
—Full Paper—

High Level Expression of Prop-1 Gene in Gonadotropic Cell Lines

Satoko AIKAWA1), Takanobu SATO1), Tetsuo ONO1), Takako KATO1) and Yukio KATO1)

1) Laboratory of Molecular Biology and Gene Regulation, Department of Life Science, School of Agriculture, Meiji University, 1–1–1 Higashi-mita, Tama-ku, Kawasaki, Kanagawa 214-8571, Japan

Abstract. Prop-1 acts as an upstream regulator for the Pit-1 gene to induce development of Pit-1 lineage pituitary cell lines, GH-, PRL-, and TSH-producing cells, in the early stage of pituitary organogenesis. Furthermore, Prop-1 is presumed to be involved in the function of FSH/LH-producing cells, gonadotropes, since the defective Prop-1 gene shows hypogonadism. Recently, we reported evidence that Prop-1 directly regulates expression of the porcine FSHβ gene, thus providing a novel advance in understanding the function of Prop-1 in FSH/LH production and hypogonadism. This study was intended to demonstrate the expressions of Prop-1 gene in pituitary tumor-derived cell lines. RT-PCR analyses were conducted of Pit-1, glycoprotein α subunit (αGSU), GnRH receptor, and cyclophilin A (a ubiquitously expressing gene). We observed expression of the Pit-1 gene in αT1-1, αT1, MtT/S, GH3, and TtT/GF cells, expression of the αGSU gene in αT1-1, αT3-1, LβT2, LβT4, TaT1, and GH3 cells, and expression of GnRH receptor gene in αT3-1, LβT2, LβT4, and GH3 cells, respectively. These expression profiles were in accord with their cell lineages, with only a few exceptions. To accurately measure the expression level of the Prop-1 gene, a quantitative analysis was performed using the real-time PCR method. This analysis demonstrated that the LβT2 and LβT4 gonadotrope cell lines, which express the FSHβ gene, express the Prop-1 gene. Taken together with our previous observation that Prop-1 is present in the adult porcine pituitary gonadotropes, Prop-1 might also be involved in development of gonadotropes and hormone production.

Key words: Glycoprotein hormone, Transcription, Pig, Pituitary, Prop-1, Dwarfism

Accepted for publication: November 9, 2005
Published online: December 28, 2005
Correspondence: Y. Kato (e-mail: yukato@isc.meiji.ac.jp)

Pituitary transcription factor Prop-1, an upstream regulator of the Pit-1 gene, was originally cloned as a gene responsible for a heritable form of murine pituitary-dependent dwarfism (Ames dwarf). The gene was confirmed as a novel, tissue-specific, paired-like homeodomain transcription factor, termed Prophet of Pit-1 [1]. A defect in the Prop-1 gene causes failure of Pit-1 gene expression, resulting in termination of development of GH, PRL, and TSH-producing cells, all of which are Pit-1-dependent lineage cells. In addition, the defect also reduces the production of FSH and LH, causing hypogonadotropic hypogonadism (Combined Pituitary Hormone Deficiency; CPHD) [2]. The molecular mechanism of CPHD is not yet fully understood. We reported that several pituitary nuclear proteins bind to the upstream region (–852/–746 bp) of the porcine FSHβ gene [3] and demonstrated that Prop-1 is one of the binding proteins that directly regulates this gene [4]. This
finding provides novel insight into better understanding the roles of Prop-1 in pituitary organogenesis, hormone gene regulation, and CPHD.

It is noteworthy that the ontogeny of murine Prop-1 gene expression was first detected at around embryonic day 10 (e10) after formation of the oral ectoderm on e8.5 and invagination leading to formation of a Rathke’s pouch on e9.5–e10 [1]. These two events preceded expression of Pit-1 gene, which is a major target of Prop-1 and a crucial stage in the progression of pituitary differentiation. Thus, the ontogeny of Prop-1 gene expression plays an indispensable role in the early stages of pituitary organogenesis. Thereafter, Prop-1 gene expression reaches its maximum level by e12, followed by a decline to an extremely low level by e14.5 that continues until birth [1]. Although postnatal expression has been confirmed in the porcine [4] and human [5] pituitary, information concerning mice, rats and other animals has yet to be elucidated.

In this study, we attempted to examine the presence of Prop-1 transcripts in various cell lines derived from pituitary tumors. Quantitative real-time PCR analyses revealed that the Prop-1 gene is expressed abundantly in gonadotrope cell lines.

