PREPARATION AND MORPHOLOGICAL CHARACTERIZATION OF PRIMARY MONOLAYER CULTURES OF HUMAN SOMATOTROPHINOMA CELLS

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An urgent problem at the present time in neuroendocrinology is that of pituitary adenomas. According to some data [3] subclinical pituitary adenomas are found in 27-30% of all autopsies. In recent years much progress has been made in the study of morphological and functional properties of human pituitary adenomas following investigations in vitro on cell cultures, derived from the same population of isolated tumor cells capable of functioning in a stable manner in vitro [2, 4, 7]. It must be pointed out, however, that many problems of the methodology of obtaining and culturing cells from brain tumors in patients undergoing operative treatment still remain unsolved.

This paper gives the results of isolation and maintenance in primary monolayer cultures of pituitary adenoma cells obtained from patients with active acromegaly during operative treatment for this disease; morphological, including immunocytochemical, characteristics of the adenoma cells are described.

METHODS

Pieces of tumor tissue were obtained from material removed at operations on three patients (A, D, and N) aged 24-31 years, with pituitary adenomas accompanied by a marked clinical picture of acromegaly with a high serum somatotrophic (STH) level. The pituitary adenoma tissue was cut into small fragments about 1 mm in diameter. The tissue was dissociated by treatment with 0.25% trypsin solution (Sigma, USA), in medium PM-16, not containing Ca^{2+} and Mg^{2+} (Serva, Germany), for 30-40 min at 37°C, using a magnetic mixer followed by mechanical dissociation into single cells. After centrifugation at 800 g the cells were resuspended in incubation medium consisting of medium 199 (Research Institute of Poliomyelitis and Virus Encephalitis, Russian Academy of Medical Sciences) with the addition of 10% fetal calf serum (Calbiochem, USA) and penicillin (50 U/ml). The cell suspension was seeded into 24-well plastic macropanels (Flow Laboratories, England), containing coverslips, at the rate of 600,000 cells per well, and grown as monolayer cultures in incubation medium in an incubator at 37°C in an atmosphere of air with 5% CO_2. The medium was changed every 48 h during culture. For morphological investigation, including immunocytochemical detection of STH and prolactin (PRL), cells of 6-day cultures growing on coverslips were fixed for 2 h with a mixture of picric acid and paraformaldehyde, by the method in [8]. After repeated rinsing with phosphate buffer (0.01 M, pH 7.4) and treatment for 10 min with glycine (50 mM) to remove the fixative, some of the coverslips were stained with hematoxylin and eosin. Two consecutive immunocytochemical tests were carried out under remaining coverslips to detect somatotrophs and lactotrophs in the same preparation, by the use of monoclonal (mouse) and polyclonal

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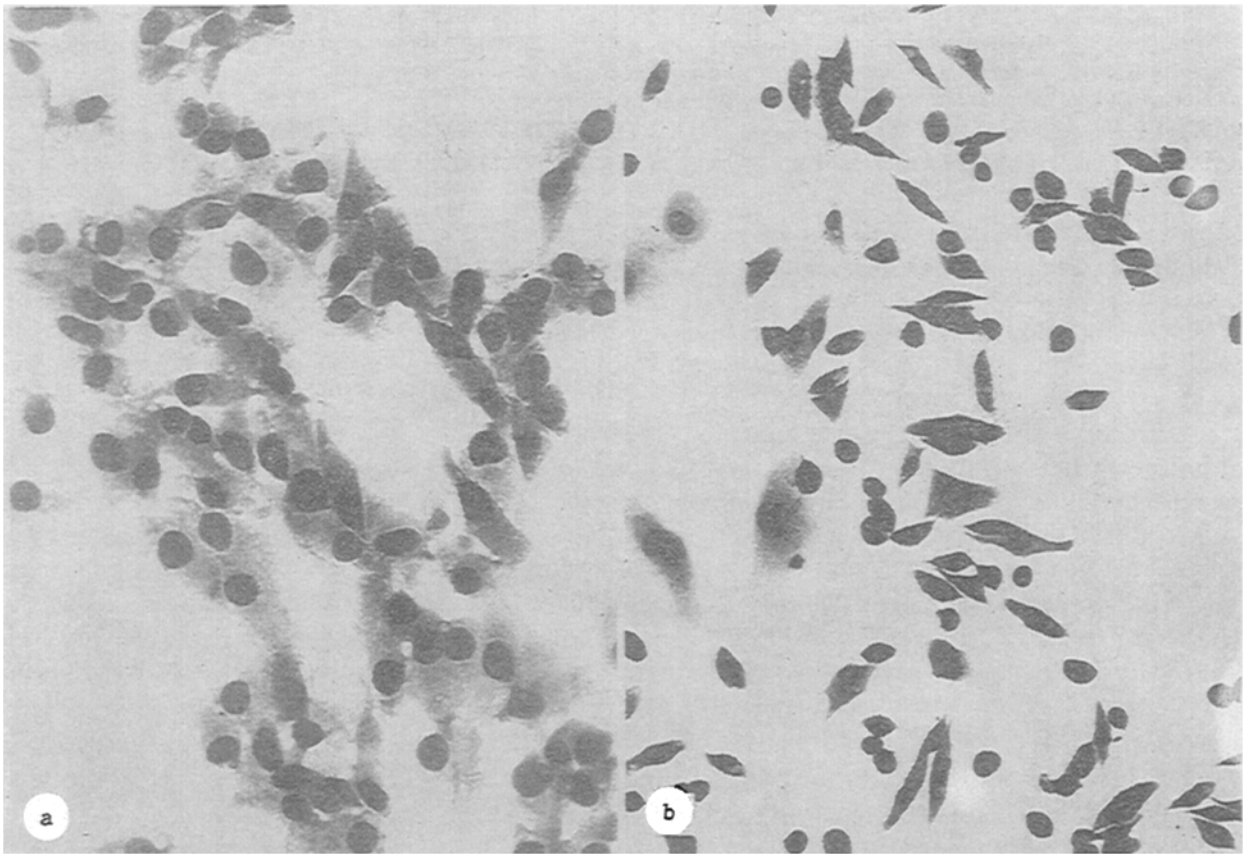

Fig. 1. Primary 6-day monolayer cultures of somatotrophinoma cells from patients N, A (a), and D (b). Stained by Giemsa's method. 100×.

