Functional Significance of Prop-1 Gene Expression in Pituitary Adenomas

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Abstract. *Prophet of Pit-1* (Prop-1), which is a paired-like homeodomain transcription factor, is capable of binding to sites in an early enhancer of the Pit-1 gene and regulating its expression. As human Pit-1 is expressed considerably in pituitary adenomas, we studied human Prop-1 gene expression in pituitary adenomas. We also sequenced the Prop-1 cDNAs in pituitary adenomas. Human Prop-1 transcript was detected in all pituitary adenomas examined by RT-PCR analysis. The expression of human Prop-1 in pituitary adenomas was confirmed by *in situ* hybridization in one of the GH-producing adenomas. The sequence analysis of human Prop-1 cDNAs in these pituitary adenomas revealed that there were no mutations except 5 silent nucleic acid substitutions, suggesting that mutations of Prop-1 gene do not represent a frequent mechanism of human pituitary tumorigenesis.

Key words: Human Prop-1, Gene expression, Pituitary adenoma, Transcription, Direct sequencing

(Endocrine Journal 47: S85–S89, 2000)

THE molecular basis for dwarfism has been revealed to be the mutations of various genes. For example, Pit-1 has been shown to be responsible for the dwarf phenotype of Snell (*dw*) and Jackson (*dwJ*) [1], GRF receptor for little mouse (*lit/lit*) [2], and Hmgi-c for pygmy (*pg*) [3], respectively. Recently, a positional cloning approach has identified a novel, paired-like homeodomain transcription factor, *Prophet of Pit-1* (Prop-1), as responsible for a heritable form of murine pituitary-dependent dwarfism (Ames dwarf, *df*) [4]. Prop-1 is capable of binding to sites in an early enhancer of the Pit-1 gene [5] and regulating its expression. In the Ames mouse, a single amino acid substitution in the first helix of the Prop-1 homeodomain seems to result in the impairment of its DNA binding. Hypoplastic anterior pituitary gland in the Ames mouse with few somatotropes, lactotropes, or thyrotropes, appears to reflect a failure of determi-

nation of the Pit-1 lineages from perinatal pituitary cells, suggesting that Prop-1 is essential to the asymmetric cell division that generates the Pit-1 lineages.

In this study, we investigated human Prop-1 expression in pituitary adenoma cells and we sequenced the Prop-1 cDNAs derived from pituitary tumors to determine the mutations that might have led or contributed to their tumorigenesis.

Materials and Methods

RT-PCR

14 Human pituitary adenoma tissues (5 GH-, 3 PRL-, 2 TSH-, 1 ACTH- producing, and 3 non-functioning adenomas) were obtained by transphenoidal surgery. cDNAs were synthesized from these tumors, and RT-PCR was performed with sets of primers. The forward and reverse primers were as follows; S1 and A1 for human Prop-1, 5'-GGAAT-

GAGGATCCAAGCTTTTACTTCGGCT-3' and 5'-GGTTCTGCAGATGACAGGAAGGAACAG-3' for human Pit-1, and 5'-CCGCGTCTCCTTTGAGC-TGTTTGCAG-3' and 5'-ACCCAAAGGGAAGTGC-AGCGAGAGC-3' for human cyclophilin [10] as an internal control.

In situ hybridization for human Prop-1

One of the GH producing adenoma tissues obtained by transsphenoidal surgery (Case 5) was fixed, dehydrated, and embedded in paraffin. The sense and antisense probes for Prop-1 and GH were labeled with digoxigenin-dUTP which was obtained from an RNA labeling kit (Boehringer Mannheim, Germany).

Sequencing of human Prop-1 expressed in pituitary adenomas

To examine whether the Prop-1 gene could be an oncogene in human pituitary tumors, we amplified and directly sequenced the entire coding region of Prop-1. cDNAs derived from pituitary adenomas were amplified by PCR with *Pfu* DNA polymerase. The PCR products were collected from 1.5% low melting agarose gel and purified by means of a QIAquick Gel Extraction Kit. The purified products were used for direct sequencing with a Dye Primer Cycle Sequence FS Ready Reaction Kit (–21M13) (Applied Biosystems, Warrington, U.K.). Sequenc-

ing was performed with a 373A DNA Sequencer (Applied Biosystems).

Results

Expression of human Prop-1 in tumoral pituitary cells

By RT-PCR analysis, it was demonstrated that human Prop-1 mRNA was expressed in all of the 14 human pituitary adenomas examined. On the other hand, human Pit-1 mRNA was expressed in only GH-, PRL- and TSH-producing adenomas, but not in ACTH-producing and nonfunctioning adenomas (Fig. 1). Expression of human Prop-1 gene in pituitary adenomas was confirmed by *in situ* hybridization analysis in one of the GH-producing adenomas (Fig. 2a). Human Prop-1 mRNA was co-localized with GH mRNA in the nuclei of pituitary adenoma cells (Fig. 2a, b).

Sequence of human Prop-1 gene in pituitary adenomas

The nucleotide sequences of PCR products were identical to those of human Prop-1 cDNA, except for 5 silent mutations (amino acid 9, Ala, GCC for GCT; amino acid 56, Phe, TTT for TTC; amino acid 111, Ala, GCG for GCC; amino acid 112, Arg, AGA for

Fig. 1. RT-PCR of RNAs from pituitary adenomas with human Prop-1 (a), Pit-1 (b) and cyclophilin (c) primers. Lanes 1–5: GH-producing adenomas; lanes 6–8: PRL-producing adenomas; lanes 9–10: TSH-producing adenomas; lane 11: ACTH-producing adenoma; lanes 12–14: nonfunctioning adenomas. M indicates a size marker of the 100 bp DNA Ladder (New England Biolabs, Beverly, MA). None of the PCRs examined without reverse transcriptase yielded any bands (data not shown).

