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Identification of a Novel Gene by EST Clustering and its Expression in Mouse Ovary and Testis

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EST Clustering 방법으로 동정한 새로운 유전자의 생쥐 난소 및 정소에서의 발현

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포천중문 의과대학교 생명과학 전문대학원[1], 경상대학교[2], 차병원 여성의학연구소[3]

목 적: 본 연구에서는 EST Clustering 방법을 이용하여 이전의 연구에서 발굴한 B357 EST의 염기서열을 포함하는 유전자를 동정하고, 이 유전자의 발현을 생쥐의 난소와 정소를 포함한 여러 가지 조직들에서 살펴보고자 하였다.

연구방법: EST Clustering으로 얻어진 전체 염기서열을 5-day-ovary-specific gene-1 (5DOS1)이라고 명명하여 GenBank에 등록하였으며 (AY751521), northern blotting, real-time RT-PCR, *in situ* hybridization, western blotting, immunohistochemistry 등의 방법을 이용하여 생쥐 난소와 고환의 발달단계에 따라 그 발현양상을 관찰하였다.

결 과: 5DOS1의 전사체는 성장한 정소, 뇌, 근육에서 높게 발현하였으며, 난소의 경우에서는 원시난포 시기부터 모든 난자에서 발현하였으며 특히 생후 5일째 높게 발현하였고 그 이후로는 점차 감소하였다. 반면에 정소의 경우는 발달과 함께 계속 증가하였으며, 정모세포를 제외한 모든 발달단계별 정자에서 발현함을 관찰하였다.

결 론: 본 연구결과는 5DOS1에 대한 발견과 동정에 대한 첫 보고로써, 유전자 및 단백질이 생쥐의 난소 및 정소의 생식세포에서 발현하는 것을 관찰하였다. 앞으로 생식세포 발생 및 분화에 관련된 5DOS1의 기능에 대한 심층 연구가 더 필요하다고 사료된다.

중심단어: EST (expressed sequence tag), EST clustering, 5DOS1(AY751521), Follicular development, Testicular development, Gametogenesis

Whole genome sequences are now available for several species including human, mouse, *Drosophila*, and *Caenorhabditis*. For these and many more species there are large, publicly available collections of ESTs. Scientists are now faced with the challenge of extracting meaningful information

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from these massive sequence resources to support and accelerate their understanding of gene function.

One of the more valuable resources for any given species is a collection of non-redundant mRNA transcripts from expressed genes, for cloning full-length cDNAs and analyzing microarray. EST sequencing is a cost-effective way to survey the expressed portions of a whole genome. Although the list of completed genome sequencing projects has expanded rapidly, sequencing and analysis of ESTs remain a rapid and inexpensive approach to novel gene discovery in many eukaryotes and a key element in genome annotation.[1,2] Because ESTs are short, error-prone sequences, clustering of ESTs that are likely to be derived from the same mRNA is used first to reduce the redundancy in the EST database, improve the sequence quality, and increase the sequence coverage for a particular cluster in genome annotation. EST clustering usually refers to the entire process of identifying and assembling sibling ESTs, which can generate gene cluster profile data.

Previously, we obtained a list of differentially expressed genes that may play important roles in the arrest and/or initiation of primordial follicular growth into primary follicles by using suppression subtractive hybridization.[3] In this list, we found 15 EST clones in the day-5-subtracted cDNA library. Clone B357 was exclusively expressed in the mouse day-5 ovary as compared to the day-1 ovary. In the present study, we report the cloning of a novel cDNA, 5DOS1, extended from clone B357 by EST clustering and the determination of the spatial and temporal expression of the gene at the mRNA transcript level, as well as at the protein level, in both male and female gonads.

MATERIALS AND METHODS

1. *In silico* identification of the novel gene

Homologous clustering was performed by building of ESTs with identical or nearly identical overlapping sequences and then bringing the related contigs together to form 'clusters'.[4] Contig member ESTs should be derived from identical transcripts, whereas cluster members might be derived from the same gene, yet represent different transcript-spliced isoforms or transcripts from multigene families with extremely high sequence identity. Ortholog analysis was performed by searching the NCBI database (http://www.ncbi.nlm.nih.gov).[5]

