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The *DAZL1* gene is expressed in human male and female embryonic gonads before meiosis

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The human *DAZL1* gene (known also as *DAZH* or *DAZLA*) is the autosomal homologue of the Y-chromosomal *DAZ* gene which has been found to be deleted in azoospermic males. Evidence suggests that the role of *DAZL1* may not be restricted to spermatogenesis, but may include oogenesis as well. In order to study the function of human *DAZL1* gene in the ovary, we observed its expression pattern during embryonic development. RNA in-situ hybridization showed that *DAZL1* transcripts were localized to a subset of cells (somatic versus germ cells) in human embryonic ovary (23 weeks of gestation) and testis (21 weeks gestation). In the ovary, *DAZL1* transcripts were found in oogonia and in oocytes and granulosa cells of primordial follicles. In the testis, *DAZL1* transcripts were identified exclusively in the germ cells. Our results demonstrate high similarity between the human *DAZL1* and the mouse *Dazl1* gene expression patterns during embryonic development, suggesting that the human gene functions at the first phase of gametogenesis, just as in the mouse, where *Dazl1* mutations cause male and female sterility.

Key words: DAZL1/embryonic gonad/meiosis/oogenesis/spermatogenesis

Introduction

The *DAZ* (deleted in azoospermia) gene on the long arm of the Y chromosome (Reijo *et al*., 1995) may be the most thoroughly studied pure male sterile locus in humans. Although no human *DAZ* point mutants have been reported, strong evidence suggests that this gene is involved in early spermatogenesis. Firstly, *Drosophila* and mouse mutants that have lost *DAZ* homologous gene function (*boule* and *Dazl1* respectively) were found to be azoospermic, just as with human azoospermia (Eberhart *et al*., 1996; Ruggiu *et al*., 1997). Secondly, *DAZ* homologous genes were found in many vertebrates (Reijo *et al*., 1995, 1996; Cooke *et al*., 1996; Saxena *et al*., 1996; Shan *et al*., 1996; Yen *et al*., 1996; Houston *et al*., 1998) in which they appear to be expressed in the testis, exclusively in spermatogonia (Menke *et al*., 1996; Niederberger *et al*., 1997).

From the evolutionary point of view, the Y chromosomal *DAZ* gene arose from the *DAZL1* gene on chromosome 3 via a series of structural transformations (Saxena *et al*., 1996). A recent study of *DAZ* and *DAZL1* sequences suggested that the Y-linked *DAZ* plays either a limited or little role in human spermatogenesis (Agulnik *et al*., 1998). These studies imply that the *DAZL1* gene is the main functional locus. Indeed, the human *DAZL1* and mouse *Dazl1* autosomal genes show higher similarity in nucleotide sequences compared with the two human genes (*DAZ/DAZL1*). Within the 82 residue RNA-binding domain, the products of human *DAZL1* and mouse *Dazl1* differ by only one amino acid substitution, while both differ from human Y-encoded *DAZ* at nine residues. In addition, *DAZ* has a variable number of copies of a 72 nucleotide repeat unit termed '*DAZ* repeat', whereas the human *DAZL1* and the mouse *Dazl1* has only one copy of this motif (Saxena *et al*., 1996; Seboun *et al*., 1997).

The *DAZ* gene expresses only in the testis, whereas the human *DAZL1* and the mouse *Dazl1* express in testes and ovaries (Cooke *et al*., 1996; Saxena *et al*., 1996; Seligman and Page, 1998). We previously showed that *Dazl1* expresses during mouse embryonic developing male and female gonads well before the onset of meiosis, suggesting that *Dazl1* functions at the first phase of gametogenesis at the differentiation, proliferation and/or maintenance of primordial germ cells and their substitutes (Seligman and Page, 1998). Our expression results are supported by recent evidence showing that the mouse *Dazl1* is essential for the differentiation of male and female germ cells. As expected, disruption of the *Dazl1* gene leads to loss of germ cells and complete absence of male and female gamete production (Ruggiu *et al*., 1997).

