Expression of Prnp mRNA (Prion Protein Gene) in Mouse Spermatogenic Cells

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Abstract. The Prnp (prion protein) gene, which encodes a soluble protein anchored to the cell surface by glycosylphosphatidylinositol (GPI), might be involved in cell-to-cell interaction. The expression of Prnp is strongly observed not only in the brain, but also in non-neuronal tissues. In order to examine the Prnp expression sites in mouse testes, we carried out Northern blot and in situ hybridization analyses. By Northern blot analysis, two kinds of Prnp transcripts (major band of 2.2 kb, and minor band of 1.1 kb) were detected in testes. The 2.2-kb transcript was observed in testes throughout the postnatal development, whereas the 1.1-kb transcript was observed in testes from 2 to 70 weeks old. In situ hybridization analysis showed that the positive signals for Prnp mRNAs were predominantly observed in spermatogenic cells, but not in somatic cells such as Sertoli cells, Leydig cells and peritubular myoid cells. The signals were observed moderately in spermatogonia, and strongly in spermatocytes and round spermatids, but not in elongate spermatids and spermatozoa. These results suggest that Prnp may be involved in germ cell differentiation during mammalian spermatogenesis.

Key words: Prnp, In situ hybridization, Northern blot, Spermatogenesis

Transmissible spongiform encephalopathy (TSEs) or prion diseases which show a transmissible neurodegenerative disease common to medical (new variant Creutzfeldt-Jakob disease etc.) and veterinary medical fields (scrapie of sheep, bovine spongiform encephalopathy, etc.) manifest as sporadic, infectious, or genetic disorders characterized by the accumulation of an abnormal isoform (PrPsc) of cellular prion protein (PrPc). PrPc expression has been investigated in most of organs, and differences in amount of expression in each organ have been reported in the hamster [1] and sheep [6]. Concerning the expression pattern and the functional analysis of PrPc, much information is available for the central nervous system. Especially, detailed analysis has been done on the hamster brain [23], and on hamster brain...
developmental stage [13, 14]. Although many studies on digestive and immune systems have also been carried out, there is little data on reproductive organs such as the testis.

PrPc is classified as a GPI-anchored glycoprotein [26]. Since the expression of PrPc is frequently seen on the cell membrane surface, it has been speculated that PrPc may participate in cell signaling or adhesion [17, 24]. PrPc also participates in regulation of copper/zinc superoxide dismutase (Cu/Zn SOD: SODI) as a copper binding protein, suggesting that PrPc may be related to copper metabolism and anti-oxidative responses [3, 4]. Since the oxidative stress gives severe damage to testes and the SodI mRNA expression is present in seminiferous tubules [7], PrPc may play an important role in spermatogenesis. Moreover, it has been found by using the Prnp+/− mouse that the prion-like protein gene (Doppel gene; Dpl) exists in a downstream region of the prion gene and is specifically expressed in wild-type mouse testes [16]. It is anticipated that the relationship between the two genes in the testes will be clarified in the future.

In order to examine the role of PrPc during spermatogenesis, we examined the Prnp expression in mouse testes by using Northern blot and in situ hybridization analyses. The present study has revealed that Prnp transcripts are abundantly expressed in spermatogenic cells, but not in somatic cells, suggesting the possible role of PrPc in germ cell differentiation during mammalian spermatogenesis.

Materials and Methods

Experimental animals

C57BL/6J male mice were used in the present study. They were maintained in our closed colony under a standardized laboratory condition. At 1, 2, 4, 8 and 70 weeks of age, male mice were sacrificed under pentobarbital anesthesia, and the testes were isolated and used for the following RNA analysis. We used the 8-week-old mouse as a representative of a mature mouse, and the 70-week-old as a representative of senescent one. For in situ hybridization, 8-week-old male mice were perfused with Bouin’s fixative, and the testes were excised and immersed in the same fixative for 12–24 h. Subsequently, they were processed for embedding in paraffin.

Generation of probes

The 495-bp DNA fragments, corresponding to the coding region of a Prnp cDNA (1–165 amino acids) were isolated from testis cDNAs by polymerase chain reaction, using the specific primers 5’-ATGGCGAACCTTGGCTACTG-3’ (forward) and 5’-ACTGGCCTGTAGTACACCTG-3’ (reverse); then they were subcloned into pCR® plasmid (Invitrogen Corp., Carlsbad, CA USA). The clone was linearised with the appropriate restriction enzyme, and antisense RNA probes were generated by in vitro transcription using α-32P UTP or DIG-labeled UTP with Sp6 or T7 RNA polymerase (Roche Diagnostics Corp., Mannheim, Germany).

Northern blot analysis

Total RNAs were extracted from testes and brains of 5 adult mice using a RNA extraction kit (Isogen; NPG, Toyama, Japan). Each 10 µg of total RNA sample was fractioned on 1% agarose formalin gel, and blotted onto a nylon membrane (Hybond N; Amersham Biosciences Corp., Piscataway, NJ USA). Blots were hybridized with the appropriate 32P-labeled RNA probe at 67°C as described previously [8]. Filters were finally washed with 0.1 × SSC-0.1% SDS at 75°C for 1 h and autoradiographed.