ORF prediction and translation were performed using the BioEdit Sequence Alignment Editor program V7.0.0 (Tom Hall Ibis Therapeutics, Carlsbad, CA).[6] Using this program, amino-acid sequences of human, rat, and mouse orthologs were aligned. Signal peptides and transmembrane domains were searched with the PSORT II program (http://psort.ims.u-tokyo.ac.jp) and domain structures were searched with the NCBI Conserved Domain (CD) Search (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), ProDom (http://protein.toulouse.inra.fr/prodom/current/html/home.php), and Pfam (http://www.sanger.ac.uk/Software/Pfam/search.shtml). The exon-intron boundaries were determined by examining the consensus sequence for exon-intron junctions ('GT-AG' rule) and codon usage within ORFs.[7] Other genes linked to the novel gene were searched for, based on the descriptions of the mouse reference genomic contigs. Localization of the novel gene in the mouse chromosome was determined based on localization of the genomic clones in the NCBI database (http://ww.ncbi.nlm.nih.gov) and the Ensemble database (http://www.ensemble.org).[8]

2. Northern blot analysis

The RNA sample for Northern blot of adult mouse tissues was purchased from Seegene Co., with each lane containing 20 μg of total RNA. To make a probe, RNA was extracted from 4-weeks ICR mouse tissues. A 911-bp fragment of the 5DOS1 cDNA (nucleotides 741-1,651) was used

as the probe for hybridization of the Northern blots. The probe was synthesized by using the Megaprime DNA Labeling System kit (Amersham Biosciences, Piscataway, NJ) and [^{32}P]-dCTP according to the manufacturer's protocol. The blot was hybridized for 3 h in the Rapid-hyb buffer (Amersham Biosciences) at 65℃ with the radio-labeled 5DOS1 probe and then washed with 2X SSC, 0.1% SDS at 65℃, followed by exposure to X-ray film.

3. Real-time RT-PCR

The amount of 5DOS1 expressed in mouse tissues (ovaries from days 1, 5, 14, 21, and 28; testes from days 5, 14, and 21, and 6 wks) was measured by real-time RT-PCR. Total RNA was isolated by Trizol (Invitrogen, Carlsbad, CA), treated with DNaseI to remove genomic DNA, and extracted by acid phenol and precipitated by ethanol.

Reverse transcription was performed using the SuperScript^{TM}III First-Strand Synthesis System (Invitrogen). Quantitative real-time RT-PCR analysis was performed as described previously.[3] All PCRs were conducted in triplicate and normalized with 18S rRNA expression.

4. *In situ* hybridization

For tissue preparation, ovaries and testes were fixed in 4% paraformaldehyde in PBS overnight at 4℃. Paraffin-embedded tissues were cut into 5-μm sections, mounted on positively charged slides (ProbeOn Plus, Fisher Scientific, Pittsburgh, PA), and analyzed by *in situ* hybridization as described.[9] The digoxigenin (DIG)-labeled antisense probe was generated as follows: 5DOS1 was linearized using SpeI. DIG-labeled riboprobes of the target gene were synthesized with an *in vivo* transcription kit (Promega), using T7 RNA polymerase for the antisense orientation and SP6 RNA polymerase for the sense orientation, along with a DIG RNA labeling mix (Roche, Indianapolis, IN).

After incubation at 37℃ for 6 h, the probes were purified on G-50 columns (Amersham Biosciences) and diluted with Harland hybridization buffer (50% formamide, 5X SSC, 1 mg/ml Torula yeast RNA, 100 μg/ml heparin, 1X Denhardt's solution, 0.1% Tween-20, 0.1% CHAPS, 0.5 mM EDTA). DIG-labeled antisense and sense probes were used in parallel in this study.

5. Anti-5DOS1 antibody production

Rabbit anti-sera were prepared against 5DOS1 peptides. Polyclonal antibodies were produced against synthetic peptides containing the internal sequence (amino-acid residues 246-258: NH2-EQDHDWPKHWRAK-COOH) of the 5DOS1 protein. The peptides were coupled to the immunogenic carrier protein keyhole limpet hemocyanin (KLH) via an additional C-terminal cysteine, by the N-γ-maleimidobutyryloxylsuccinimide (GMBS) conjugation method. Immunization with the conjugated peptides and sampling of the anti-sera from rabbits were performed by a commercial facility (Peptron Inc., Daejeon, Korea).

The antibodies were purified using a peptide-linked affinity resin, which was prepared by linking the peptide to the activated affinity resin. Crude serum was applied to the affinity column and anti-5DOS1 IgG was eluted with 100 mM glycine (pH 2.5) and neutralized with 1 M Tris-HCl (pH 8.0).

