Mammomatotroph Adenoma Cells Secrete Both Growth Hormone and Prolactin

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Abstract

Pituitary adenoma cells from a mammomatotroph adenoma obtained from a 21-year-old female presenting with acromegaly and amenorrhea were investigated by sandwich cell immunoblot assay, immunohistochemistry, and electron microscopy. The new, simple technique of sandwich cell immunoblot assay could detect two hormones secreted in the same one cell, and found that 89% of mammomatotrophs secreted both growth hormone (GH) and prolactin (PRL). Immunohistochemistry showed that the tumor cells were positive for both GH and PRL. Electron microscopy showed cells contained granules ranging in size from 150 to 500 nm. This is the first demonstration of both GH and PRL in the same mammomatotroph cell. Sandwich cell immunoblot assay can measure the amount of secreted hormone, allowing a new approach to the investigation of mammomatotroph adenomas.

Key words: growth hormone-secreting adenoma, mammomatotroph adenoma, cell immunoblot assay, prolactin

Introduction

The pathophysiology of acromegaly can be divided into three types: mixed growth hormone (GH) cell-prolactin (PRL) cell adenoma, acidophil stem cell adenoma which expresses both GH and PRL, and mammomatotroph adenoma which is thought to be a mature type of acidophil stem cell adenoma.²⁶⁾ The two hormones may be produced by the same^{5,9,11,15-17,29,30)} or different cells^{8,10,24,25,31)} in human pituitary adenoma.

Mammomatotroph cells are said to contain secretory granules of both GH and PRL in the same cell. Several methods have been used to investigate colocalization of GH and PRL, such as the double staining method and gold particle labeling technique.^{5,7,12,21,23,27,29,32)} Morphological studies have demonstrated cells that colocalize both GH and PRL in normal bovine¹⁹⁾ and rat pituitary gland,^{14,28)} and human pituitary adenoma,^{5,12,32)} but cosecretion of GH and PRL has not been shown. These methods are not simple to perform and take many hours.

Recently, the reverse hemolytic plaque assay (RHPA)^{7,23)} was developed to show the presence of hormone secretion rather than the secretory granules. RHPA has showed that one cell can secrete two hormones at a single cell level, but this method is again not simple or suitable for quantification of hormone secretion.

We have developed a sandwich technique cell immunoblot assay which permits simultaneous identification of two antigens on two transfer membranes⁴⁾ and is simple and suitable for the simultaneous quantification of hormone secretion on the single cell level. We then measured the secretion of GH and PRL in mammomatotroph adenoma cells from a patient with acromegaly.

Subject and Methods

I. Patient and endocrinological examination

A 21-year-old female noticed progressive enlargement of her chin, hands, and feet from age 16 years. On admission, her height and weight were 178 cm

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and 72 kg. She had been in good health except for a 1-year history of amenorrhea. She did not have galactorrhea. Computed tomography and magnetic resonance imaging showed a macroadenoma with slight suprasellar extension. She was not receiving any medication known to affect GH and PRL secretion. Her basal GH and PRL levels were 70 ng/ml (normal <1 ng/ml) and 18 ng/ml (normal <20 ng/ml). Thyrotropin-releasing hormone (TRH) increased the plasma GH level to 500 ng/ml, and the plasma PRL level to 187 ng/ml. Both GH and PRL levels showed a dramatic and prompt decrease after the oral administration of bromocriptine. Her serum somatomedin-C level was 9.17 U/ml (normal 0.64–1.68 U/ml). Acromegaly was diagnosed on the basis of clinical features and elevated plasma GH levels.

The adenoma was removed subcapsularly *via* the transsphenoidal route. One month after surgery, her serum GH and PRL levels were 1 and 1.9 ng/ml. Her menstruation recovered 2 months after surgery.

II. Immunohistochemical examination

Surgical specimens were fixed for 12 hours at room temperature in 10% buffered formalin, and embedded in paraffin. Sections (4 μ m thick) were prepared for the indirect immunoperoxidase staining technique using antisera to GH, PRL, luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and adrenocorticotrophic hormone (ACTH): L1814, L1837, L1827, L1810, L1847, and L1801, respectively (DAKO Co., Carpinteria, Cal., U.S.A.). Visualization of reaction used 3,3'-diaminobenzidine.