In situ hybridization

In situ hybridization was performed as described by Nam et al. [18] and Kanai et al. [9]. In short, deparaffinized sections were pretreated with 0.3% Triton X-100 in 10 mM phosphate buffer saline and 20 µg/ml proteinase K in Tris-HCl buffer (pH 7.5) containing CaCl2, and then hybridized with DIG-labeled RNA probes in solution containing 50% formamide, 10% dextran sulfate, 5 × SSC, 1 × Denharts’ solution, 1% SDS, 100 µg/ml heparin, 10 mM DTT, and 1 mg/ml denatured tRNA and ssDNA at 55°C for 12–16 h. After treatment with RNase (20 µg/ml; Sigma-Aldrich, St. Louis, MO, USA) at 37°C for 1 h, the specimens were finally washed twice with 0.1 × SSC at 65°C for 1 h. The signals were detected by an immunohistological method using alkaline phosphatase conjugated anti-DIG antibody and nitro blue tetrazolium as the chromogen (Roche Diagnostics).
Results

In order to examine the Prnp expression in mouse testes, we first carried out Northern blot analysis using testes isolated from mice of various ages. We detected two kinds of the Prnp transcripts (major band of 2.2 kb and 1.1 kb) in the testes isolated from 2- to 70-week-old mice. The 2.2-kb major band is observed throughout the postnatal development and aging. In contrast, the 1.1 kb band is observed in the testes isolated from 2- to 70-week-old mice. Lower panels show the 28 S and 18 S rRNA bands stained with ethidium bromide (EtBr).

Discussion

In Northern blot analysis, a 1.1 kb band was observed in testes. This band was observed from 2-week-old and older mice, and was also accepted at 70-week-old. Previous studies showed that two Prnp mRNAs were found in peripheral tissues of rodents as a result of alternative polyadenylation [5, 11, 21]. Although there was no detailed analysis about Prnp transcripts in testes, the 1.1 kb band we detected in mouse testes may be a mature transcript produced by the same mechanism. The probe which includes PrP ORF (open reading frame) hybridized with the prominent 2.2 kb Prnp mRNA expressed in wild-type adult mice testes, and also in Prnd–/– (prion-like protein gene, doppel) mice [2]. Prnd is located 16 kb downstream from Prnp and generates major transcripts of 1.7 kb and 2.7 kb as well as some unusual chimeric transcripts generated by intergenic splicing with Prnp [16]. To detect the chimeric transcripts in Northern blot analysis, we should use the probe consisting of Prnp exon1 and 2 cDNA, not Prnp exon3 including PrP ORF [10]. Because we used a probe which include PrP ORF in this study, the possibility that the 1.1 kb band is Prnd or chimeric ones may be low.

In situ hybridization analysis showed that a strong signal was detected from spermatocytes to round spermatids in adult mouse testes. Little signal was observed in Sertoli cells, Leydig cells, and myoid cells. Although previous studies showed that reactivities of Prnp mRNA were detected in rat myoid cells [27] or negative in mouse testes [12], our finding was different from these studies. The strongest expression was observed in pachytene spermatocytes and round spermatids (Fig. 2D-G). No signals were detected in elongate spermatids and spermatooza. Such strong expression in spermatocytes and round spermatids may be partially due to the additional expression of the 1.1 kb transcript obtained by Northern blot analysis (Fig. 1). In contrast to high expression in spermatogonic cells, Prnp-positive signals could not be detected in interstitial cells such as Leydig cells (Fig. 2C), Sertoli cells and myoid cells.

Reactive oxygen species are highly toxic agents that have an important role in male infertility. Copper/zinc superoxide dismutase (SODI) is one
of the reactive oxygen scavengers. Its mRNA is determined in testes as two transcripts, and is localized in seminiferous tubules of rats [7]. Brown et al. [3, 4] and Wong et al. [28] reported that Prnp\textsuperscript{−/−} mice have depleted levels of copper, and have shown a reduction in the activity of SODI, indicating that PrP may play a role in copper homeostasis, and that PrP itself can act as a superoxide dismutase in the brain. When the testicular copper concentration is higher than normal, degenerative changes, such as vacuolation and pyknosis, can be found in pachytene spermatocytes and early spermatids [29]. A copper metabolism system also exists in the testes. Therefore, PrP is one of the factors maintaining the system with SODI or other copper binding proteins within seminiferous tubules.

It has been reported that the Prnp\textsuperscript{−/−} mutant mice are fertile both in male and female mice [22]. Moreover, our unpublished histological observation also showed no appreciable defect in spermatogenesis in Prnp\textsuperscript{−/−} mice, suggesting that Prnp is not essential for spermatogenesis \textit{in vivo}. While Prnd is strongly expressed in adult testes [16], these findings suggest that no defect in Prnp\textsuperscript{−/−} mice is due to the complementation of Prnd in testicular function. In fact, Doppel is expressed in both Sertoli cells and spermatozoa in human testes [20], and Prnd deficiency resulted in male sterility [2]. A recent study showed that there was no evidence that Doppel compensates for the loss of PrP function in Prnp\textsuperscript{−/−}/Prnd\textsuperscript{−/−} mice (Prion/Doppel double knockout mice), but that it has an important anti-oxidant function necessary for sperm integrity.
PRNP mRNA IN MOUSE TESTIS

and male fertility [19].

PrPc is classified as a GPI-anchored glycoprotein, which exists on the cell membrane surface via GPI [26]. It has been shown that the structure of PrPc in mature spermatozoa differs from immature ones [25]. PrPc was not separated from epididymal and ejaculated spermatozoa processed by PIPLC (phosphatidylinositol phospholipase C). Moreover, PrPc in ejaculated spermatozoa had a truncated C terminus. A sperm antigen is classified broadly into three sorts, an acrosome membrane antigen, a sperm membrane (SM) antigen, and a sperm coating (SC) antigen. We surmise that PrPc of spermatozoa is in the form of a SM or SC antigen.

In conclusion, the present study revealed that Prnp transcripts are apparently expressed in spermatogenic cells, not in testicular somatic cells, which indicates that Prnp may have a possible role in germ cell differentiation during mammalian spermatogenesis.

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References


