IN VITRO CELL CULTURE AND HORMONE RADIOIMMUNOASSAY OF HUMAN PITUITARY ADENOMAS

Lu Hankui (陆汉魁), Lin Xiangtong* (林祥通), Yang Detai (杨德泰), Zhang Kaili (张凯莉), Zhang Fulin (张福林) and Bao Weimin (鲍伟民)

(*Huashan Hospital, Shanghai Medical University, Shanghai 200040, China*)

ABSTRACT

Tissues from 30 human pituitary adenomas are monolayer-cell-cultured in vitro. Hormone secretion of GH, PRL, TSH, LH and FSH by cells into medium is detected by radioimmunoassay. The pattern and amount of hormone(s) in the medium are used to determine the nature of the cells and thus to establish functional classification of pituitary adenomas. The results show that cell culture technique provides an easy and suitable mode for investigating the nature of pituitary adenomas. Hormone radioimmunoassay of culture medium is precise and reliable and represents the whole adenoma tissue. Further studies can lead to clearer understandings of the pathology of pituitary adenomas.

Keywords Pituitary adenomas, In vitro, Radioimmunoassay, Classification

1 INTRODUCTION

Human pituitary adenomas have long been classified according to their tinctorial quality as acidophil, basophil, chromophobe. In recent years this classification has been developed and gradually replaced by functional classifications which are primarily based on the techniques of immunocytochemistry and electron microscopy[1]. However, new studies have shown that in vitro cell techniques provide an ideal mode for studying human pituitary adenomas. The pattern of hormone secretion by adenoma cells in culture has shown to be of value in establishing the nature of the adenoma tissue. There is a complete agreement between the diagnoses reached by immunocytochemical techniques and by examining hormone secretions in culture media and, as Adams and Mashiter suggested[3], the latter techniques have some advantages over the former ones.

To test these hypotheses, we performed in vitro monolayer cell culture from 30 pituitary adenoma tissues obtained from pituitary adenomectomy. Hormone secretions in the culture medium were detected by radioimmunoassay. The pattern and amount of hormone(s) secreted by cultured cells were used to determine the nature of each adenoma tissue and the results were compared with the clinical data and histological findings.

* Lu Hankui's mentor

Manuscript received date: 1994-06-02

2 MATERIALS AND METHODS

Pituitary adenoma tissues from 30 patients (12 men and 18 women, age range of 22–56 a) are obtained by transphenoidal operation. The clinical diagnosis of the patients

Table 1

Results of serum pituitary hormones from 30 patients, grades of hormone secretion in media 5th day in culture, and histological examinations

Patient No.	Serum hormones					Hormones in medium*					Histological examinations**
	GH ng/l	PRL ng/l	TSH Iu/l	LH Iu/l	FSH Iu/l	GH	PRL	TSH	LH	FSH	
1	2.1	396	0.8	nd	nd	++	+++	+++	nd	nd	PRL adenoma
2	5.5	285	1.2	nd	nd	++	+++	++	+++	+	PRL adenoma
3	<1.0	408	0.7	nd	nd	++	+++	+++	++	+	PRL adenoma
4	5.9	89	1.1	4.2	5.8	–	+++	–	–	–	PRL adenoma
5	1.9	354	1.0	4.2	5.8	–	+++	–	–	–	PRL adenoma
6	1.1	203	1.1	<2.0	3.5	–	+++	–	–	–	Basophil
7	<1.0	109	0.6	nd	nd	–	+++	–	–	–	Basophil
8	74	89	0.9	10.1	3.0	+++	–	–	–	–	GH adenoma
9	100	36	0.5	8.9	<3.0	+++	–	–	–	–	GH adenoma
10	29	82	0.7	11.5	4.3	+++	–	–	–	–	GH adenoma
11	205	500	1.4	<2.0	<3.0	+++	–	–	–	–	GH adenoma
12	166	284	1.2	2.5	<3.0	+++	+++	–	–	–	GH adenoma
13	208	39	0.8	<2.0	4.6	+++	+++	–	–	–	Acidophil
14	368	164	0.6	nd	nd	+++	–	–	–	–	Acidophil
15	267	432	0.8	15.4	11.4	+++	–	–	–	–	Acidophil
16	64	49	1.1	nd	nd	+++	–	–	–	–	Acidophil
17	1.5	24	0.9	114	32	–	–	–	–	–	Nonfunction⁻
18	2.5	1.2	0.5	54	408	–	+	–	++	+++	Nonfunction
19	<1.0	243	0.4	242	463	–	–	–	+++	+++	Chromophobe
20	4.4	33	1.2	132	49	–	+	–	+++	+++	Chromophobe
21	<1.0	21	1.1	19.0	32	–	+	–	+	++	Nonfunction
22	5.9	43	1.4	18.0	11.3	–	–	–	+	++	Nonfunction
23	11.5	104	0.5	15.0	14.2	–	–	–	–	–	Chromophobe
24	<1.0	25	0.7	8.5	19.4	–	–	–	++	–	Chromophobe
25	2.1	17	1.3	15.3	24.8	–	–	–	–	–	Chromophobe
26	5.4	14	1.1	14.3	3.0	–	–	–	++	++	Chromophobe
27	1.3	114	1.2	10.8	<3.0	–	–	–	++	–	Chromophobe
28	<1.0	22	0.7	<2.0	<3.0	–	–	–	+	++	Chromophobe
29	5.4	15	0.8	5.4	4.1	–	+	–	+	+	Chromophobe
30	6.4	98	0.3	29	10.2	–	–	–	+	+	Chromophobe

