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Expression of mPOU Protein in the Human Pituitary Adenomas

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[Objective] mPOU is a POU protein classified as class VI. It is present in the pituitary gland as well as the brain, heart muscle, skeletal muscle, lung, and lymphocytes. In our previous investigation, mPOU bound to the Pit-1-binding DNA elements of the rat PRL gene, and promoted transcription of the GH and PRL genes. In this study, we immunohistologically investigated the expression of mPOU in pituitary adenomas. [Methods] 17 patients with pituitary adenoma underwent tumor excision by transsphenoidal approach at our hospital (PRL: 5, GH: 4, FSH: 1, non-functioning: 7). The expression in the tissue sections was investigated using immunostaining (ABC method). [Results] In all GH-producing and PRL-producing adenomas, mPOU protein was specifically expressed, particularly in the nuclei. [Discussion] Pit-1 has been considered to be a factor determining the expression of GH and PRL genes, but mPOU may also be involved in the expression.

Pituitary adenoma occurs at a relatively high frequency and accounts for about 10% of incidences of all intracranial tumors in adults, but the tumorigenesis of the majority of pituitary adenomas remains unknown. Since Hermann et al. showed that pituitary adenomas are monoclonal in origin using X chromosome inactivation analysis[5)], investigations to identify gene mutations in tumor cells have been performed. Landis et al. detected subunit of Gs alpha protein (gsp)-activating mutation in about 40% of growth hormone-producing adenoma[7)]. The molecular alterations in oncogenes such as ras, c-myc, and c-myb and tumor suppressor gene such as Rb and p53 have been investigated. However these alterations do not seem to contribute to tumorigenesis[9)].

A pituitary-specific transcription factor, Pit-1, is the transcription factor activating the expression of growth hormone (GH), prolactin (PRL), and TSH genes. It has been reported that Pit-1 not only regulates tissue-specific expression of the GH and PRL genes, but is also involved in regulation of the GH and PRL gene expression by diverse hormones[1),6)].The expression of Pit-1 has been investigated in pituitary adenomas[2),4),10)].

We have proposed a possibility that a protein other than Pit-1 binds to 1P, the closest Pit-1-binding DNA sequence to the transcription initiation site in the PRL gene, and

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promotes expression of the PRL gene[8]. We attempted cloning using the yeast one-hybrid system, and obtained a POU protein, mPOU as a candidate for the proposed protein[3]. mPOU is classified as class VI of the POU protein family[11] and has homologies with Pit-1 and Oct-1. It has been shown that in addition to the pituitary gland, mPOU is present in the brain, heart muscle, skeletal muscle, lung, and lymphocytes[11]. The function has not been fully understood, and there has been no report of mPOU expression in pituitary adenomas although we have revealed that mPOU has a stimulatory effect on the PRL gene expression in the presence of Pit-1. In this study, we immunohistologically investigated the expression of mPOU in pituitary adenomas.

SUBJECTS AND METHODS

The subjects were 17 patients with pituitary adenoma who underwent tumor excision by transsphenoidal approach at the Department of Neurosurgery, Kobe University (Table 1). There were 9 males and 8 females ranging in age from 20 to 67 years (mean: 44.7 years old). The clinical and histological diagnoses included 5 PRL-producing adenomas, 4 GH -producing adenomas, 1 FSH-producing adenoma and 7 non-functional adenomas. Five patients presented with microadenomas, and 12 with macroadenoma.

TABLE 1. Patients characteristics

Parameters	Values
male ; female	9 ; 8
age range(mean)	20-67(44.7) yrs
producting hormone	
growth hormone(GH)	4
prolactin(PRL)	5
follicle stimulating hormone(FSH)	1
non-functioning	7

Anti-mPOU Antibody

For obtaining anti-mPOU antibody, a 15-amino acid peptide, VRKPSTPESPAKSEV, corresponding to residues 70- 84 of mPOU sequence was synthesized. The peptide was selected as an antigen because it is located on the transactivation domain of mPOU and has no homology with transactivation domains of other POU proteins. The peptide conjugated with keyhole limpet hemocyanin was subcutaneously injected to rabbits. Anti-mPOU antiserum was obtained from the rabbits, and the specificity of the antibody was tested by enzyme immunoassay.

Tissue

The excised tissues were fixed with 10% buffered formalin and embedded in paraffin, and 5 μm-thick sections were prepared.

Immunohistochemical staining

Deparaffinized sections were kept in 2 N HCl for 30 minutes, and 0.1M $Na_2B_4O_7$ (pH 7.5) for 10 minutes. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide-containing methanol.

The sections were reacted with 300-fold diluted rabbit anti-mPOU antibody at 4°C overnight, and avidin-biotin staining was performed using Vectastain Elite detection system (Vector Lab.) and 3,3´-diaminobenzidine for color reaction.

