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SHORT COMMUNICATION

Localization and expression of selenoprotein S in the testis of *Psammomys obesus*

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Summary Selenium is an essential trace element and selenoprotein S is a member of the selenoprotein family that has the non-standard amino acid selenocysteine incorporated into the polypeptide. Dietary selenium has been shown to play an important protective role in a number of diseases including cancer, immune function and the male reproductive system. In this study, we have observed high levels of selenoprotein S gene expression in the testis from *Psammomys obesus*. Real-time PCR and immunofluorescence demonstrate that selenoprotein S expression is low in testes from 4-week-old animals but increases significantly by 8 weeks of age and remains high until 17 weeks of age. Selenoprotein S protein is detected in primary spermatocytes, Leydig and Sertoli cells of 8, 12 and 17-week-old animals. These results suggest that selenoprotein S may play a role in spermatogenesis.

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Introduction

Selenium is a non-metallic trace element that is now recognised as having protective effects in a number of physiological systems and pathological conditions including cancer, cardiovascular disease, immune function, aging, mammalian development and fertility (Kryukov et al. 2003). Dietary selenium is incorporated co-translationally into selenoprotein as the amino acid selenocysteine and the selenium content of endocrine organs (thyroid, adrenals, pituitary, testis and ovary) is higher than in other organs (Kohrle et al. 2005). Twenty-five selenoproteins have been identified in humans (Kryukov et al. 2003) and they are thought to be responsible for most of the biological effects of selenium. Several selenoproteins have antioxidant properties; others are associated with either production of active thyroid hormone or the synthesis, transport or storage of selenium whilst others have no known functions (Kryukov et al. 2003).

In our laboratory, selenoprotein S (SEPS1) was initially identified as being differentially regulated by fasting in liver of the obese/diabetic animal model *Psammomys obesus* (Walder et al. 2002). Hepatic SEPS1 gene expression was markedly increased after fasting in diabetic but not in non-diabetic animals. We have further shown using in vitro studies that glucose deprivation and endoplasmic reticulum stress inducers such as tunicamycin and thapsigargin cause an increase in the levels of SEPS1 mRNA and protein. These results would indicate that SEPS1 is a member of the

glucose-regulated protein family, and therefore, a member of the heat shock protein superfamily (Gao et al. 2004). More recently, genetic variations in SEPS1 have demonstrated significant associations with cytokine levels of interleukin-6, interleukin-1β and tumor necrosis factor-α suggesting that SEPS1 has a role in mediating inflammation (Curran et al. 2005).

Real-time PCR has demonstrated ubiquitous expression of SEPS1 in a range of tissues from *P. obesus* including the testis (Walder et al. 2002). In male germ cells and spermatozoa, there is a requirement for selenium for proper development and maintenance of spermatogenesis. The major testicular selenoprotein is phospholipid hydroperoxide glutathione peroxidase (PHGPx). PHGPx has been identified as a structural component of sperm but is also involved in antioxidant defence. It produces oxidative cross-links of structural proteins in spermatids as well as itself becoming oxidatively cross-linked in mature sperm (Ursini et al. 1999). Recently, another selenoprotein thioredoxin/glutathione reductase (TGR) has been implicated to play a role in isomerization of disulfide bonds of proteins that form structural components of sperm (Su et al. 2005).

Some members of the heat shock protein superfamily have been shown to play a crucial role in spermatogenesis and to protect cells from environmental damage. Heat shock protein 70-2 is synthesized during the meiotic phase of spermatogenesis, is abundant in pachytene spermatocytes and has been shown to act as a chaperone for cdc-2 to enable cell cycle progression (Eddy 1998). Heat shock protein 70-2 knockout in mice leads to spermatogenic arrest and male infertility but has no phenotype in the female. Given that we have shown previously that SEPS1 has antioxidant properties, is expressed in the testis and knowing that oxidative stress plays an important role in male infertility, it was of interest to examine SEPS1 testicular expression and localisation.

Materials and methods

To examine the expression of SEPS1 in testis from *P. obesus*, we used both real-time PCR and immunohistochemical analysis. The use of all animals in this study was approved by Deakin University Animal Ethics Committee and a colony of *P. obesus* was maintained as previously described (Walder et al. 2002). In this study, only lean, non-diabetic animals were used.

For real-time PCR quantification of SEPS1 mRNA, animals of 4, 8, 12 and 17 weeks (n = 5–6 per age group) were sacrificed and testicular tissue immediately frozen in liquid nitrogen. Total RNA was extracted using Trizol (Invitrogen, Rockville, MD) and RNA concentration was determined using an Agilent 2100 Bioanalyser (Agilent Technologies, Palo Alto, CA). cDNA was synthesised using Superscript II (Invitrogen). SEPS1 transcript was quantified using real-time PCR technology on an ABI Prism 7700 sequence detector using the forward primer 5′-GATGCGTTCAATGATGTCTTCCT-3′ and the reverse primer 5′-GAAGCAAACCCCATCAACTGT-3′. PCR conditions were 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. SEPS1 mRNA content was normalized against cyclophilin transcript in the same samples quantified under the same PCR conditions.