Materials and Methods

Cell lines

Mouse pituitary tumor cell lines, αT1-1, αT3-1, LβT2, LβT4, and TaT1, were kindly supplied by Dr. P. L. Mellon [6–8]. MtT/S and TtT/GF cell lines were established from rat estrogen-induced mammotrophic pituitary tumors [9] and mouse thyrotropic pituitary tumors [10], respectively, by Dr. K. Inoue. AtT-20 [11] and GH3 [12] cell lines derived from mouse and rat pituitary tumor, CHO cells established from Chinese hamster ovaries [13], and mouse L929 fibrosarcoma cells [14] were obtained from the RIKEN Cell Bank (Tsukuba, Ibaraki, JAPAN). The characteristics of the cell lines used are summarized in Table 1.

Preparation of total RNAs and cDNA synthesis

Total RNAs were prepared from 60–70% confluent cells of each cell line using ISOGEN (Nippon Gene Co., Ltd., Toyama, Japan) according to the instruction manual. The cDNA synthesis from the total RNAs was carried out using a SMART cDNA Synthesis Kit (BD Bioscience Clontech, Palo Alto, CA) with ReverTra Ace reverse transcriptase (TOYOBO, Tokyo, Japan).

PCR

The specific PCR primer sets for Pit-1, glycoprotein hormone α subunit (αGSU), GnRH receptor (GnRH-R) and cyclophilin A shown in Table 2 were synthesized. PCR was essentially performed as described previously [15]. Each cDNA was amplified in a reaction mixture (5 µl) containing the two required primers (5 pmol each) and 0.125 U AmpliTaq Gold DNA polymerase (PE Applied Biosystems, Foster City, CA), with 32 to 36 cycles of denaturation (94 C, 30 s), annealing (55 C, 30 s), and extension reaction (72 C, 2 min). The resulting PCR products were analyzed on 2% agarose gels.

Table 1. Characteristics of the cell lines used

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>Sex</th>
<th>Cell lineage</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>αT1-1</td>
<td>mouse</td>
<td>?</td>
<td>thyrotrope</td>
<td>[8]</td>
</tr>
<tr>
<td>αT3-1</td>
<td>mouse</td>
<td>male</td>
<td>gonadotrope</td>
<td>[7]</td>
</tr>
<tr>
<td>LβT2</td>
<td>mouse</td>
<td>male</td>
<td>gonadotrope</td>
<td>[6]</td>
</tr>
<tr>
<td>LβT4</td>
<td>mouse</td>
<td>male</td>
<td>gonadotrope</td>
<td>[6]</td>
</tr>
<tr>
<td>TaT1</td>
<td>mouse</td>
<td>?</td>
<td>thyrotrope</td>
<td>[8]</td>
</tr>
<tr>
<td>MtT/S</td>
<td>rat</td>
<td>female</td>
<td>somatotrope</td>
<td>[9]</td>
</tr>
<tr>
<td>GH3</td>
<td>rat</td>
<td>female</td>
<td>somatotrope/mammotrope</td>
<td>[12]</td>
</tr>
<tr>
<td>TtT/GF</td>
<td>mouse</td>
<td>male</td>
<td>folliculostellate cell</td>
<td>[10]</td>
</tr>
<tr>
<td>CHO</td>
<td>Chinese hamster</td>
<td>female</td>
<td>ovary cell</td>
<td>[13]</td>
</tr>
<tr>
<td>L929</td>
<td>mouse</td>
<td>male</td>
<td>fibrosarcoma</td>
<td>[14]</td>
</tr>
</tbody>
</table>
Comparative quantification by real-time PCR

For real-time transcript quantification, cDNAs were subjected to a fluorogenic 5’–3’ exonuclease assay utilizing the chemistry of the TaqMan system on an ABI Prism 7500 Sequence Detector (Applied Biosystems, Foster City, CA). Gene-specific primers (forward and reverse) and TaqMan Minor Groove Binder (MGB) probe sets were created for Prop-1, FSHβ, and cyclophilin A using the Primer Express Software v.1.5 (Applied Biosystems) as shown in Table 3. The TaqMan MGB probe for cyclophilin A was labeled with VIC and used as a control for normalization. The TaqMan MGB probes for Prop-1 and FSHβ were labeled with 6-carboxyfluorescein (FAM). The experimental PCRs included 900 nM of each primer, 250 nM MGB probe, 1 × TaqMan buffer A (50 mM KCl, 10 mM Tris-HCl, 0.01 mM EDTA), 5 mM MgCl₂, dATP (200 µM), dCTP (200 µM), dGTP (200 µM), dUTP (400 µM), 0.5 U AmpErase UNG, and 1.25 U AmpliTaq Gold DNA Polymerase. The real-time PCR consisted of one cycle at 50 C for 2 min and 95 C for 10 min, followed by 40 cycles at 95 C for 15 s and 60 C for 1 min. All data were calculated by the comparative Ct method (ΔΔCt method) [16] according to the instruction manual to estimate the relative gene copy numbers using the same threshold line for Prop-1, FSHβ, and cyclophilin A. Reactions that exhibited a low amplification profile resulting in a low relative content against cyclophilin A of less than 0.02 were not included.