RESULTS

In GH-producing and PRL-producing adenomas, mPOU protein was specifically expressed, particularly in the nuclei (Table 2). Nuclear expression of mPOU protein was observed in 4 of 4 GH-producing tumors (Fig.1) and 5 of 5 PRL-producing tumors (Fig.2). However, in immunohistochemical investigation, no staining of the nuclei or cytoplasm was observed in 7 nonfunctional adenomas and one FSH-producing adenoma, showing no expression of mPOU (Fig.3).

TABLE 2. Expression of mPOU

Adenoma Type	No.(%)
growth hormone(GH)	4(100)
prolactin(PRL)	5(100)
Clinically non-functioning	
follicle stimulating hormone(FSH)	1(0)
non-functioning	7(0)

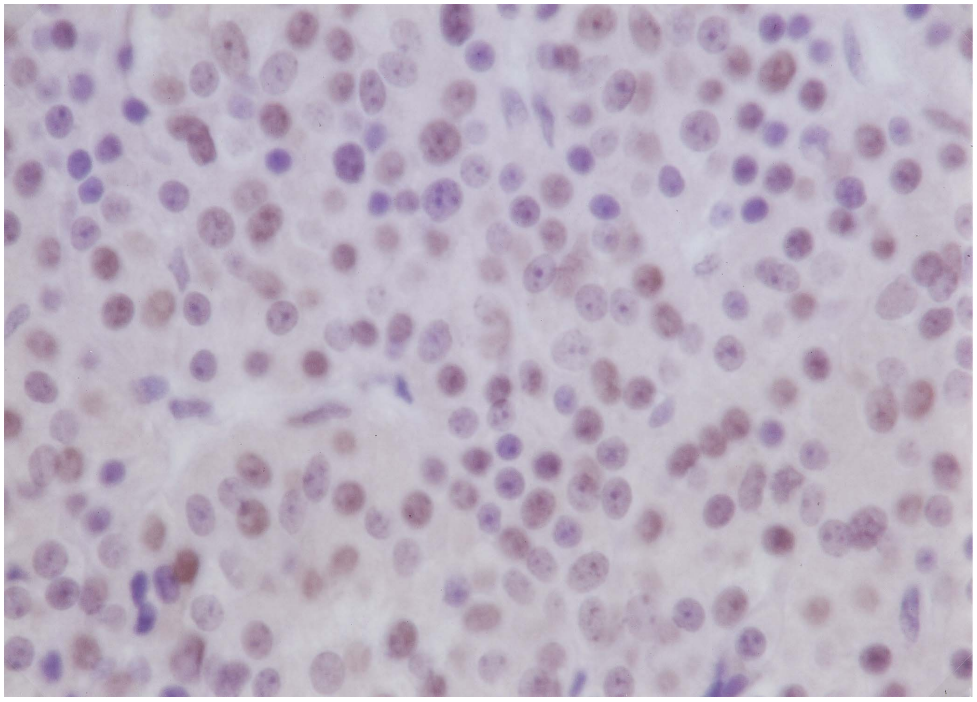

Figure 1. Immunohistochemical localization of mPOU protein in a growth hormone producting adenoma indicated that mPOU protein was specifically expressed, particularly in the nuclei (original magnification X400)

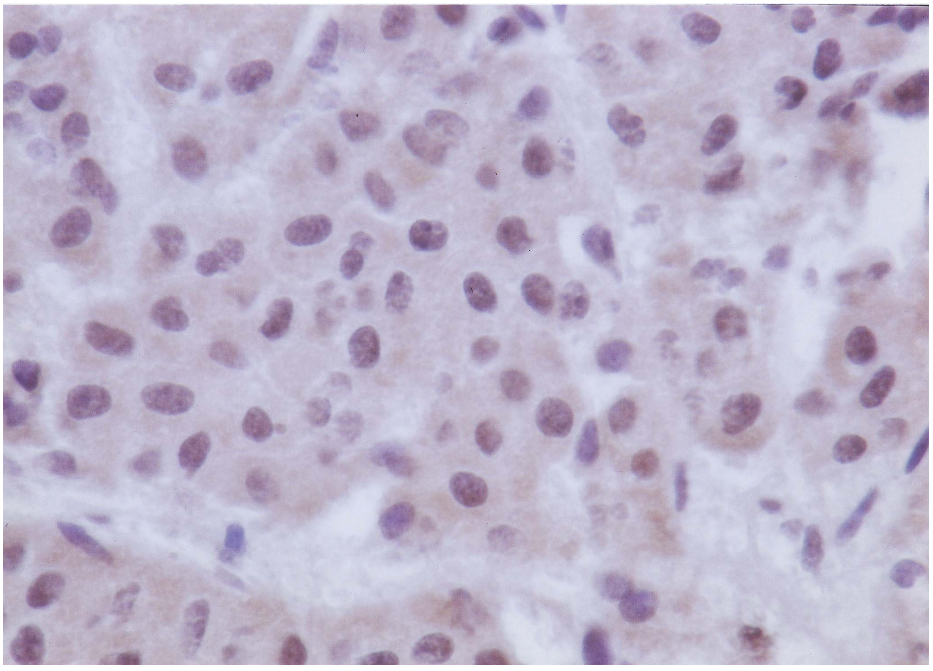

Figure 2. Immunohistochemical localization of mPOU protein in a prolactin producting adenoma indicated that mPOU protein was specifically expressed by the same pattern as a growth hormone producting adenoma (original magnification X400)