TABLE 1. Basal Secretion of STH and PRL by Monolayer Cultures of Pituitary Adenoma Cells at Different Times after Seeding (n = 4)

Days of culture	STH in medium (μg/ml/24 h), M ± m	PRL in medium (ng/ml/24 h), M ± m
1-	6,02±0,24	116,8±7,0
3-	2,85±0,07	71,5±3,8
5-	2,48±0,14	59,3±1,5
6-	2,39±0,23	68,4±2,9

(rabbit) antibodies to human STH or PRL (Biogenesis, England). Biotinylated antibodies against mouse or rabbit immunglobulins, conjugated with peroxidase (ABC kits from Vector, USA) were used as second antibodies. The preparations were examined in a light microscope with magnification of 100 and 900×. Aliquots of medium were collected periodically during six days of culture, frozen, and kept at —40°C until required for analysis. Concentrations of STH and PRL in the incubation medium were determined by the use of specific radioimmunologic systems developed in the Protein Hormone Standards Laboratory, All-Union Endocrinologic Scientific Center, Russian Academy of Medical Sciences [1].

RESULTS

The study of the basal secretion of immunoreactive STH and PRL by pituitary adenoma cell cultures obtained from the patients demonstrated their viability and preservation of their functional activity, at least during culture in vitro for 6 days. Table 1 gives the results of hormonal secretion by adenoma cells from patient A. Clearly the character of functioning of the somatotrophs and lactotrophs in vitro was quite similar, despite the fact that the quantity of PRL released into the medium was many times less than that of STH. The concentrations of both hormones in the medium, which were depressed in the initial period of culture, remained sufficiently stable throughout the subsequent period of observation. The character of basal secretion of STH and PRL by pituitary adenoma cell cultures from the other two patients was similar. Thus cell cultures of human pituitary adenomas can be used at least for 6 days during culture in vitro in order to investigate the regulation of STH and PRL secretion, and also for various types of morphological analysis.

The study of histological preparations by light microscopy revealed two types of cell cultures (Fig. 1). The first type (patients N and A) was characterized by the presence of connected cell aggregates with free spaces between them (Fig. 1). Most cells were epithelioid in type and were oval, rhomboid, or triangular in shape. The second type (patient D), on the other hand, was characterized by accumulation of isolated cells, not aggregated into colonies (Fig. 1b). Most of the cells were rhomboid in shape with pointed ends, although this cannot be said of the fibroblast-like type of structure, which is typical of several cells grown in vitro.

A characteristic feature of both types of pituitary adenoma cell cultures was the presence of binuclear and multinuclear cells, and the absence of figures of mitotic division and of fibroblast-like cells.

Comparison of the morphological properties of the primary cell cultures of the pituitary adenomas investigated and of other assemblies of hormone-secreting cells, such as primary cell cultures of rat adenohypopheses [6], revealed the following characteristic features of human pituitary adenoma cell cultures: 1) they contained virtually no fibroblast-like cells, typical of cells of the rat adenohypophysis growing in vitro. 2) Despite the tumor nature of the human cells in culture there were virtually no mitotic figures or signs of proliferative activity. On the contrary, the primary cultures of rat pituitary cells were characterized by growth of the cells with the formation of a confluent monolayer although, however, mainly on account of multiplication of undifferentiated cells.

According to the results of immunocytochemical investigation, the predominant cells in the cultures of all three pituitary adenomas studied were somatotrophs, in agreement with the clinical picture of the disease (acromegaly), and also with the results of investigation of the functional activity of the cell cultures. Accordingly, the tumors whose investigation is described above could be identified as somatotrophinomas.

Immunocytochemical detection of STH and PRL in the same preparations showed that a small proportion of the somatotrophinoma cells may consist of lactotrophs, and also of lactosomatotrophs, exhibiting double strains of the cytoplasm brown and blue, and synthesizing the two hormones simultaneously. These results are in agreement with those obtained by other workers [5] by means of immunoelectron microscopy.

It must be pointed out, however, that if light microscopy is used for quantitative analysis of bifunctional cells, certain doubts may arise when some of them are categorized, especially if the same enzyme is used for subsequent detection of STH and PRL by the immunocytochemical method, for this may be accompanied by an increase in the intensity of background staining. Nevertheless, performing several immunocytochemical tests on the same preparation, preferably with the use of conjugates with different enzymes (for example, with peroxidase and alkaline phosphatase), is highly promising for the quantitative analysis of secreting cells of pituitary adenomas under basal conditions and under the influence of various exogenous factors, including inhibitors of hormonal secretion and of tumor growth.

Thus the method of isolating human pituitary adenoma cells and culturing them in vitro makes it possible to obtain many different parallel cell cultures, functioning under standard conditions, and derived from the same population of isolated tumor cells, and to study in detail their cell composition, cytoarchitectonics, and function in culture.

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