FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 44, No. 2, 2006
pp. 123-126

Localization of the *DAZ* gene expression in seminiferous tubules of patients with spermatogenic disorders

Anna Szczerba, Anna Jankowska, Mirosław Andrusiewicz and Jerzy B. Warchoł

Department of Cell Biology, University of Medical Sciences, Poznan, Poland

Abstract: The research on the expression and mutations of *DAZ* and its homologues in human and other mammals suggests that protein products of these genes can mainly affect development of germinal cells. The aim of the present study was to analyze the expression of the *DAZ* gene in seminiferous tubules of six men with spermatogenic disorders (hypospermatogenesis and spermatogenic arrest). The results based on the RT-PCR IS technique demonstrated that the *DAZ* product was present only in some seminiferous tubules and the fluorescence intensity was different within individual tubules. The most intense fluorescence characterised the spermatogonia, especially these organised in small groups inside separate tubules. In the patients with spermatogenic arrest at the spermatocyte stage, the *DAZ* gene transcripts were also found in primary spermatocytes. However, the fluorescence intensity of primary spermatocytes, except the fluorescence of the spermatocytes localised upon the lumen, was weaker than the fluorescence of spermatogonia. The results of our study showed that *DAZ* gene activity seems to correspond to the proliferative activity of stem cells of germinal epithelium. (www.cm-uj.krakow.pl/FHC)

Key words: *DAZ* - *In situ* RT-PCR - Spermatogenesis - Disorders

Introduction

Male factor infertility accounts for about half of the cases of couple infertility. The potential of assisted reproduction techniques to transmit genetic defects causing male infertility raises questions concerning the need for a systematic genetic screening and relevant genetic counseling of the affected patients. With the advancement of molecular cloning techniques, Y chromosome genes associated with spermatogenesis have been identified. Deletions of these genes, especially located on the long arm of Y chromosome are frequently linked with the spermatogenic defects [12]. Molecular techniques employing Y chromosome-specific markers subsequently revealed that 5-10% of severely oligozoospermic or azoospermic men have cytogenetically invisible Yq microdeletions [8]. These fall into three classes mapping to discrete subintervals of the Yq region, termed Azoospermia Factor - AZFa, b and c. Only AZFc deletions were associated with a variable testicular pathology (spermatogenic arrest, lack of the germ cells, Sertoli cell only syndrome - SCOS) and in rare cases AZFc deletions were even found inherited from father to son [10]. The best-characterized gene family of this region is the Deleted in AZoospermia (DAZ) gene family, which is present in four nearly identical copies. Although *DAZ* is not the only gene present in this region, it is the major AZFc candidate [15]. This possibility is supported by high homology of *DAZ* with *Drosophila melanogaster* male infertility gene, *boule*, which mutation causes spermatogenic arrest [3]. Furthermore, more recent evidence of the spermatogenic role of the *DAZ* gene product arose from the observation that a human *DAZ* transgene is capable of partially rescuing the sterile phenotype of mouse knockout for the homologous gene *Dazl* [13]. The critical role for DAZ in human male germ cell development is also supported by the evidence that this gene is testis-specific, with transcription limited to germ cells [7], even if its function remains unclear, and the postulated RNA binding property of the gene has not yet been demonstrated [11].

The aim of our study was to examine the distribution and concentration of *DAZ* transcripts in the seminiferous epithelium of testis of men with spermatogenic disorders.

Materials and methods

Testicular biopsies were obtained from six men with defective spermatogenesis: hypospermatogenesis (4 patients) and spermatogenic arrest (2 patients). The tissue was fixed in 4% formaldehyde in

Correspondence: A. Szczerba, Dept. Cell Biology, University of Medical Sciences, Swiecickiego 6, 60-781 Poznan, Poland;
e-mail: abosacka@amp.edu.pl

phosphate buffered saline (PBS), dehydrated and embedded according to standard procedure [2]. All cases were evaluated on hematoxylin-eosin stained sections. For the research the approval was obtained from the institutional ethical committees.