Results

Characterization of pituitary cell lines

RT-PCR was first performed for cyclophilin A, a housekeeping gene, to determine the quantity of each cDNA sample needed to obtain the same amount of PCR product (Fig. 1A). RT-PCRs for Pit-1, αGSU, and GnRH-R were then performed using individual quantities of each cDNA sample having the same amount of cyclophilin A cDNA. The other non-pituitary cell lines, CHO and L929 cells, were also negative. Figure 1C shows the results of PCR for αGSU. Products of αGSU were observed in αT1-1, αT3-1, LβT2, LβT4, and AtT-20 did not yield any Pit-1 product. The other non-pituitary cell lines, CHO and L929 cells, were also negative.
pituitary cell lines, MtT/S, AtT-20, and TtT/GF cells, and non-pituitary cell lines, CHO and L929 cells, failed to yield any αGSU product.

Therefore, the RT-PCR for αGSU and Pit-1 confirmed that these genes express in a cell-type specific manner as expected. Since GnRH-R is known to be a specific receptor for gonadotrope lineage cells, RT-PCR for GnRH-R was performed. Products of GnRH-R were observed in αT3-1, LβT2, and LβT4 cells, and a small amount (invisible in the figure) was present in GH3 cells (Fig. 1D). It is worth noting that the product amount of GnRH-R was higher in αT3-1 cells than in LβT2 and LβT4 cells, which are thought to be part of a later stage of the development for gonadotrope lineage than αT3-1 because of acquisition of ability to express LHβ and FSHβ genes.

LβT2 and LβT4 cells express Prop-1 gene

Real-time PCR was employed for quantitative estimation of Prop-1 gene expression. The results for Prop-1 were arranged in comparative values against those of cyclophilin A using the ΔΔCT method (Fig. 2A). LβT4 cells showed the highest content of Prop-1 (about 59% of that for cyclophilin A), and another gonadotropic cell line, LβT2 cells, contained about 57% of that for cyclophilin A. Other cell lines had low contents of Prop-1 as follows: 3% (αT3-1), 2% (TαT1), 2% (AtT-20), and 3% (TtT/GF) of that for cyclophilin A. No products were found in αT1-1, MtT/S, and GH3 cells, or in the non-pituitary cell lines of CHO and L929 cells.

Real-time PCR analysis of FSHβ was also carried out (Fig. 2B). Expression of FSHβ gene was observed in LβT2 and LβT4 cells at a level 13 and 15% of that for cyclophilin A, respectively, which were lower amounts than for Prop-1 (Fig. 2A).
Only a small amount of FSHβ gene expression was observed in αT1-1(4%) and αT3-1 cells (3%), respectively.

**Discussion**

Prop-1 is a crucial pituitary-specific transcription factor for pituitary organogenesis. This factor appears at an early stage of pituitary development and disappears before completion of differentiation of hormone producing cells [1]. Expression of Prop-1 gene during the postnatal period was confirmed on P0 for the mouse in the report of Sornson *et al.* [1], but Cushman *et al.* reported that no Prop-1 expression was evident at or beyond birth in the mouse [17]. However, by transient transfection and immunohistochemical analysis, we observed that Prop-1 directly regulates the porcine FSHβ subunit gene and is present in adult porcine gonadotrope cells [4]. Furthermore, by the RT-PCR analysis for porcine ontogeny, we observed expression of Prop-1 gene during the embryonic period and the expression increased after birth [18]. We hypothesize that Prop-1 might play a role in regulation of specific hormone genes after accomplishment of its primary role in the early stage of pituitary development. Our purpose in this study was to determine whether the Prop-1 gene is continues to express in hormone producing cells during the course of the developmental stage, especially in Pit-1 dependent lineage cells and FSH-producing cells.