6. Western blot analysis

Protein lysates of ovarian tissues were prepared by incubating in 200 μl lysis buffer (150 mM NaCl, 50 mM Tris-HCl pH7.4, 1% Triton X-100, 0.1% SDS, and 5 mM EDTA) with freshly added 1 mM phenylmethylsulphonylfluoride (PMSF; Sigma-Aldrich, St. Louis, MO) for 30 min on ice. Western blotting was performed as follows. Protein concentration was measured by Bradford assays (Bio-Rad, Hercules, CA) and equal amounts of total protein (15 μg) were separated by SDS-PAGE in 10% polyacrylamide gels. The blots were incubated overnight

with rabbit polyclonal anti-5DOS1 antibody (1:500; Peptron, Inc.) or mouse monoclonal anti-beta actin antibody (1:500; A5316, Sigma-Aldrich) in blocking buffer, and then incubated with HRP-conjugated goat anti-rabbit IgG (1:2000; sc-2030, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) or goat anti-mouse IgG (1:2000; sc-2030, Santa Cruz Biotechnology, Inc.) in blocking buffer for 1 h at room temperature. After three washes of 15 min each with TBS-Tween, peroxidase activity was visualized using the Western Blotting Luminol Reagent System (Santa Cruz Biotechnology, Inc.).

7. Immunohistochemistry

Ovaries and testes were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned into 5-μm thicknesses, and mounted on positively charged slides (ProbeOn Plus, Fisher Scientific). Immunohistochemistry was performed as follows. Tissue sections were deparaffinized and treated with 3% H_2O_2 in methanol for 20 min to quench endogenous peroxidase activity, preincubated with blocking buffer (DAKO, Carpenteria, CA) for 15 min at room temperature, followed by incubation with primary antibodies against each target. After incubation for 1 h at room temperature, the sections were rinsed in PBS, treated for 20 min at room temperature with a biotinylated secondary antibody, and treated for 20 min at room temperature with a streptavidin-biotin-peroxidase complex. Peroxidase activity was developed with AEC^+ and hematoxylin was used for counterstaining. In each experiment, control sections were incubated with dilution buffer lacking the primary antibody.

8. Statistical analysis

Differences between groups were analyzed for statistical significance by using one-way analysis-of-variance (ANOVA) and a log linear model. Data were presented as mean ± SEM derived from at least three separate, independent experiments.

RESULTS

1. *In silico* cloning and identification of mouse 5DOS1 cDNA

Previously, in order to identify differentially expressed genes that might play important roles in the arrest of primordial follicles and/or initiation of their growth into primary follicles, we compared gene expression profiles from mouse day-1 and day-5-ovaries, using suppression subtractive hybridization techniques.[3] Among the ESTs, clone B357 was predominant and specifically abundant in the day-5-subtracted cDNA library. By searching with mouse EST BLAST programs and using the EST clustering method, a 2,965-bp extended cDNA fragment of clone B357 was obtained. This sequence was named 5DOS1 and submitted to GenBank (accession number **AY751521**). Mouse ESTs **BY709345**, **BQ929605**, **BG800014**, **CA945582**, **BY709844**, **BB612078**, and **BB622953** might be derived from the 5DOS1 gene (Figure 1A). The sequence was used as a reference for searching genomic databases to find homologous genes in different species, especially human and rat, thereby using ontology to judge the existence of the gene.

Precise exon-intron boundaries of the mouse 5DOS1 gene, consisting of 15 exons, were determined based on the GT-AG rule. The gene is located at about nucleotides 122721389-122710295 of mouse chromosome 8 genomic contig **NT_078575.2**, in reverse orientation, and between the CDK10 and Zfp276 genes (data is not shown).

The mouse 5DOS1 cDNA consists of a 262-bp 5'-UTR, a 753-bp 3'-UTR, and a 1949-bp ORF encoding 649 amino acids containing a coiled-coil motif and a vacuolar sorting protein 9 (VPS9) domain (Figure 1B). The VPS9 domain is known to be involved in endosome organization and biogenesis. In addition, a protein domain database search indicates that the ORF has other potential