For immunohistochemical analysis, a selection of animals of various ages (4, 8, 12 and 17 weeks; n = 3 per age group) were sacrificed and testes dissected and placed in Zamboni's fixative for 15 h at 4°C. Tissues were processed into paraffin before 14 μm thick sections were cut and mounted onto silanised slides (Sigma Chemical Company, St Louis, MO). After dewaxing and rehydration, the sections were blocked with 2% buffered BSA/0.3% Triton X–100 for 30 min and then treated with affinity purified SEPS1 antibody (1:500) overnight at 4°C. Characterisation of the polyclonal SEPS1 antibody has been described previously (Gao et al. 2003). Sections were subsequently washed 3 × 5 min with TBST and then incubated for 1 h in the dark with Alexa Red 594 goat anti-rabbit (Molecular Probes, Eugene, OR). Following incubation with the secondary antibody, sections were washed 3 × 5 min with TBST before the nuclear counterstain DAPI dilactate (Molecular Probes, Eugene, OR) was incubated for 5 min. Sections were washed 2 × 5 min with TBST and finally with TBS (1 × 5 min). All sections were mounted with Dako-Cytomation fluorescent mounting media (Dako Corporation, Carpinteria, CA). Negative control sections incubated with buffered BSA, in place of the primary antibody, produced negligible background staining.

Results

Real-time PCR analysis demonstrated that SEPS1 gene expression was relatively low in 4-week-old prepubertal *P. obesus* animals but increased approximately 4-fold by 8 weeks of age and remained at this level until at least 17 weeks of age (Fig. 1). SEPS1 gene expression was significantly greater in testis from 8, 12 and 17-week-old animals compared to expression levels from 4-week-old animals ($P < 0.05$).

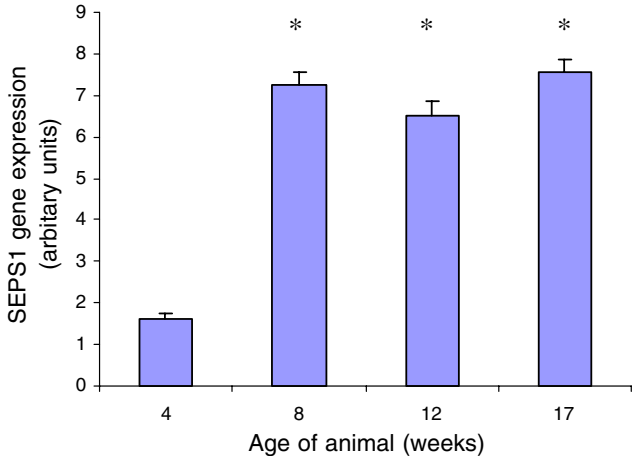

Fig. 1 SEPS1 gene expression in testis from Psammomys obesus of various ages (n = 5–6 animals per group).*indicates significantly different from 4-week-old animals ($P < 0.05$)

Using immunofluorescence, SEPS1 protein was detected at varying levels in the testes of *P. obesus* at all ages examined (Fig. 2). The level of SEPS1 protein expression in immunohistochemical sections appeared to correlate with the SEPS1 gene expression data, levels were lowest at 4-weeks of age compared to the older animals (8, 12 and 17 weeks). In the 4-week-old animals, SEPS1 staining was minimal (Fig. 2A) whereas in the 8-week-old animals, SEPS1 staining was observed in the nuclei of primary spermatocytes with low levels of staining present in the Sertoli cells (Fig. 2B). By 12 weeks of age, a full complement of cell types was seen in the seminiferous tubules and SEPS1 immunoreactivity was predominant in nuclei of pachytene spermatocytes with some staining also present in the Sertoli and Leydig cells (Fig. 2C). A similar staining pattern was seen in the sections of testis from 17-week-old animals, positive SEPS1 immunostaining in the pachytene spermatocytes and Leydig cells as well as some minor SEPS1 expression in the Sertoli cells (Fig. 2D).

Discussion

In this study, we have shown that SEPS1 gene expression in the testis of *P. obesus* is relatively low in immature 4-week-old animals but increases just prior to puberty and stays at this level until at least 17 weeks of age. In a similar pattern, PHGPx mRNA is first detected at 3 weeks of age in mouse testis, increases greatly by 8 weeks of age and remains high until at least 80 weeks of age (Nam et al. 1998). Similarly, TGR was not detected by immunoblot in testes from 20-day-old mice but was highly expressed in 7-month-old mice (Su et al. 2005). It is unknown at this stage whether the increase in SEPS1 gene expression at 8 weeks of age is due to a direct effect of steroid hormones or simply the age-dependent development of the primary spermatocytes. A previous study has shown that seminiferous tubules from *P. obesus* are similar to other rodent species with a full complement of spermatogonia, spermatocytes, round spermatids and elongate spermatids well-established by 2.5 months of age (Spando