The DAZL1 protein was found in the cytoplasm of follicular human oocytes (Nishi *et al*., 1999). However, little is known about the embryonic expression, transcription units and cellular mechanisms of the human *DAZL1* gene. Here, we wanted to examine the localization of *DAZL1* transcripts during gonad embryonic development and compare the human and the mouse gene expression. We have localized *DAZL1* transcription units in human gonads (testes and ovaries) from embryos at 15–25 weeks gestation by RNA in-situ hybridization. We observed high similarity between the human *DAZL1* and mouse *Dazl1* gene expression during early embryonic development; they both express in early developing male and female germ cells (prospermatogonia and oogonia respectively). Our results suggest that the human and the mouse genes exhibit similar,

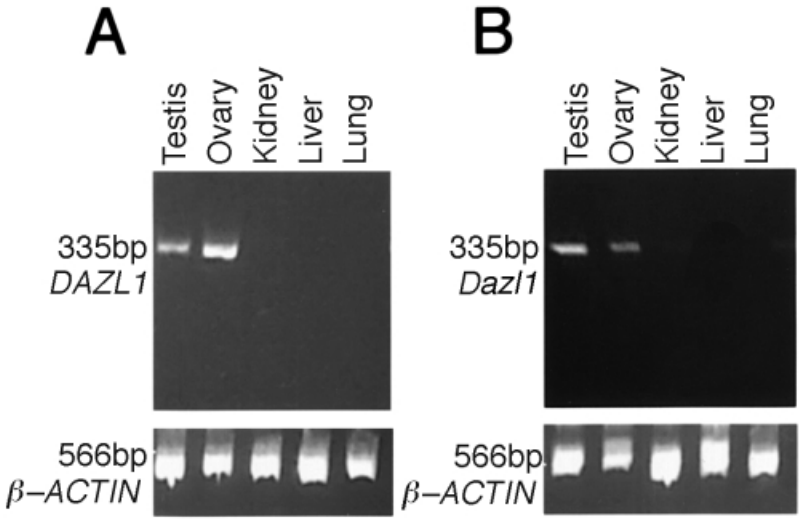

Figure 1. Analysis of human *DAZL1* (**A**) and mouse *Dazl1* (**B**) expression in embryonic testis, ovary, kidney, liver and lung by reverse transcription–polymerase chain reaction (RT–PCR). RNA samples were isolated from human fetuses at ~23 weeks gestation and from C57BL/6J mouse at 14.5 days post-coitum. A 335 bp fragment represents the human *DAZL1* and mouse *Dazl1*. The gene encoding β-actin was used as a reference reaction.

if not identical, function at the first phase of male and female gametogenesis.

Materials and methods

Embryo tissue dissections

Human gonads were obtained from aborted fetuses after the patients had signed a concession agreement. The human embryonic gonad work was approved by the Institutional Ethics Committee. Gonads from embryos at 15–25 weeks gestation were isolated and fixed immediately in 4% formalin to prepare paraffin-embedded cross-sections or frozen in liquid nitrogen for RNA extraction. Mouse embryonic gonads were obtained from C57BL/6J mating mice. For all mouse embryo dissections the appearance of the vaginal plug was calculated as 0.5 days post-coitum.

RNA extraction and reverse transcription–polymerase chain reaction (RT–PCR)

RNA samples were extracted from various human and mouse embryonic tissues using Trizol reagent (Gibco BRL, Grand Island, NY, USA) according to the manufacturer's instruction. Briefly, gonads were suspended in 1 ml Trizol, and 0.2 volumes of chloroform were added to each sample. After centrifugation to remove cell debris, RNA in the supernatant was precipitated with isopropanol, rinsed with ethanol, and resuspended in deionized water. These RNA samples were used to amplify the 335 bp fragment of human *DAZL1* and mouse *Dazl1* by RT–PCR (cDNA cycle kit; Invitrogen) by using a forward primer F1: 3′ AGCCACGTCCTTTGATTTT 5′ and a reverse primer R1: 3′ TAAGCACTGCCCGACTTCTT 5′. The gene encoding β-actin was used as a reference reaction using a forward primer: 3′ CGACGAGGCCCAGAGCAAGAGAGG 5′ and a reverse primer: 3′ CGTCAGGCAGCTCATAGCTCTTCTCCAGGG 5′.

In-situ hybridization

To identify human *DAZL1* and mouse *Dazl1* we used a 335 bp fragment from the mouse *Dazl1* cDNA including *Dazl1* repeat region. This 335 bp fragment was amplified by PCR using a forward primer F1 containing SP6 promoter sequence at its 3′ end and a reverse primer R1 containing T7 promoter sequence at its 3′ end. We labelled the probes with SP6 and T7 digoxygenin-RNA labelling kit (Roche Molecular Biochemicals) for antisense and sense probe respectively. Hybridization to gonad sections was performed according to a well-established method (Komminoth *et al*., 1992). Slides were reacted with anti-digoxygenin antibodies and developed with NBT/BCIP reagents following Fast red staining.