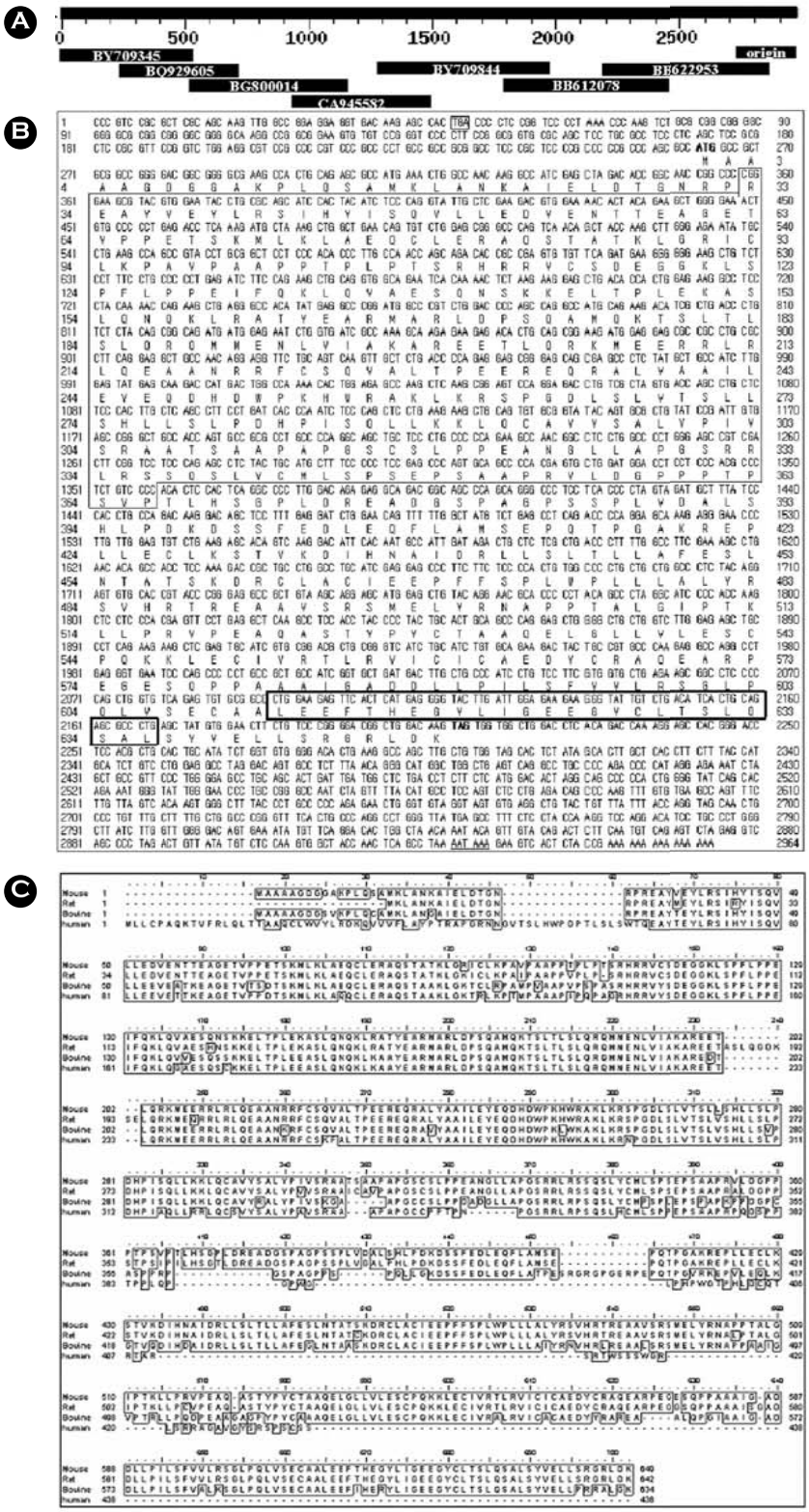

Figure 1. Clustered 5DOS1 sequences, amino acid sequence, and orthology. **(A)** Schematic diagram of EST clustering of mouse 5DOS1 starting from the origin (right end). The complete coding sequence of the mouse 5DOS1 cDNA was determined by assembling BB622953, BB612078, BY709844, CA945582, BG800014, BQ929605, and BY709345. Nucleotides 263-2212 constitute the coding region of 5DOS1. **(B)** The complete coding sequence of the mouse 5DOS1 cDNA and its amino-acid sequence. Nucleotides and amino-acid residues are numbered on the right. The mouse 5DOS1 gene encodes the 649-amino-acid 5DOS1 protein. The conserved amino-acid residues forming the coiled-coil (thin box) and vacuolar sorting protein 9 (bold box) domains are indicated. **(C)** Amino-acid sequence alignment of the mouse, human, and rat 5DOS1 orthologs. Amino-acid residues are numbered on the right. Mouse 5DOS1 shows 43.6% total amino-acid identity with human 5DOS1, and 93.8% total amino-acid identity with rat 5DOS1. Conserved amino-acid residues are shown by thin boxes.

domains such as those found in E-MAP-115 (epithelial microtubule-associated protein) and Apo-VLDL-II (apolipoprotein very-low-density lipoprotein) with low E values. The coding region of the cDNA clone is preceded by a consensus sequence (AGCGCCATGG) and a 5'-UTR of 262 nucleotides, and followed by a 3'-UTR of 753 nucleotides, including a polyadenylation signal sequence (AATAAA) downstream of the TGA stop codon.