In order to detect the *DAZ* mRNA, the sections (3 μm) were prepared according to the procedure described earlier [14]. Reverse transcription *in situ* was performed using Expand Reverse Transcriptase (Roche Molecular Biochemicals, Mannheim, Germany) with the primer specific for the *DAZ* gene (reverse primer: 5'-ATG-CAAAGCTCAATACTGA-3', complementary to nucleotides 36319-36337, PubMed AC00021). The 460 bp fragment of cDNA of the *DAZ* gene transcript was amplified by *in situ* PCR, using the following primers: sense 5'-TGTTACCAGAAGGCAAAATC- 3' (nucleotides 1413-1432, PubMed AC00021) and antisense 5'-GCAACTGACATCCAGTGATG-3' (complementary to nucleotides 4613-4632, PubMed AC00021). Fluorescence-labeled nucleotides CyTM3-dUTP (Amersham Biosciences, Little Chalfont Buckinghamshire, UK) were used in order to identify the amplified product. *DAZ* mRNA distribution was examined using Carl Zeiss LSM 510 confocal microscope.

The use of computer-assisted image transformation allowed for semi-quantitative assessment of RT-PCR IS results. Greyscale format of the images was created as described earlier [9, 14]. Images generated in .lsm true color file format from Zeiss LSM Image Browser Version 3.1.0.99 for Carl Zeiss Laser Scanning System LSM 510 (Carl Zeiss GmbH) were exported to Microsoft Windows® Bitmap File Format and saved. In Adobe Photoshop® 5.5 CEEA (Adobe Systems Inc.1989-1999), the color mode was changed to greyscale format (8-bit per channel; 256 grey levels) and stored as a Windows® 8 Bitmap File Format. Using Scion Image® (Release beta 4.0.2) for Windows software (Scion Corporation© 2000), the greyscale was changed into 32-colour table mode and stored as a pseudocolors.

Results

The analysed group included both men with hypospermatogenesis and spermatogenic arrest. The results of the research based on the RT-PCR IS technique demonstrated that the localisation of *DAZ* mRNA in all studied patients was similar to these obtained in the control group, especially in case of hypospermatogenesis, characterised by the presence of mature spermatids [14]. The active *DAZ* gene was present only in part of examined seminiferous tubules (Fig. 1). The intensity of RT-PCR IS product fluorescence was different within individual tubules, as was clearly shown by expressing the intensity of the fluorescence as levels of greyscale images. The most intense fluorescence both in case of hypospermatogensesis and spermatogenic arrest characterised the spermatogonia, especially those organised in small groups inside separate tubules (Fig. 2). The fluorescence intensity measured on 256-grade greyscale exceeded 100 (data not shown). A weaker fluorescence was detected in primary spermatocytes, mostly located in the nearest neighbourhood of positively stained spermatogonia (Fig. 2). The fluorescence intensity of primary spermatocytes ranged from 68 to 75 of greyscale and was weaker than the corresponding intensity of spermatocytes in the control group [14]. A very weak fluorescence (around 60) was noted for the other cells of seminiferous tubules, *i.e.* secondary spermatocytes and spermatids.

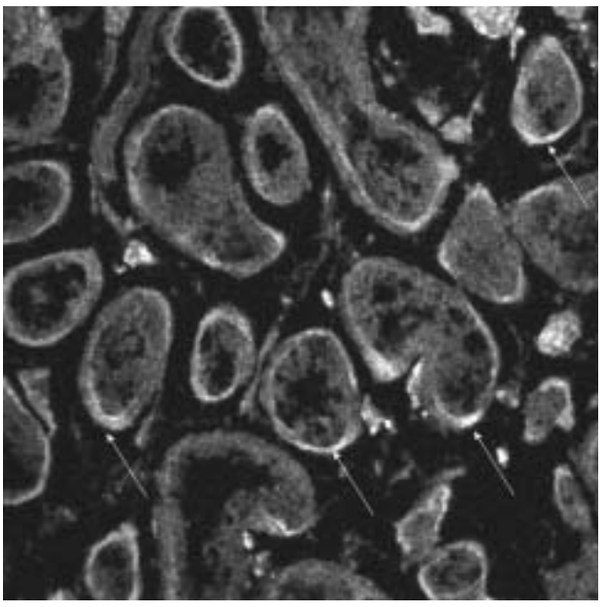

Fig. 1. Detection of the *DAZ* transcript in testis of men with hypospermatogenesis. Bright fluorescence shows the RT-PCR IS products. The places of accumulation of *DAZ* mRNA corresponding to the groups of spermatogonia are indicated by arrows. × 100.