III. Electron microscopy examination

Surgically removed specimens were fixed at 4°C in 2% glutaraldehyde, postfixed in 1% OsO₄, dehydrated, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under an electron microscope.

IV. Sandwich cell immunoblot assay

Pituitary cells were dispersed as described previously²⁻⁴⁾ with some modifications. Briefly, the fragments were incubated with shaking at 37°C for 60 minutes in Hanks' balanced solution containing 0.4% collagenase (Sigma, St. Louis, Mo., U.S.A.) and 0.005% deoxyribonuclease (Sigma). During incubation, trituration was performed every 15 minutes using a flame-polished siliconized Pasteur pipette. The fragments were then mechanically dispersed in Ca²⁺- and Mg²⁺-free Hanks' solution containing 0.02% ethylenediaminetetra-acetic acid until monodis-

persed cells were obtained. The monodispersed cells were resuspended at 2×10^5 cells/ml in Dulbecco's modified Eagle's medium containing 0.1% bovine serum albumin.

The sandwich cell immunoblot assay was performed as described previously.¹⁾ Polyvinylidene difluoride transfer membrane (Immobilon; Millipore, Bedford, Mass., U.S.A.) was prewetted in methanol for 20 seconds, rinsed in distilled water for 5 minutes, and equilibrated with Earle's balanced salt solution supplemented with 20 mM HEPES for 60 minutes. A small volume (30 μ l) of cell suspension was placed on a transfer membrane then covered with a second, overlapping transfer membrane. The cells were incubated in the membrane sandwich for 1 hour. The two transfer membranes were then separately stained with different primary antisera (anti-GH: A570 [DAKO] 1:15,000, anti-PRL: A569 [DAKO] 1:15,000). A cell secreting both hormones can be identified by cell blots stained with the different antisera at the mirror-image positions on the different transfer membranes. The transfer membranes were incubated with standard blots of GH and PRL at six known concentrations.

Cell blots immunopositive for GH or PRL were quantified with a computerized microscopic image analysis system (SPICCA; Nippon Avionics, Tokyo) by reference to the GH and PRL standard blots. The amounts of GH and PRL secreted from single mammosomatotroph cells were calculated by multiplying the area of cell blots by the difference between the mean gray value of cell blots and the background value. We examined 887 blots for identification of immunopositivity for both GH and PRL or GH only or PRL only. Quantification of amounts of GH and PRL were obtained from 40 mammosomatotroph cells. Mammosomatotroph cells were classified into three types (GH blot parallel to PRL blot, larger GH blot than PRL blot, larger PRL blot than GH blot) according to the size and gray level of the blot.

Results

I. Immunohistochemical examination

The specimen showed positive staining for both GH and PRL (Fig. 1). Almost all cells of the specimen were stained. However, there was no staining for the other hormones (ACTH, TSH, LH, FSH).

II. Electron microscopy

The specimen showed densely granulated adenoma. Many granules had diameters ranging from 150 to 500 nm (Fig. 2). The small granules may be PRL granules and the large granules may be GH

Fig. 1 Photomicrographs showing immunohistochemical staining for GH (*upper*) and PRL (*lower*) in the same area. $\times 200$.

Fig. 2 Electron micrograph of mammosomatotroph cells showing numerous small and large granules. Bar = 500 nm.

granules. The tumor could not be diagnosed as an acidophil stem cell adenoma because there were no giant mitochondria in the cytoplasm.

III. Sandwich cell immunoblot assay

The immunoblot assay showed that 88.8% of cells (788 of 887) showed immunostaining for both GH and PRL (mammosomatotroph cells), 9.9% (88)

Fig. 3 Sandwich cell immunoblotting assay transfer membranes showing cosecretion of GH and PRL in mammosomatotroph cells. Most of the GH-immunoreactive cell blots (*upper*) and PRL-immunoreactive cell blots (*lower*) are located at the same positions with reference to the two pin hole marks. Some blots showed immunopositivity for only GH or PRL.

showed staining for only GH (somatotroph cells), and 1.2% (11) showed staining for only PRL (lactotroph cells) (Fig. 3). Quantification of hormone secretion showed that about one third of mammosomatotroph cells demonstrated parallel secretion of GH and PRL. The other mammosomatotroph cells showed dominant GH or PRL secretion (Fig. 4).