Neither a N-terminal signal peptide nor a transmembrane domain was identified within the mouse 5DOS1 protein using the Kyte and Doolittle hydrophobicity analysis (http://arbl.cvmbs.colostate.edu/molkit/hydropathy/index.html). A nuclear localization signal was not identified within the protein using the PSORT program (http://psort.nibb.ac.jp/form.html). Alignment of mouse with rat, bovine, and human orthologous proteins indicated that mouse 5DOS1 (**AAV30827**) showed 92.2% total amino-acid similarity with the rat protein (**XP_226551**), 79.1% with the bovine ortholog (**XP_592406**), and 43.6% with the human ortholog (**NP_004904**). Human protein showed homology only to the N-terminal part of the mouse, bovine, and rat orthologs (Figure 1C).

2. Expression of 5DOS1 transcript and protein

To determine the tissue distribution of 5DOS1 expression, we performed Northern and Western

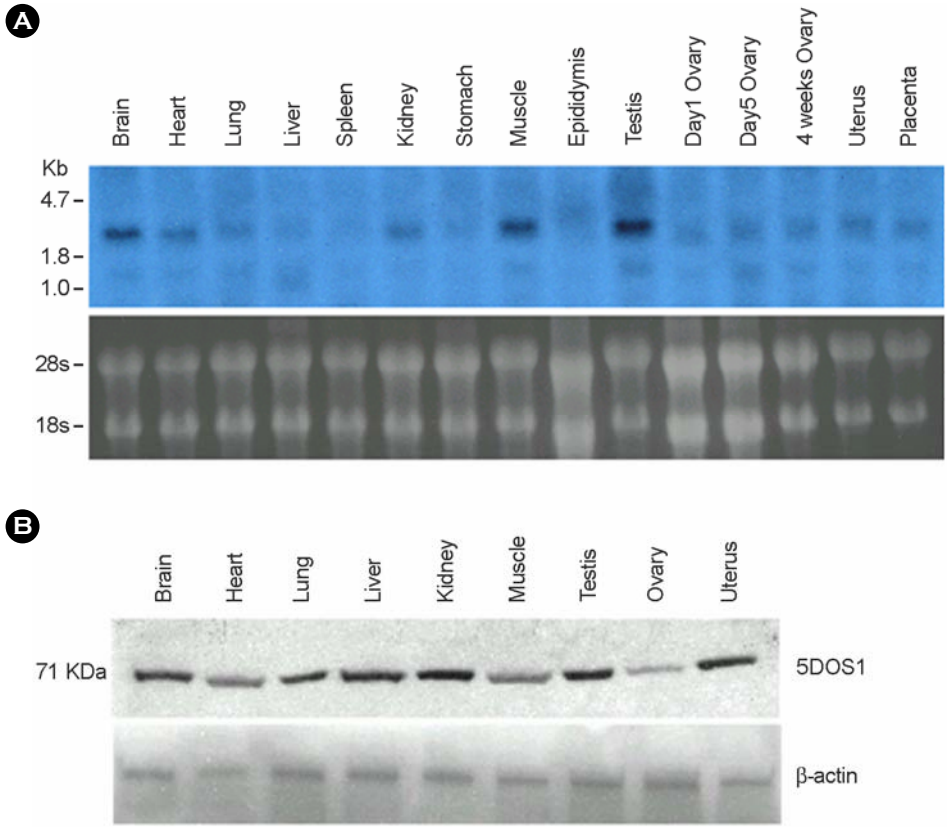

Figure 2. Expression of 5DOS1 mRNA and protein in various mouse tissues. **(A)** Northern blot analysis of 5DOS1 mRNA. Total RNA (20 μg) from each of the mouse tissues was subjected to northern blot analysis with the [^{32}P]-dCTP-labeled cDNA of mouse 5DOS1. The 5DOS1 mRNA was about 3.0 Kb in the various mouse tissues. The 28S and 18S ribosomal RNAs are indicated on the left. **(B)** Western blot analysis of 5DOS1 protein expression in adult tissues. A polypeptide of 71 kDa was detected in the tissues.

blot analyses. Northern blots revealed the expression of an approximately 3.0-kb transcript in all tissues. Interestingly, the 5DOS1 transcript was highly expressed in adult testis, brain, and muscle, as compared to the other tissues (Figure 2A). Western blots detected a protein of about 71 kDa in all tissues (Figure 2B).

We investigated the temporal expression profiles of 5DOS1 during ovarian (Figures 3A-3C) and testicular (Figures 3D-3F) development by real-time RT-PCR. The amplification profiles of 5DOS1 were obtained from ovaries and testes at various developmental stages (Figures 3A, 3D). Profiles of melting curves confirmed the absence of non-specific gene products during real-time RT-PCR (Figures 3B, 3E). Figures 3C and 3F depict the threshold cycle (C_T) values at the various stages after normalization. In the ovary, 5DOS1 was highly expressed at day 5 after birth and decreased thereafter. In contrast, expression of 5DOS1 in the testis showed a gradual increase during testicular development.