Notes: *: ranges of hormone grades (ng or Iu/(L·24h·10^5cells)): -<5; +5–10; ++ 10–50; +++>50. **: PRL, GH adenomas and nonfunction (-ing adenoma omitted) clasified by immunocytochemistry, and basophil, acidophil and chromophobe by HE stains. nd: not detected.

was on the basis of symptoms and signs and imaging features of X-CT, MRI as well as results of serum pituitary hormone assays. GH, PRL, LH and FSH in serum were

detected by radioimmunoassay (RIA) and TSH by immunoradioassay (IRMA)(RIA and IRMA kits from DPC company, USA). A small part of adenoma tissue was meted out for histological studies. Tissue from 14 cases was examined by both conventional HE stains and immunocytochemical techniques[4]. The rest 16 cases were only examined by HE stains.

In vitro cell culture protocol as follows[5]. Adenoma tissue was divided into small pieces and dissociated by incubation with 1% trypsin in PBS(-) for 30 min. Cells were then dispersed and rinsed by PBS(-) three times and transfered into culture medium RPMI-1640 containing 10% fetal calf serum and antibiotics. Aliquot of the cell suspension was used to count cells using a hemocytometer. Viability of cells was determined by trypsin blue exclusion and was never less than 90%. Cells were distributed into 24 well plastic plate with 1×10^5 cells and 2 ml media each well. The wells were precoated with the collagen that was derived from diabetic rat tails. The plastic plate was placed in incubator with $5\%O_2$ and 95% CO_2. The gross morphology of the cultured cell was observed on microscope everyday and the culture medium was changed every 24 h. The used medium was centrifugalized and the suspension was preserved at -20° for future hormone assay.

The methods for the medium hormone assay were the same as those for serum hormone radioimmunoassay as described above. The results of hormone assay in the fifth day of culture were chosen to represent the hormone secretion function of the adenoma cells in culture and were expressed in four grades as shown in Table 1. The cross reaction by each hormone to others in radioimmunoassay was tested and all were below 1%.

3 RESULTS AND DISCUSSION

30 adenoma tissues were all successfully dispersed and maintained in vitro monolayer cell culture. Generally, the cells attached to the collagen surface firmly after 1 to 3 d incubation and no obvious cell proliferation nor severe cell loss occured during the whole culture periods. It proves that cell culture techniques for human pituitary adenoma tissues can be relatively easy to master, provided that, from our experiences and other authors'[6], some suitable collagen could be used to support cells in culture.

Results of hormone assay in the media showed that from all but 2 adenoma tissues, cells in culture secreted certain detectable hormone(s), with the amounts varying greatly from one another. From table 1, it is obvious to notice that the pattern and amount of hormone(s) in media coordinated well with whether the presence of the patient's serum hormone excess (except for hyperprolactinemia in some patients due to mass effects of tumors in pituitary) and also were comparable with the results of histological examinations. However some discrepancies could be easily perceived. In three patients (No.1–3), only hyperprolactinemia were found in clinic and immunocytochemical stains only found PRL immunoreactive in adenoma cells, but in culture media by those cells were detected not only PRL, but other hormones as GH, TSH, LH (and/or FSH) also detected in large quantities. Consecutive hormone assays were performed and the results exhibited that

hormones other than PRL in the media declined rapidly and most of them became undetectable after 10 days of culture (Fig.1) while, on the other hand, secretions of PRL in the media persisted at high levels for the duration of culture. This pattern of multihormone secretion in the media resembled that of nontumorous pituitary cells in culture as reported by other authors[2,7]. Concerning with the facts that these adenoma tissues were from patients with microadenomas in vivo (and the only three ones) and nontumous pituitary tissues might well be within the surgical specimens and then co-dispersed into cells in culture. Nontumorous pituitary cells are less likely to maintain its secretory activities in vitro, apparently due to lack of some necessary nutrients in conventional medium.