Fig. 2. *In situ* hybridization of paraffin sections of a GH-producing adenoma. (a) Positive hybridization signal is present in the nuclei of many tumor cells with Prop-1 antisense probe. The hybridization with the sense probe resulted in only a background signals (data not shown). (b) A positive hybridization signal with the GH antisense probe is present in the same cells in which the hybridization signal with the Prop-1 antisense probe exists. (a) and (b) are serial sections.

CGA; amino acid 203, Ala, GCG for GCC), which were all heterozygotes. Amino acid 142 was a homozygote of Thr in 8 cases and Ala in one case, and a heterozygote of Thr and Ala in 5 cases (Table 1).

Discussion

We examined human Prop-1 mRNA expression in

pituitary adenomas by RT-PCR analysis. As shown in Fig. 1, Prop-1 mRNA was expressed in all the pituitary adenomas we examined, whereas Pit-1 mRNA expression was restricted to GH-, PRL- and TSH-producing adenomas. Previous reports on Pit-1 mRNA expression have been controversial. Some investigators have reported that Pit-1 mRNA is expressed in pituitary adenomas other than GH-, PRL- and TSH-producing adenomas [6-9], and others have reported that in most cases it is expressed only in

Table 1. Nucleic acid substitutions in human Prop-1 in pituitary adenomas

Case No.	Tumor type	Immunohistochemistry	Mutations (wild to mutaut)	Condon 142
1	Somatotropinoma	GH	Condon 112 Arg (CGA to AGA)	GCC (Ala)/ACC (Thr)
2	Somatotropinoma	GH	Condon 112 Arg (CGA to AGA)	ACC (Thr)/ACC (Thr)
3	Somatotropinoma	GH	No mutation	ACC (Thr)/ACC (Thr)
4	Somatotropinoma	GH	Condon 112 Arg (CGA to AGA)	ACC (Thr)/ACC (Thr)
5	Somatotropinoma	GH	No mutation	GCC (Ala)/ACC (Thr)
6	Lactotropinoma	PRL	Condon 56 Phe (TCC to TTT) Condon 111 Ala (GCC to GCG) Condon 112 Arg (CGA to AGA)	ACC (Thr)/ACC (Thr)
7	Lactotropinoma	PRL	No mutation	GCC (Ala)/GCC (Ala)
8	Lactotropinoma	PRL	No mutation	GCC (Ala)/ACC (Thr)
9	Thyrotropinoma	TSH, GH, PRL	Condon 112 Arg (CGA to AGA)	ACC (Thr)/ACC (Thr)
10	Thyrotropinoma	TSH	Condon 9 Ala (GCT to GCC)	GCC (Ala)/ACC (Thr)
11	Corticotropinoma	ACTH	Condon 56 Phe (TTC to TTT) Condon 203 Ala (GCC to GCC)	ACC (Thr)/ACC (Thr)
12	Nonfunctioning adenoma	Negative	No mutation	GCC (Ala)/ACC (Thr)
13	Nonfunctioning adenoma	Negative	No mutation	ACC (Thr)/ACC (Thr)
14	Nonfunctioning adenoma	Negative	No mutation	ACC (Thr)/ACC (Thr)

GH-, PRL- and TSH-producing adenomas [10, 11–14]. The expression of Pit-1 in ACTH-producing and clinically nonfunctioning adenomas might be explained by the existence of tumor cells that are focally differentiated into Pit-1 lineage cells or trapped, intermingled normal pituitary cells in the adenomas [15]. No evidence of Pit-1 mRNA in ACTH-producing and clinically nonfunctioning adenoma cells in this study indicates that these tumor tissues were not contaminated with normal pituitary cells or tumor cells which have characteristics of Pit-1 lineage cells. Recently it has been reported that some patients who have human Prop-1 gene mutations have not only GH, PRL and TSH deficiencies but also have lower LH, FSH and ACTH levels in serum than normal subjects [16, 17, 18], which are not observed in patients who have Pit-1 gene mutations. Therefore, the expression of Prop-1 gene in ACTH-producing and clinically nonfunctioning adenomas, many of which are reported to secrete gonadotropin subunits [19], suggests that human Prop-1 might have functions other than as a Pit-1 gene enhancer,

such as regulating cell division which is independent of Pit-1 lineage directly or regulating pituitary specific transcription factor(s) other than Pit-1.

No mutations except 5 silent nucleic acid changes were found in human Prop-1 cDNA in GH-, PRL-, TSH- and ACTH-producing pituitary adenomas, suggesting that abnormalities of the Prop-1 gene might not be frequent mechanisms of human pituitary tumorigenesis. Further studies are required to elucidate its relation to the pathogenesis of pituitary adenomas.

Acknowledgements

We thank Ms. Keiko Matsuda for her excellent technical assistance. This work was supported in part by research grants from the Naito Foundation; the Uehara Memorial Foundation and the Japanese Society for the Promotion of Science "Research for the Future" program (JSPS-RFTF 96100204).

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