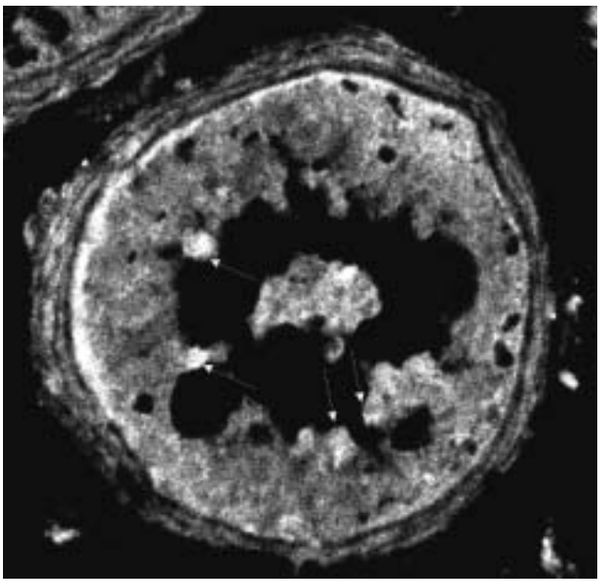

Fig. 2. Localization of *DAZ* transcripts in seminiferous tubule of men with spermatogenic arrest. Arrows indicate single primary spermatocytes with high accumulation of RT-PCR IS products. × 400.

The most characteristic feature of the studied group was the presence of single primary spermatocytes with high accumulation of RT-PCR IS products, localised

upon the lumen level (Fig. 2) The fluorescence intensity corresponding to the level of *DAZ* expression for about 5% spermatocytes reached 150th greyscale level (data not shown).

In some seminiferous tubules only Sertoli cell were observed. The intensity of RT-PCR IS product fluorescence in the tubules of studied patients corresponded to the fluorescence of the background, thus it was assumed, that there was no expression of the *DAZ* gene.

Discussion

The results of the present study showed that there was no significant difference of *DAZ* activity between control and study groups. The *DAZ* mRNA was found in most spermatogonia, but its amount, measured in levels of greyscale, was lower that in the control group. A weaker fluorescence corresponding to minor amount of *DAZ* transcripts was noted for the other cells of seminiferous tubules, *i.e.* spermatocytes and spermatids.

The precise biological role of the *DAZ* gene in spermatogenic process is still unknown. There are almost no data about the expression of the *DAZ* gene in men with defective spermatogenesis and without deletions of the studied gene. Kuo *et al.* using quantitative PCR showed that expression of the *DAZ* gene in patients with spermatogenic disorders is significantly lowered. The authors suggested that the decreased number of spermatogonia is responsible for the lowered level of *DAZ* transcripts [5, 6]. The hypothesis is acceptable only when assumed that the amount of mRNA among the spermatogonia is constant. This amount, however, is clearly lowered in the spermatogonia of men with spermatogenic disorders, as shown in our study. The active gene present in spermatogonia could be involved in the regulation of their proliferation. The observations are in agreement with the results of Bar-Shira and Maymon, who assessing PCNA immunoreactivity showed the proliferating potential of spermatogonia [1]. The absence of *DAZ* gene deletions in case of spermatogenic disorders does not exclude a decreased expression of this gene. Low expression of *DAZ* could be responsible for the lower amount of transcripts in spermatogonia noticed during our experiments. Since there were no significant differences in *DAZ* activity between the control and study groups, it seems that morphological changes in testis of men with spermatogenic defects can be related to disturbed meiotic divisions. Mutation of other genes located in Y chromosome, especially those involved in DNA repair could be responsible for the impaired spermatogenesis [4]. The results of our study support such hypothesis. The spermatocytes with high accumulation of *DAZ* transcripts were observed upon the lumen of seminiferous tubules. Very likely in these spermatocytes meiosis was blocked at the first stages of division.

