Journal of Equine Veterinary Science 45 (2016) 1–7

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Protein Gene Product 9.5 Expression in Stallion Testes

Heejun Jung [a,*], Minjung Yoon [a,b]

[a] Department of Animal Science and Biotechnology, Kyungpook National University, Sangju, Republic of Korea
[b] Department of Horse, Companion and Wild Animal Science, Kyungpook National University, Sangju, Republic of Korea

A R T I C L E I N F O

Article history:
Received 25 November 2015
Received in revised form 5 February 2016
Accepted 8 February 2016
Available online 21 February 2016

Keywords:
PGP9.5
Germ cell
Leydig cell
Spermatogonia
Stallion

A B S T R A C T

The expression of protein gene product 9.5 (PGP9.5) in testes has been reported in domestic species, including pigs, sheep, and goats. The main objectives of this study were to: (1) investigate the expression pattern of PGP9.5 in stallion testes and (2) identify stage-dependent immunolabeling of PGP9.5 in stallion testes. Samples were categorized based on the age of the horses as follows: prepubertal (<1 year), pubertal (1–1.5 years), postpubertal (2–3 years), and adult (4–8 years). Immunohistochemistry was performed to investigate the expression pattern of PGP9.5 at different reproductive stages. For double staining, the goat anti-human DAZL and GATA4 antibody were used as markers for germ cells and Sertoli cells, respectively. The Western blot procedure was used to determine the cross-reactivity of the PGP9.5 antibody in horse testis tissues. During the prepubertal and pubertal stages, germ cells, which were located in the center of the seminiferous tubules, were positive for PGP9.5. During the postpubertal and adult stages, expression of PGP9.5 was found in the cytoplasm of germ cells adjacent to the basement membrane. PGP9.5-positive cells were immunolabeled with DAZL but not immunolabeled in Sertoli cells at all reproductive stages. Interestingly, Leydig cells were stained with PGP9.5 at all reproductive stages. In conclusion, PGP9.5 antibody can be used as a tool to identify and isolate PGP9.5-positive germ cells only after Leydig cells were separated from the seminiferous tubules.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Spermatogenesis is a key process for maintenance of male fertility. Spermatogonial stem cells (SSCs) form the basis of spermatogenesis [1]. SSCs undergo self-renewal to sustain the stem cell population and differentiation to produce spermatozoa [1]. In stallions, limited studies have investigated the biological process of spermatogenesis because of the absence of stage-specific markers of germ cells in cell culture. Recently, Costa et al [2], demonstrated that GFRa1, PLZF, and CSF1R can serve as molecular markers for SSCs in stallions. We reported that UTF1 is a putative marker for undifferentiated SSCs [3], and DAZL is a marker for differentiated spermatogonia and primary spermatocytes in stallions [4]. In addition, c-kit was suggested to be a putative marker for differentiating spermatogonia [5]. Discovery of additional molecular markers for germ cells at specific stages will be beneficial in the study of the biology of SSCs in stallions.

Protein gene product (PGP) 9.5 is encoded by a gene of ubiquitin–carboxyl hydrolase [6]. The expression of PGP9.5 was observed in neuroendocrine cells [7–10] and neuroendocrine tumors [11]. However, the expression of PGP9.5 in testes has been reported in other species, including pigs [12], sheep [13], and goats [14]. The results of these studies suggested that PGP9.5 is expressed in spermatogonia, and its expression is conserved across species. However, the expression of PGP9.5 in horse testes has not been examined.

The main objectives of this study were to: (1) monitor the expression pattern of PGP9.5 and (2) evaluate the stage-dependent expression of PGP9.5 in stallion testicular cells.

* Corresponding author at: Minjung Yoon, Department of Horse, Companion and Wild Animal Science, Kyungpook National University, Sangju 742-711, Republic of Korea.
E-mail address: mjyoonemail@gmail.com (H. Jung).

0737-0806/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2016.02.227

We performed immunohistochemistry and Western blot procedures. The results of this study will be applicable to identify specific spermatogonial subpopulations and monitorization of normal spermatogenesis in stallions.

2. Materials and Methods

2.1. Testicular Samples

Testes were obtained during routine field castrations of several breeds of stallions, including Thoroughbreds and Quarter Horses. The reproductive stages of the horses were classified based on the age of horses as follows: prepubertal (<1 year, n = 3), pubertal (1–1.5 years, n = 3), postpubertal (2–3 years, n = 3), and adult (4–8 years, n = 3). A single testicular tissue sample per colt or stallion was used. At least three samples or sections per testis were used to observe the staining patterns.

2.2. Preparation of Testicular Tissue Samples

Testicular tissue samples were prepared as previously described [15]. Briefly, after castration, the testes were transported directly on ice. For tissue fixation, approximately 1 cm^3 of testicular parenchyma was removed and fixed in 4% paraformaldehyde for at least 24 hours. After dehydration, tissues were embedded in paraffin blocks and processed for immunohistochemistry. Testicular tissues were snap-frozen in liquid nitrogen and stored at −80°C for the Western blotting procedure.