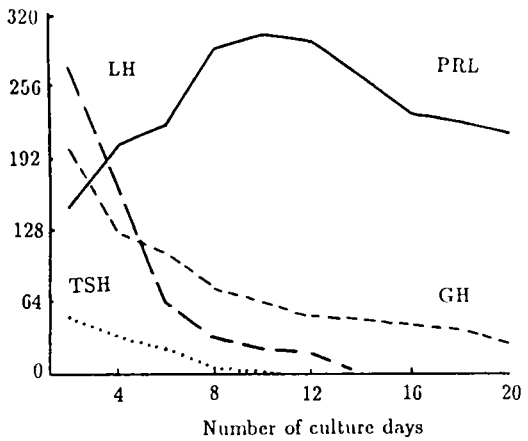

Fig.1 Daily hormone productions by PRL adenoma cells in culture from patient No.2
TSH, LH (Iu/(1·24h·10^5 cells));
PRL, GH(ng/(1·24h·10^5 cells))

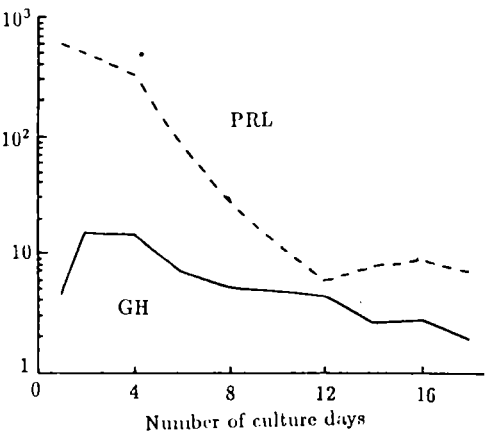

Fig.2 Daily hormone productions by GH adenoma cells in culture from patient No.12
PRL, GH (ng/(1·24h·10^5 cells));

In case of No.12, however, though immunocytochemical stains revealled no positive PRL in adenoma cells, in the culture media the amount and duration of PRL secreted matched with those of GH (Fig.2) and we assumed the adenoma tissue was actually GH-PRL mixed adenomas. (Cells from case 13 had the same secretion pattern). Also in cases of No.17-20, cells from those four adenoma tissues produced large amounts of LH and FSH into media and we classified them as GH adenomas, combined with the clinical evidences of hypergonadotrophnemia in these patients. The facts that immunocytochemical studies failed to find PRL or LH and FSH immunoactive stains within those cells might reflect that only some subpopulations of adenoma cells possess hormone secretion functions[8] and these cells unevenly distributed in adenoma tissues. Since only a very small part of adenoma tissues could practically be available for histological examinations,

other parts be ignored. Besides, the degree of tumor differatiation and other technical problems during fixing, embedding, and immunoreaction procedures might affect final stain colours[4].

Cell culture techniques make use of whole adenoma tissues and, in addition, hormone secreted in the media can be accurately and quantitively detected by hormone radioimmunoassay with high sensitivity. This advantage could be clearly seen in the studies of non-functioning adenomas. Non-functioning adenomas present no serum hormone excess in vivo, and not any positive hormone immunoreactivity in immunocytochemistry, as shown in cases of No.21–30 in our studies. But by cell cultures, certain amounts of LH and FSH (and PRL occasionally) were detected in the media by cells from 8/10 of these adenoma tissues, though much smaller than those from functional (especially GnH) adenomas. This finding indicates that most non-founctioning adenomas are in fact capable of producing glycoprotein hormones by some cells[9], at least. These cells may also secrete hormones in vivo, but low amounts cannot significently increase the hormone levels peripherally.

In conclusion, In vitro cell culture has been found useful for investigating the nature of pituitary adenomas. Together with hormone radioimmunoassay of the media, the secretory activities of pituitary adenoma cells can be effectively investigated and better relationships could be established to clinical hormone excess. By in vitro cell culture technique, more prevalent GH-PRL mixed adenomas and GnH (LH and/or FSH) adenomas could be identified. Also most non-founctioning ademonas are found capable of producing some pituitary hormones. Further studies could result in more understandings of structure-function relationships of pituitary adenomas.

REFERENCES

1 Horvath E, Kovacs K. Path Res Pract, 1988; 183:129
2 Adams E F, Mashiter K. Neurosurg Rev, 1985; 8:135
3 Asa S L. Path Res Pract, 1988; 183:561
4 Zhang Fulin, Yang Detai, Zhang Y L *et al.* Chinese J Path, 1990; 19:97
5 Lu Hankui, Lin Xiangtong, Yang Detai *et al.* Chinese J Nucl Med, 1994; 14:28
6 Weiner R I, Bethen C L, Jaquet P *et al.* Methods in Enzymology, 1983; 103:287
7 Kohler P O, Bridson W E, Rayford P L *et al.* Metabolism, 1969; 18:782
8 Yamada S, Asa S L, Kovacsk *et al.* J Clin Endocrinol Metab, 1989; 68:73
9 Arafah B M. J Clin Endocrinol Metab, 1986; 62:1173