Pituitary cell lines, which are immortalized at discrete stages of pituitary development, were used in this study in addition to non-pituitary cell lines, CHO cells derived from Chinese hamster ovary cells, and L929 cells derived from mouse fibrosarcoma (Table 1). The pituitary cell lines used are classified into 7 cell lineages, including folliculostellate cells, and covered all the cell lineages of the anterior pituitary. The characteristics of the cell lines used are summarized in Table 1. The cell lineages reported are characterized by specific marker genes. The αT1-1 cell line, which expresses the αGSU gene, belongs to a thyrotrope cell lineage. The αT3-1, LβT2, and LβT4 lines, which express the αGSU and GnRH-R genes, belong to a gonadotrope cell lineage. The TaT1 cell line, which expresses the αGSU and TSHβ genes, belongs to a thyrotrope cell lineage. The MtT/S cell line, which expresses the GH gene, belongs to a somatotrope cell lineage, and GH3, which expresses both the GH and PRL genes, belongs to a somato/mammotrope cell lineage. The AtT-20 cell line is known as a corticotrope cell line, and TtT/GF is a non-hormone producing pituitary cell line with the characteristics of folliculostellate cells [10]. The CHO and L929 cell lines are non-pituitary cell lines derived from Chinese hamster ovaries and mouse fibrosarcoma, respectively.

The results of real-time PCR for Prop-1 and FSHβ and RT-PCR for Pit-1, αGSU, and GnRH-R are summarized in Table 4. It is noteworthy that the cell lines expressing the Pit-1 gene, which is regulated by Prop-1 in the early stages of pituitary development, did not express the Prop-1. The LβT2 and LβT4 cells, which express αGSU and FSHβ genes but not the Pit-1 gene, expressed the Prop-1 gene. Previously, Pernastetti *et al.* found expression of FSHβ gene in LβT2 cells [19], and we confirmed that a detectable amount of FSHβ transcripts are present in both LβT2 and LβT4 cells. This result corresponds to our previous finding that Prop-1 regulates the expression of FSHβ gene [4], and supports our hypothesis described above.

On the other hand, αT3-1 cells, which are

<table>
<thead>
<tr>
<th>Table 4. Summary of RT-PCR and real-time PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>αT1-1</td>
</tr>
<tr>
<td>Pit-1*</td>
</tr>
<tr>
<td>αGSU*</td>
</tr>
<tr>
<td>GnRH-R*</td>
</tr>
<tr>
<td>FSHβ**</td>
</tr>
<tr>
<td>Prop-1**</td>
</tr>
</tbody>
</table>

The mRNA quantity of each gene observed by RT-PCR (*) and real-time PCR (**) are arbitrarily indicated with an order of +++>++>+>++. Hyphens (−) indicate undetectable amounts.
classified as being of gonadotrope lineage by the characteristics of producing αGSU, GnRH-R, and SF-1 [7], failed to express the Prop-1 gene. The other αGSU-producing cell lines, αT1-1 and TαT1, which produce Pit-1 and are classified as being of thyrotrope lineage, also failed to produce Prop-1.

We recently found that Prop-1 also participates in regulation of gene expression of αGSU (Sato et al., unpublished observation), and that the distal and proximal upstream regions of the porcine αGSU gene contain profound regulatory elements [20]. In this context, the regulation system for expression of αGSU gene in LβT2 and LβT4 cells is distinguished from the systems in other αGSU producing cells, αT3-1, and thyrotripe lineage cells.

The accumulation of these data probably confirms our view that synthesis of FSH is involved in Prop-1 function. It has already been established that a Prop-1 gene defect causes CPHD, although its molecular mechanism is not yet fully understood. Since Prop-1 definitely regulates expression of the Pit-1 gene, a loss of Prop-1 function by mutation does not result in the differentiation of Pit-1 lineage cells, GH, PRL, and TSH producing cells. However, this disruption in the development of Pit-1 lineage cells failed to explain the hypogonadism symptoms involved in CPHD caused by Prop-1 defect. However, two possibilities may be considered. One is that Prop-1 participates in differentiation of gonadotropes in a manner specific to its role and similar to Pit-1 lineage cells. The other is that it directly contributes to activation of gonadotropin subunit gene expression. The experimental evidence described above supports this later possibility.

Acknowledgments

This research was supported in part by Grant-in-Aid for Scientific Research (B) No. 12470217 and No. 12557087 from the Ministry of Education, Culture, Sports, Science and Technology and by a Grant-in-Aid for Research Grant (A) from the Institute of Science and Technology at Meiji University to Y. K. This study was also supported by a matching fund subsidy (2001–2005) from the “High-Tech Research Center” Project for private universities of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References


10. Inoue K, Matsumoto H, Koyama C, Shibata K, Nakazato Y, Ito A. Establishment of a folliculo-stallete-like cell line from a murine thyrotropic