DAZ gene activity seems to correspond to the proliferative activity of stem cells of germinal epithelium. Since the amount of *DAZ* mRNA was clearly lowered in the spermatogonia of men with spermatogenic disorders, it seems that the active gene present in most spermatogonia observed by us could be involved in the regulation of division of these cells.

The analysis of *DAZ* expression could be helpful in diagnosis of spermatogenic disorders.

Acknowledgments: The study was supported by a grant from Poznan University of Medical Sciences no. 501-01-4410519-07300.

References

[1] Bar-Shira Maymon B, Paz G, Elliott DJ, Hammel I, Kleiman SE, Yogev L, Hauser R, Botchan A, Yavetz H (2002) Maturation phenotype of Sertoli cells in testicular biopsies of azoospermic men. Hum Reprod 15: 1537-1542

[2] Duda T, Venkataraman V, Krishnan A, Nagele RG, Sharma RK (2001) Negatively calcium-modulated membrane guanylate cyclase signaling system in the rat olfactory bulb. Biochemistry 40: 4654-4662

[3] Eberhart CG, Maines JZ, Wasserman SA (1996) Meiotic cell cycle requirement for a fly homologue of human deleted in azoospermia. Nature 381: 783-785

[4] Fox MS, Reijo-Pera RA (2001) Male infertility, genetic analysis of the DAZ genes on the human Y chromosome and genetic analysis of DNA repair. Mol Cell Endocrinol 184: 41-49

[5] Kuo PL, Lin YH, Teng YN, Hsu CC, Lin JS, Lin YM (2004) Transcriptional levels of four Y chromosome-linked AZF genes in azoospermic men and their association with successful sperm retrieval. Urology 63: 131-136

[6] Kuo PL, Wang ST, Lin YM, Lin YH, Teng YN, Hsu CC (2004) Expression profiles of the DAZ gene family in human testis with and without spermatogenic failure. Fertil Steril 81: 1034-1040

[7] Menke DB, Mutter GL, Page DC (1997) Expression of DAZ, an azoospermia factor candidate, in human spermatogonia. Am J Hum Genet 60: 237-241

[8] Najmabadi H, Huang V, Yen P, Subbarao MN, Bhasin D, Banaag L, Naseeruddin S, Dekretser DM, Baker HWG, McLachlan RI (1996) Substantial prevalence of microdeletions of the Y-chromosome in infertile men with idiopathic azoospermia and oligozoospermia detected using a sequence-tagged site-based mapping strategy. J Clin Endocrinol Metab 81: 1347-1352

[9] Nowak-Markwitz E, Jankowska A, Szczerba A, Andrusiewicz M, Warchol JB (2004) Localization of human chorionic gonadotropin beta subunit transcripts in ovarian cancer tissue. Folia Histochem Cytobiol 42: 123-126

[10] Pryor JL, Kent-First M, Muallem A, Van Bergen AH, Nolten WE, Meisner L, Roberts KP (1997) Microdeletions in the Y chromosome of infertile men. N Engl J Med 336: 534-539

[11] Reijo R, Lee TY, Salo P, Alagappan R, Brown LG, Rosenberg M, Rozen S, Jaffe T, Straus D, Hovatta O (1995) Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nat Genet 10: 383-393

[12] Simoni M, Kamischke A, Nieschlag E (1998) Current status of the molecular diagnosis of Y-chromosomal microdeletions in the work-up of male infertility. Initiative for international quality control. Hum Reprod 13: 1764-1768

[13] Slee R, Grimes B, Speed RM, Taggart M, Maguire SM, Ross A, McGill NI, Saunders PT, Cooke HJ (1999): A human DAZ

transgene confers partial rescue of the mouse Dazl null phenotype. Proc Natl Acad Sci USA 96: 8040-8045

[14] Szczerba A, Jankowska A, Andrusiewicz M, Karczewski M, Turkiewicz W, Warchol JB (2004) Distribution of the DAZ gene transcripts in human testis. Folia Histochem Cytobiol 42: 119-121

[15] Wong J, Blanco P, Affara NA (1999) An exon map of the AZFc male infertility region of the human Y chromosome. Mamm Genome 10: 57-61

Received: July 25, 2005
Accepted after revision: November 29, 2005