3. Localization of 5DOS1 in gonads

The cellular distribution of 5DOS1 mRNA was checked by *in situ* hybridization in adult mouse ovaries (Figures 4A-4D) and testes (Figures 4E-4H). In the ovary, positive signals were detected exclusively in oocytes, from primordial follicle to antral follicle, with maximum expression in the nucleoli of oocytes (Figures 4A-4D).

5DOS1 mRNA was not detected in day-5 or day-14 testes (Figures 4E, 4F), but was detected in secondary spermatocytes or round spermatids in the testes of 21-day-old mice (Figure 4G). Expression of 5DOS1 mRNA also was detected in spermatid

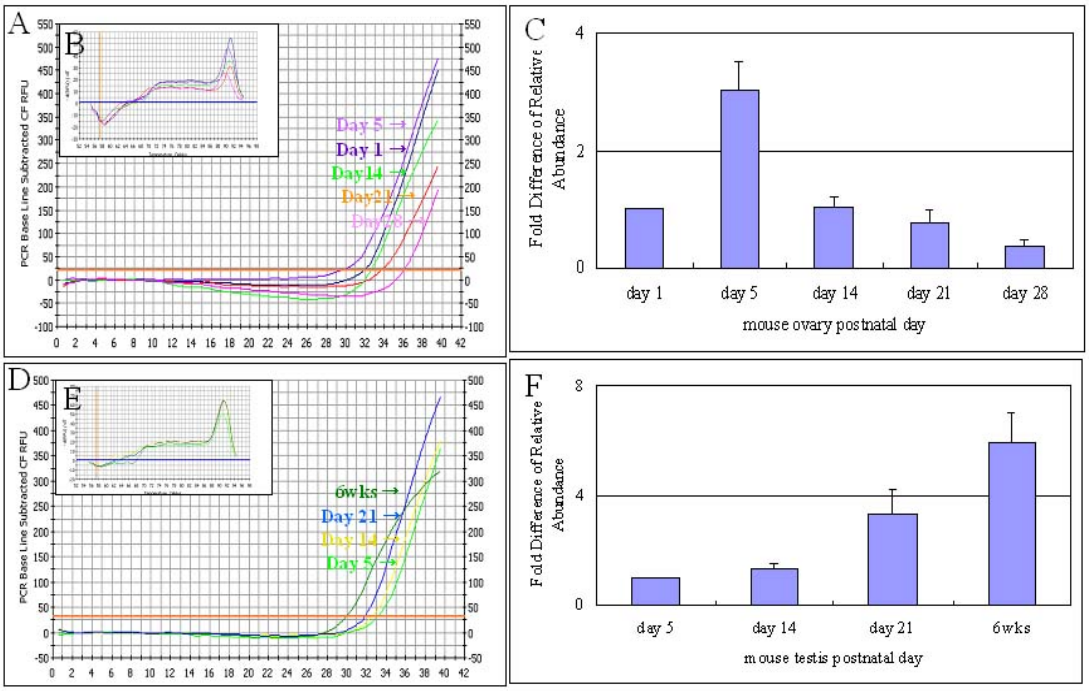

Figure 3. Expression of 5DOS1 in developing gonads. **(A, D)** Amplification profiles of 5DOS1 expression at various stages of ovarian and testicular development. 5DOS1 levels were normalized with 18S rRNA. **(B, E)** Melting curves for amplified 5DOS1. **(C, F)** Expression was calculated from CT values and the fold-differences in relative mRNA abundance were measured against those of day-1 ovaries **(C)** and day-5 testes **(F)**. Experiments were repeated three times and the data expressed as mean ± SEM.