2.3. Immunohistochemistry

Immunohistochemical staining of the PGP9.5 molecule was performed in a manner similar to that previously described [5]. Testicular tissue embedded in paraffin wax was stored on slides at 4°C, and the paraffin was removed using xylene. Tissues were dehydrated with a graded series of 100%, 95%, 80%, 50%, and 25% ethanol baths. For antigen retrieval, tissues were treated in a citrate buffer at 97.5°C for 30 minutes and then allowed to cool for 30 minutes to room temperature. Slides were incubated in a blocking buffer of 5% donkey serum (Sigma, St. Louis, MO), diluted in a phosphate-buffered saline (PBS) solution. The rabbit anti-human PGP9.5 antibody (7863-0504, Serotec, Oxford, UK) was diluted at 1:1,500 in the blocking buffer (PBS containing 5% donkey serum) and incubated for 1.5 hours in a humidity chamber. After washing for 5 minutes three times with PBS containing 0.1% tween-20, the primary antibody was detected using donkey anti-rabbit immunoglobulin G (IgG) Alexa Fluor 488 (1:1,000 dilution, Life Technologies, Grand Island, NY). Tissues were mounted with Vectashield mounting medium containing 4,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA). Immunolabeling of PGP9.5 was observed using a Leica DM2500 microscope (Leica Microsystems, Wetzlar, Germany), and images were captured by using a Leica DFC450C digital camera (Leica Microsystems, Wetzlar, Germany). Goat anti-human deleted in azoospermia-like (DAZL, sc-27333; Santa Cruz Biotechnology, Santa Cruz, CA) antibody and goat anti-human GATA4 antibodies (sc-1237, Santa Cruz Biotechnology, Santa Cruz, CA) were used as a double staining at 1:50 and 1:200 dilution in the blocking buffer, respectively. Donkey anti-goat IgG Alexa Fluor 594 (1:1,000 dilution; Life Technologies, Grand Island, NY) was used as a secondary antibody for double staining. The tissues were mounted with VECTASHIELD mounting medium containing 4,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA). The immunolabeling was examined with ×400 magnification under a fluorescent microscope (Leica DM2500; Wetzlar, Germany) by a well-trained investigator, who was not blinded. For general analysis of PGP9.5, DAZL, and GATA4 staining on germ cells, at least 30 microscopic fields per testicular tissue were observed. To calculate the number of PGP9.5-positive germ cells on the round cross section of seminiferous tubule, approximately 100 microscopic fields on a testicular tissue section of postpubertal (n = 3) and adult stallion (n = 3) were examined. To calculate the percentage of spermatogonia stained with PGP9.5 only, PGP9.5 and DAZL, or DAZL only, approximately 1,000 spermatogonia in a testicular tissue section of postpubertal (n = 3) and adult stallion (n = 3) were analyzed.

2.4. Western Blotting

Western blot analysis was carried out to verify the specificity of the PGP9.5 antibody for stallion PGP9.5. Western blots were performed using a previously reported protocol [15]. Briefly, testicular tissues of stallions were snap frozen in liquid nitrogen and stored in a freezer at −80°C. For preparation of protein samples, frozen samples were thawed in a water bath at 38°C. Thawed specimens were homogenized with a Polytron PT 1200 CL homogenizer (Kinematica AG, Littau-Lucerne, Switzerland) in a radioimmunoprecipitation assay buffer for 5 minutes. The protein concentration of each sample was measured with the Bradford Bio-Rad Total Protein assay (Bio-Rad Laboratories, Inc, Hercules, CA). The homogenized tissues were diluted to a concentration of 1 mg/mL in the sample preparation buffer (0.5 M of Tris-HCL [pH 6.8], glycerol [0.1% w/v], 10% [w/v] SDS, 2-β-mercaptoethanol [0.05% w/v], and bromophenol blue in distilled water). The protein samples were heated in a boiling water bath for approximately 15 minutes and loaded (15 μL) into a 10% SDS-polyacrylamide gel and separated using the Mini-protean II electrophoresis system (Bio-Rad, Hercules, CA). The proteins were electro-transferred to a membrane (Millipore, Bedford, MA) and blocked with Blotto milk (Santa Cruz Biotechnology, Inc, Santa Cruz, CA) for 1 hour on the shaker. The membrane was treated with PGP9.5 antibody diluted to 1:300 in the Blotto overnight at 4°C. For the negative control, normal rabbit serum was used as the primary antibody at the same IgG concentration. The secondary antibody was donkey anti-rabbit IgG horseradish peroxidase (Santa Cruz Biotechnology, CA) diluted to 1:10,000 for 1 hour at room temperature. DEVELOPER and HYPAM solutions (ILFORD, Cheshire, England) were used to develop the film at 1:10 and 1:5 dilutions, respectively.

2.5. Statistical Analysis

The PGP9.5-positive cell populations on the round cross section of seminiferous tubules and the percentage of

Download English Version:

https://daneshyari.com/en/article/2394329

Download Persian Version:

https://daneshyari.com/article/2394329

Daneshyari.com