Results

To compare the human *DAZL1* and mouse *Dazl1* expression pattern during development, RNA samples were extracted from various human embryonic tissues from ~week 23 of gestation and from mouse embryonic tissues of 14.5 days post-coitum. These RNA samples were used to amplify a 335 bp fragment of human *DAZL1* and mouse *Dazl1* by RT–PCR. As shown in Figure 1, the human *DAZL1* and the mouse *Dazl1* are both expressed in the human embryonic testis and ovaries. No amplification was found in the embryonic kidney, liver and lung (see Figure 1).

To characterize more precisely the expression of *DAZL1* by specific germ cell types, we performed direct in-situ hybridization on sections of human embryonic ovaries and testes. We used the 335 bp PCR product from *DAZL1* cDNA as a probe. This fragment was amplified by PCR using a forward primer F1 containing SP6 polymerase promoter sequence (for antisense probe) and a reverse primer R1 containing T7 polymerase promoter (for sense probe). Digoxygenin-labelled *DAZL1* probes (sense and antisense) were allowed to hybridize within sections of normal embryonic ovary isolated from a fetus at 23 weeks gestation (Figure 2) and a testis from a fetus at 21 weeks gestation (Figure 3). As shown in Figure 2A,C, *DAZL1* transcripts were detected in oogonia and in oocytes and granulosa cells of primordial follicles by *DAZL1* antisense probe. In some of the oocytes, *DAZL1* was abundant in the nucleus and in others *DAZL1* was predominantly present in the cytoplasm or in both nucleus and the cytoplasm. As shown in Figure 2B,D, no reaction was observed in oogonia and

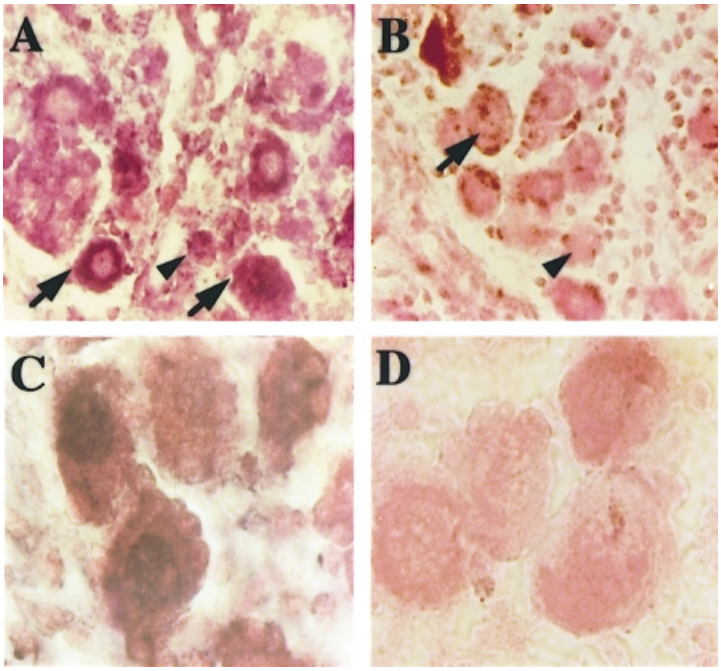

Figure 2. Localization of *DAZL1* transcripts in embryonic ovary. Digoxygenin-labelled sense and antisense *DAZL1* probes were allowed to hybridize with cross-sections of an ovary from a fetus at 23 weeks gestation. (**A**,**C**) Hybridization with antisense probe; (**B**,**D**) hybridization with sense probe (control); (**A**,**B**) low power magnification (×400); (**C**,**D**) high power magnification (×1000). Arrow = primordial follicle; arrowhead = oogonia.