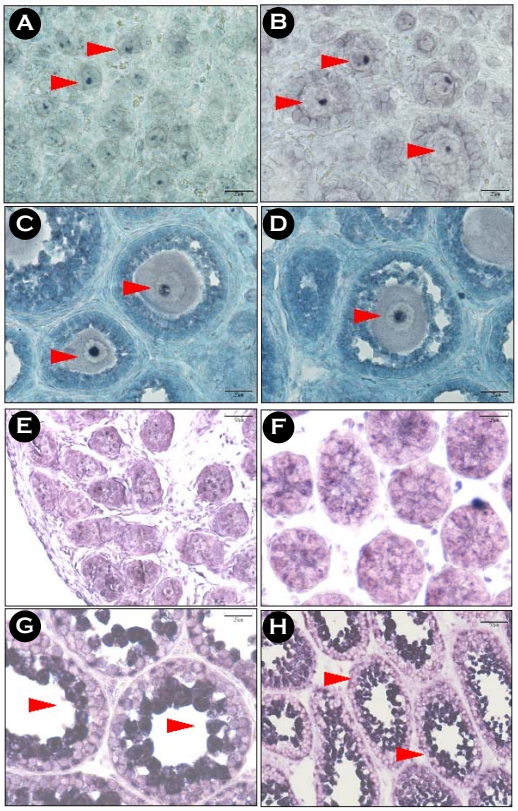

Figure 4. *In situ* hybridization of 5DOS1 mRNA in developing mouse ovaries and testes. **(A-D)** 5DOS1 mRNA expression in mouse postnatal ovaries (A: day 1; B: day 5; C: day 14; D: day 21). **(E-H)** 5DOS1 mRNA expression in mouse postnatal testes (E: day 5; F: day 14; G: day 21; H: 6 weeks). Scale bars represent 25 μm, except E, H where bars represent 50 μm.

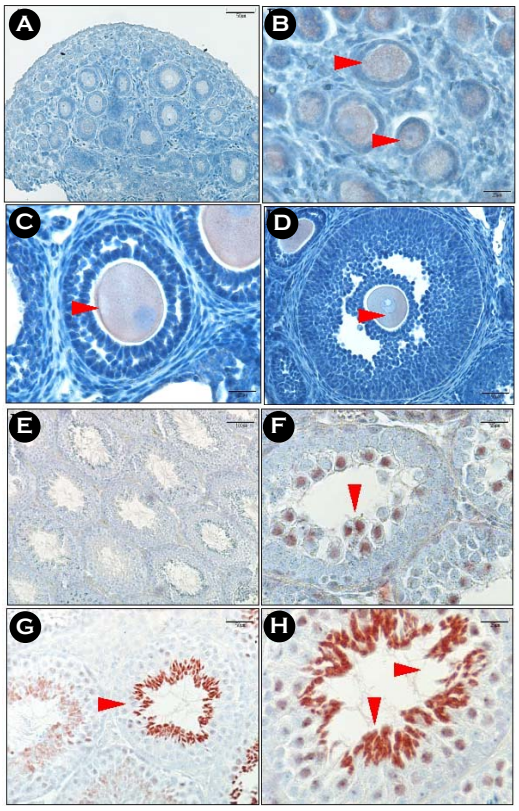

Figure 5. Immunohistochemistry of the 5DOS1 protein in developing mouse ovaries and testes. **(A-D)** 5DOS1 protein expression in mouse postnatal ovaries (A, B: day 5; C: day 14; D: day 21). Most oocytes showed cytoplasmic 5DOS1 expression from primordial stages through antral follicles. Granulosa cells in the developing follicles were weakly positive. **(E-H)** 5DOS1 protein expression in mouse postnatal testes (E, F: day 21; G, H: 6 weeks). Six-week-old testes revealed immunopositive signals in all stages of germ cells, except primary spermatogonia. Expression was predominant in elongated spermatid. No significant signals were detected in the negative controls **(A, E)**. Scale bars represent 25 μm **(B, C, F,** and **H)**, 50 μm **(A, D,** and **G)**, and 100 μm **(E)**.

in the testes of 6-week-old mice, with maximum expression in elongated spermatid and mature spermatozoa (Figure 4H).

The cellular distribution of 5DOS1 protein was checked by immunohistochemistry of the mouse ovary (Figures 5A-5D) and testis (Figures 5E-5H) at various stages. Expression of the 5DOS1 protein was detected in oocytes of primordial through antral follicles and weak expression was detected in granulosa cells (Figures 5B-5D).

Adult testicular sections showed strong signals in well-differentiated seminiferous tubules, mainly in round spermatids through mature spermatozoa. The protein expression exactly matched the *in situ* hybridization results. No immunoreactivity was detected in interstitial testicular spaces including Leydig cells. Interestingly, 5DOS1 protein expression was detected in male germ cells at all stages except spermatogonia in the adult mouse testis (Figures 5F-5H).

DISCUSSION

In the present study, we have identified the mouse 5DOS1 gene by the EST clustering method in the NCBI database. The nucleotide sequence of the mouse 5DOS1 cDNA was determined *in silico* by assembling mouse ESTs. We have demonstrated the expression of this novel 5DOS1 gene in mouse gonads. The protein product lacks a signal peptide and is predicted to be a soluble protein without membrane domains (http://sosui.proteome.bio.tuat.ac.jp/sosui_submit.html).

EST clustering is a method that combines a collection of EST sequences originating from the same transcript. Unlike whole EST assembly that includes both 5' and 3' ends using several publicly available EST databases, our approach relied on emphasizing the true 3' end containing VPS9 and the 3'-UTR in the correct orientation. In this study, we used the 3'-EST as the 3'-UTR source with a poly-A signal to find transient genes harboring a VPS9 domain. Thus, we used a conservative approach for collecting and clustering true 3'-ends containing a VPS9 domain that is both evolutionarily conserved and functionally associated with biological processes.