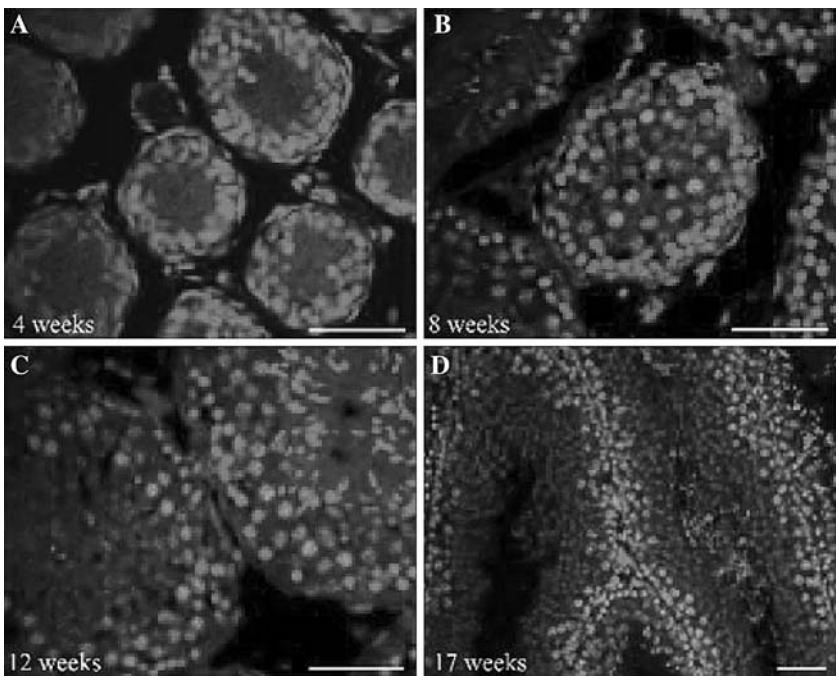

Fig. 2 Immunolocalization of SEPS1 in the testis of Psammomys obesus of different ages. SEPS1 protein is abundant in the pachytene spermatocytes of 8, 12 and 17-week-old animals. Leydig cells in testis sections from 8, 12 and 17-week-old animals demonstrate positive staining for SEPS1 whilst there are low levels of SEPS1 staining in Sertoli cells. Testis sections from 4-week-old animals show low levels of SEPS1 staining. Positive SEPS1 immunostaining is detected by Alexa Red 594 whilst cell nuclei are visualised with the blue DAPI dilactate nuclear counterstain. Scale bars = 50 μm

et al. 1999). Our results support this observation with our 12-week-old animals demonstrating the full range of spermatogenic cell types.

Few studies to date have addressed the testicular immunolocalisation of selenoproteins. In this study, we have shown the testicular expression of SEPS1 protein in animals ranging in age from 4 weeks to 17 weeks (Fig. 2). In young 4-week-old animals, SEPS1 immunostaining was at low levels whereas by 8 weeks of age, SEPS1 staining was clearly observed in primary spermatocyes with some minor immunostaining in Sertoli cells. Pubertal and older animals demonstrated SEPS1 staining predominantly in pachytene spermatocytes and Leydig cells whilst Sertoli cells displayed lower levels of SEPS1. Other studies examining testicular expression of selenoproteins have shown the presence of PHGPx in the Leydig cells of 17 day old mice whilst in 21-day old mice, staining was present in Leydig cells and the post-meiotic round spermatids (Nayernia et al. 2004). TGR expression was observed in the cytoplasm and nuclei of both spermatocytes and spermatids (Su et al. 2005). PHGPx has been shown to be hormonally regulated, to have an age-dependent enzymatic activity and to play a role in spermatogenesis and spermiogenesis (Tramer et al. 2002).

Reactive oxygen species (ROS) are known to be released during spermatogenesis and Fisher and Aitken (1997) demonstrated that a range of cells such as pachytene spermatocytes, round and elongating spermatids and mature spermatozoa from the epididymis were capable of spontaneously generating ROS. It is thought that the generation of ROS by mature spermatozoa is important in the redox regulation of capacitation however there is a fine balance between the levels required for physiological function and those that cause oxidative stress and cellular damage. Excessive ROS are capable of causing damage to membrane phospholipids and DNA and thus contribute to abnormalities in semen parameters. Interestingly, inflammatory cytokines are present in the testis and are tightly regulated to prevent any inflammatory or immune responses within the testis (Lysiak 2004). TNFα has been described in the round spermatids and pachytene spermatocytes as well as testicular interstitial macrophages. Similarly, IL-1 is produced in the testis under normal conditions and both TNFα and IL-1 are thought to play a role in testicular homeostasis (Lysiak 2004). Given that SEPS1 has been described to play a role in protecting the cell against oxidative stress as well as mediating inflammation, it is possible that SEPS1 may play an important role in maintaining the balance between physiological and pathological ROS or cytokine levels within the testis and this warrants further investigation.

In conclusion, we have shown that SEPS1 mRNA expression in the testis is low in pre-pubertal animals but increases more than 4-fold during and after puberty. In addition, we have demonstrated using immunofluorescence that SEPS1 is expressed in specific cell types during spermatogenesis and it is possible that SEPS1 may play a protective role during conditions of oxidative stress within the testis or a specific role in spermatogenesis.

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