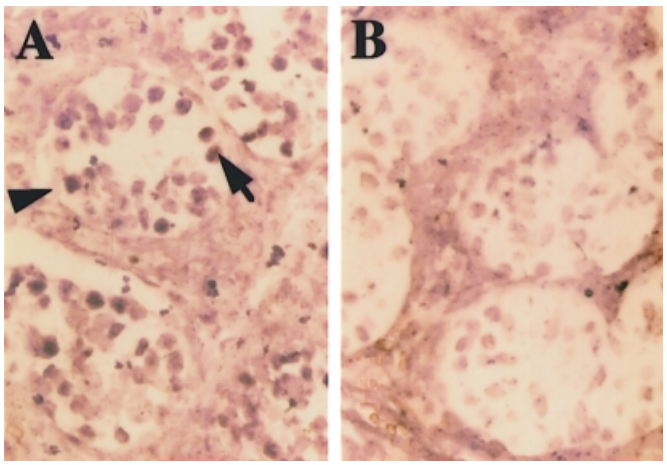

Figure 3. Expression of *DAZL1* in embryonic testis. Digoxygenin-labelled sense and antisense *DAZL1* probes were allowed to hybridize with cross-sections of a testis from a fetus at 21 weeks gestation. (**A**) Hybridization with antisense probe; (**B**) hybridization with sense probe (control). Arrow = germ cell; arrowhead = Sertoli cell.

follicles by hybridization with the sense probe (control reaction). In the testis, transcripts were identified exclusively in germ cells (Figure 3A). As shown in Figure 3B, no staining was observed in germ cells by hybridization with the sense probe (control reaction).

Discussion

Once the germ cells initiate sexual differentiation at ~7 weeks gestation in the human (13.5 days post-coitum in the mouse), the subsequent kinetics of germ cell development show a dramatic sexual dimorphism: oogonia enter meiotic prophase, while prospermatogonia continue to divide mitotically (McCarrey, 1993). In the ovarian sections at 15–25 weeks gestation, some oogonia have not entered meiosis, some oogonia have started meiosis and primordial follicles (arrested in meiotic prophase I). Prospermatogonia are present at this time in the testis, most of them are quiescent in mitosis. Here, we show that *DAZL1* transcripts are localized in germ cells before meiosis and at the time meiosis commences. We could not study earlier stages of gonad development because of the difficulty in obtaining these tissues from humans. However, it is most likely that the human *DAZL1* gene is expressed earlier during development before germ cell sexual differentiation, such as in the mouse in primordial germ cells (Seligman and Page, 1998).

Numerous genes are known to be expressed exclusively in male or female germ cells, mainly in meiotic or post-meiotic cells, but not in these earliest stages of gametogenesis (Hecht, 1993). The expression of human *DAZL1* gene in both male and female germ cells so early during embryonic development is unusual. In the mouse, few genes are known to be expressed exclusively in male and female germ cells early during gametogenesis, but no human homologous genes were studied. The mouse germ cell nuclear antigen (GCNA1) expresses in primordial germ cells, and later in oogonia and prospermatogonia, such as the *DAZL1* gene, but no DNA sequences of this antigen are available (Enders and May, 1994). The *TIAR* gene, which is also an RNA-binding protein such as *DAZL1*, was found to be expressed in primordial germ cells (Beck *et al*., 1998). Male and female mutant mice for *TIAR* are sterile, similar to *Dazl1* mutant mice. However, it seems that *TIAR* does not function exclusively in gonads since mice

mutant for this gene also exhibit other abnormalities such as growth deficit and high mortality (Beck *et al*., 1998).

The *DAZL1* transcripts in the ovary are not restricted to germ cells, but include the somatic cells of the primordial follicle (granulosa cells). A similar expression of the mouse *Dazl1* protein in the ovary has been shown previously by using antibodies (Ruggiu *et al*., 1997). The granulosa cells are in tight connection with the oocyte throughout gap junctions which allow the passage of proteins and RNA samples between the oocyte and granulosa cells. That is why it is not surprising that *DAZL1* is also localized in granulosa cells. There is not much information about *DAZL1* transcription units in the ovary. It is possible that different transcripts are present in ovarian germ cells and in the granulosa cells. We have previously identified in the human ovary a major 4.5 kb *DAZL1* transcript, while in the testis a 3.5 kDa transcript is the most abundant transcript. Only very low levels of the 4.5 kb transcript were identified in the testis (Seligman and Page, 1998). The biological significance and function of the 3.5 and 4.5 kb transcription units remain to be studied.

It is probable that human DAZL1 and the mouse Dazl1 proteins perform similar functions in early gametogenesis processes. Our results suggest that the *DAZL1* locus may be involved in infertility cases in humans. Mutation analysis and linkage studies of this locus in families with recessive inheritance pattern of infertility are needed to evaluate the role of the *DAZL1* gene in infertility incidences in humans.

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