Discussion

The original cell immunoblot assay developed by Kendall and Hymer¹⁸⁾ could not show two hormones secreted from the same cell. Our sandwich cell immunoblot assay can show the secretion of two hormones from the same cell sandwiched between two transfer membranes. We previously showed using this technique that the percentage of mammosomatotroph cells in adenomas causing acromegaly (11 cases) ranged from 0.9% to 39.8% (mean $\pm$ SE $17.9 \pm 3.8\%$) (unpublished data). The percentage of mammosomatotroph cells in human

Fig. 4 GH and PRL secretion from the same cells. 35% of mammosomatotroph cells show a good correlation between size and gray level of GH blots and PRL blots (parallel secretion). 40% of mammosomatotroph cells have larger GH blots in size and gray level than PRL blots (dominant GH secretion). 25% of mammosomatotroph cells have larger PRL blots in size and gray level than GH blots (dominant PRL secretion).

acromegalic patients has been reported as 0–53%,³²⁾ 4–20%,²³⁾ and 0–80%.⁵⁾ The percentage of mammosomatotroph cells in the present patient was 89%, rather higher than in our other acromegalic patients. Immunohistochemistry and electron microscopy confirmed that this adenoma colocalized both GH and PRL. We therefore agree with Bassetti *et al.*⁵⁾ that the frequency of mammosomatotrophs in adenomas from acromegalic patients is higher than that previously estimated using various immunohistochemical methods.

In this patient, almost all cells showed immunostaining for PRL even if the serum PRL levels were not substantially elevated. Bassetti *et al.*⁵⁾ reported that five of 13 acromegalic patients with normoprolactinemia had adenomas which showed positive immunostaining for PRL, and suggested that the parallel responses of serum GH and PRL levels to TRH and bromocriptine are characteristic of acromegalic patients with mixed GH- and PRL-secreting adenomas. The presence of many mammosomatotroph cells in such adenomas would explain these parallel responses if the cells have receptors for both GH and PRL secretion.

The high proportion of mammosomatotroph cells found within the tumor in contrast to the normal

PRL values in our patient shows that it is impossible to detect the presence of mammosomatotrophs using only the basal plasma hormone levels. However, the presence of mammosomatotrophs might be demonstrated by a parallel increase in both GH and PRL concentrations in response to TRH infusion. But the GH and PRL responses to TRH and bromocriptine are very similar in patients with tumors containing only somatotrophs and those with tumors containing a high proportion of mammosomatotrophs.⁶⁾

Quantification of PRL and GH secretion from the same mammosomatotroph cells showed that about one third of mammosomatotroph cells secreted both GH and PRL, one third secreted mainly GH, and one third mainly PRL. Clearly, heterogeneity is present in human mammosomatotroph adenoma as well as in the rat.²²⁾ This heterogeneity implies that most mammosomatotrophs will secrete predominantly GH or PRL.

Numerous studies^{13,26)} have shown that most somatotrophs arise from mammosomatotroph cells during the development of the pituitary gland. Therefore, the mammosomatotroph cell can be considered the precursor cell in the differentiation of mammotrophs and somatotrophs. However, the general incidence and secretory characteristics of mammosomatotroph cells in human pituitary adenomas is not known. Pituitary adenomas showing high serum GH concentrations are classified according to the differentiation of the precursor cell into acidophil stem cell adenoma, mammosomatotroph adenoma, and somatotroph adenoma. This classification can be performed using immunohistochemical examinations,^{5,12,20,27,29)} electron microscopy examinations,^{9,10,13,16,30)} reverse hemolytic plaque assays,^{7,23)} and cell immunoblot assays,^{1–4,18)} but the sandwich cell immunoblot assay will be especially helpful in the diagnosis of mammosomatotroph adenoma.

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