The coiled-coil motif and the VPS9 domain were identified within the mouse 5DOS1 protein by domain database searches. The VPS9 domain with guanyl-nucleotide exchange factor activity is known as a tyrosine-based and/or dileucine-based sorting signal within mammalian secretary pathways, in which selected membrane proteins are sorted from the *trans*-Golgi network (TGN) through the endosomal pathway to the major hydrolytic compartment, mammalian lysosomes.[10] The tyrosine-based signals are generally present as YXXZ, where X is any amino acid and Z represents a bulky hydrophobic amino acid. Dileucine-based signals consist of an invariant leucine in the first position and a hydrophobic residue, L, I, V, or M, in the more tolerant second position.[11]

In testes, 5DOS1 was abundantly expressed in germ cells, predominantly in the post-meiotic germ cells of 21-day-old mice testes. The abundant expression of 5DOS1 at such a restricted stage suggests a regulatory role for 5DOS1 in germ cells during spermatogenesis, perhaps closely related to meiosis. The second meiotic division occurs in rapid succession to produce spermatids. These haploid spermatids are then converted into spermatozoa by spermatogenesis.

In ovaries, 5DOS1 is detected in most oocytes, from the primordial to antral follicles. In contrast to sperm production, oocytes have two points of arrest during meiosis.[12] Oocytes at the primordial follicular stage are already arrested at prophase of the first meiosis and this arrest continues until the surge of luteinizing hormone for ovulation. Therefore, 5DOS1 expression in oocytes through all the follicular stages before ovulation may be related to maintaining the meiotic arrest of the follicular oocytes.

In oocytes, maximum expression of 5DOS1 was observed in the nucleoli of oocytes (Figures 4A-4D). The nucleolus is known to play a key role in ribosome biogenesis as well as various cellular processes such as RNA-processing steps. Primary RNA transcripts can undergo site-specific adenosine to inosine (A-to-I) base conversions. Because the newly introduced inosine residue is decoded as a guanosine by the translation apparatus, this post-transcriptional RNA processing event, RNA editing, plays a significant role in generating diversity of gene expression. RNA editing is catalyzed by adenosine deaminases (ADARs) that are dynamically associated with the nucleolus.[13] It is suggested that nucleolar-associated functions of the editing enzymes might regulate RNA editing, although a functional role of the nucleolus in the editing process has not been precisely characterized yet.[14]

The results of the present study strongly suggest that male and female germ cells express 5DOS1 at the mRNA and protein levels and developmentally regulated during folliculogenesis in the ovary and spermatogenesis in the testis. We suggest that 5DOS1 is a functional protein that may play a role in early folliculogenesis as well as late spermatogenesis. Although the exact role of 5DOS1 in the mouse ovary and testis is unknown, the restricted expression of 5DOS1 in germ cells suggests that it has a role in germ-cell growth and differentiation during normal gonadal development. Further investigation of the function and molecular regulatory mechanisms of 5DOS1 gene expression may provide information for addressing various procedures involving reproductive organs, such as contraception and infertility.

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= Abstract =

Objective: Identification of the regulatory mechanism for arrest and initiation of primordial follicular growth is crucial for female fertility. Previously, we found 15 expressed sequence tags (ESTs) that were specifically abundant in the day-5-subtracted cDNA library and that the B357 clone was novel. The present study was conducted to obtain the whole sequence of the novel gene including B357 and to characterize its mRNA and protein expression in mouse ovary and testis.

Methods: The extended sequence of the 2,965-bp cDNA fragment for the clone B357 was named 5-day-ovary-specific gene-1 (5DOS1) and submitted to GenBank (accession number AY751521). Expression of 5DOS1 was characterized in both female and male gonads at various developmental stages by Northern blotting, real-time RT-PCR, *in situ* hybridization, Western blotting, and immunohistochemistry.

Results: The 5DOS1 transcript was highly expressed in the adult testis, brain, and muscle as compared to the other tissues. In the ovary, the 5DOS1 transcript was detected in all oocytes from primordial to antral follicles, and highly expressed at day 5 after birth and decreased thereafter. In contrast, expression of 5DOS1 showed a gradual increase during testicular development and its expression was limited to various stages of male germ cells except spermatogonia.

Conclusions: This is the first report on the expression and characterization of the 5DOS1 gene in the mouse gonads. Further functional analysis of the 5DOS1 protein will be required to predict its role in gametogenesis.

Key Words: EST (expressed sequence tag), EST clustering, 5DOS1 (AY751521), Follicular development, Testicular development, Gametogenesis