulum and the Golgi apparatus were poorly developed. These two cell types formed junctional complexes between them. The cilia showed the 9+2 fibril configuration. These ultrastructural characteristics are thought to be similar between the microvillus and ciliated cells on the surface of the explant, and follicular and marginal cells in the anterior pituitary.

Similarities were also shown in the immunohistochemical studies. The brain specific S-100 protein, isolated from the bovine cerebral cortex (16, 17) was identified immunohistochemically in the folliculo-stellate cells and marginal cells of the rat anterior pituitary (4, 18, 27). Thus, S-100 protein is considered to be a marker for folliculo-stellate cells and marginal cells of adult rat pituitaries.

Some of the surface microvillous cells and ciliated cells of the cultured explants showed positive immunoreactivity for anti-S-100 protein serum. Thus the possibility is raised that these cells are originated from folliculo-stellate and marginal cells.

Tixier-Vidal suggested that microvillous and ciliated cells were nonsecretory elements corresponding to follicular cells or stellate cells that had migrated to the explant surface (28). The appearance of microfilaments in the microvillous cells during the early period supports the suggestion of Tixier-Vidal, because microfilaments are considered to be associated with cell movement (1, 8).

Microvillous and ciliated cells can migrate, come in contact with the growth medium, and surround the surface of the explant rapidly. Furthermore, these cells often extend cytoplasmic projections, surround glandular cells, and can synthesize the basement membrane along their basal surface. The basement membrane is located in various parts of the pituitary gland. A thin basement membrane covers the basal surface of the anterior marginal cells (5). A thick basement membrane separates the posterior marginal cells from glandular parenchyma of the pars intermedia (2). Furthermore, the basement membrane separates cytoplasmic processes or sheet of follicular and glandular cells from the perivascular spaces or from the connective tissue septa (30).

Connective tissue is scarce in the intact anterior pituitary of the rat. Although necrosis occurred inside the cultured explants, the amount of connective tissue increased, and follicular structures survived in the necrotic region. These follicular structures were composed of follicular cells and glandular cells which were enveloped by the basement membrane.

It is probable that there is a survival unit in the pituitary gland. On the surface of the explant, the unit is composed of surface cells, glandular cells and basement membrane, and inside the explant it is represented by the follicular structure. Although the exact function of microvillus and ciliated cells is still unknown, the present findings seem to suggest that they are involved in the survival of the glandular cells.

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