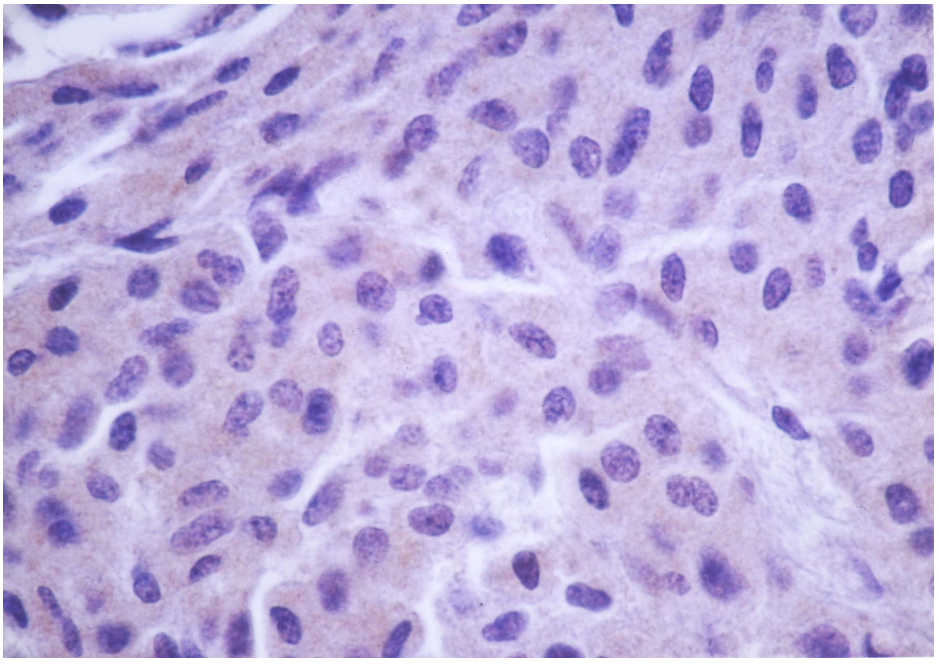

Figure 3. In a nonfunctioning adenoma, adenoma cells were negative for mPOU protein (original magnification X400)

DISCUSSION

This is the first report showing the presence of mPOU in GH-producing and PRL-producing adenomas. mPOU has been cloned from human muscle and pituitary cDNA library. mPOU is a member of the POU protein family and classified as class VI[11]. mPOU is present in the pituitary gland as well as the brain, skeletal muscle, lung, and lymphocytes, but the function has not been fully understood[11]. In this study, we synthesized a peptide corresponding to a part of the mPOU sequence, prepared anti-mPOU antibody, and used it for investigation of mPOU expression in various types of pituitary adenoma. mPOU protein was expressed in GH-producing and PRL-producing adenomas, particularly in the nuclei of the adenomas. However, no expression was observed in nonfunctional or FSH-producing pituitary adenoma, showing a specific expression pattern of mPOU protein in pituitary adenoma. These findings suggested the role of mPOU in GH-producing and PRL-producing adenomas as a transcription factor.

We have revealed that mPOU promotes PRL gene expression synergistically with Pit-1 and that the effect increases in the presence of cAMP[3]. Bromocriptine that inhibits cAMP production is used for treatment of prolactinoma. Since cAMP is considered to play a major role in PRL production, elucidation of the synergistic effect of mPOU may lead to development of a new therapeutic method for prolactinoma.

A pituitary gland-specific transcription factor, Pit-1, is the transcription factor for the GH, PRL, and TSH gene expressions. Immunohistochemical investigation of Pit-1 expression in pituitary adenoma has been performed, and specific expression has been observed in GH-producing, PRL producing-, and TSH-producing pituitary adenomas[2),4),10)]. Since the expression of mPOU is exclusively in GH-producing and PRL producing-adenomas and mPOU synergistically promoted PRL gene expression with Pit-1 in our previous investigation, in addition to Pit-1, mPOU may be involved as a factor determining expression of the PRL genes in PRL-producing adenomas. Although the synergistic action of mPOU and Pit-1 was evident in the PRL but not in the GH gene expression in our previous study, the possibility that mPOU may play a role in GH-producing adenomas could not be excluded since the specific expression of mPOU was observed in GH-producing adenomas in addition to PRL- producing adenomas. To clarify the role of mPOU in pituitary adenomas, more extensive investigation including functional experiments will be necessary.

In conclusion, immunoreactivity for mPOU was observed in all the GH-producing and PRL-producing adenomas examined. However,no immunoreactivity for mPOU was found in all the nonfunctional adenomas and FSH-producing adenoma examined. Since mPOU has a synergistic action with Pit-1on PRL gene expression in vitro study, it may be involved in the exaggerated PRL production in PRL-producing adenoma.

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