

Heat Shock Protein A2

(hs pA2) mRNA

1, 2, 3, 4.
1,4, 4, 4, 1, 1,2, 1,3

Repression of Hs pA2 mRNA Expression in Human Testes with Abnormal Spermatogenesis

W.Y. Son, S.H. Hwang, C.T. Han, J.H. Lee, S. Kim, Y.C. Kim.

¹Center for Reproduction and Genetics, ²Dept. of OB/GY, ³Dept. of Urology, Pundang Jesaeng General Hospital, Kyungki-Do, 463-050, Korea, ⁴Dept. of Biological Science, Sogang University, Seoul, Korea.

Objective: Heat shock protein 70.2 (Hsp70.2) gene knockout mice are found to have premeiotic arrest at the primary spermatocyte stage with a complete absence of spermatids and spermatozoa. This observation led to the hypothesis that *hspA2* may be disrupted in human testes with abnormal spermatogenesis. To test this hypothesis, we studied the mRNA expression of *hspA2* in infertile men with azoospermia.

Design: The mRNA expression were analyzed by competitive RT-PCR among testes with normal spermatogenesis, pachytene spermatocyte arrest, and sertoli-cell only syndrome.

Materials and methods: Testicular biopsy was performed in men with azoospermia (n=15). Specimens were subdivided into three groups: (group1) normal spermatogenesis (n=5), (group 2) spermatocyte arrest (n=5), (group 3) Sertoli-cell only syndrome (n=5). Total RNA was extracted by Trizol reagent. Total extracted RNA was reverse transcribed into cDNA and amplified by PCR using specific primers for *hspA2* target cDNAs. A competitive cDNA fragment was constructed by deleting a defined fragment from the target cDNA sequence, and then coamplified with the target cDNA for competitive PCR. Internal control was used glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene.

Results: On Competitive RT-PCR analyses for *hspA2* mRNA, significant *hspA2* expression was observed in group 1, whereas a constitutively low level of *hspA2* was expressed in groups 2 and 3.

Conclusion(s): The study demonstrates that the *hspA2* gene expression is down-regulated in the human testes with abnormal spermatogenesis, which in turn suggests that *hspA2* gene may play a specific role during meiosis in human testes.

Key Words: Spermatogenic arrest, testes, *hspA2* gene, Sertoli-cell only syndrome, and competitive RT-PCR.

가 ,
가 .
.

10-20%

가 (Meyer , 1988)

(Kretser , 1974; Giraldo , 1981; Leonard , 1979).

(Johnson , 1970) 1

heat shock protein 70 (HSP70)

HSP70

folding, transport, assembly (Georgopoulos ,

1993). , HSP70 가 HSP70

P70 (Allen ,

1988) HSC70t (Matsumoto , 1990)

P70 *hsp70.2*

(Rosario , 1992), *hspA2* 98.2%

*hspA2*가 P70 (Bonnycastle ,

1994).

Dix (1996) *hsp 70.2* gene targeting
1 *hsp70.2*
가
hsp70.2 *hspA2*
가 competitive RT-PCR
mRNA *hspA2* 가 *hspA2*

1. Testes

Bouin's 가 RNA
RNA 5 (group 1),
5 (group 2),
5 (group 3)

2. Total RNA

Total RNA (Gibco BRL, USA)
glass tissue grinder 1ml Trizol
eppendorf tube 5 incubation
Chloroform tube 가 samples 4 °C, 12,000g 20
tube 1 vol isopropanol 가 10
incubation RNA 4 °C, 12,000g 20
75% RNA 20ul DEPC-
sample 2ul DEPC-

600ul OD260
 DNA DNase I (Gibco BRL, USA) RNA
 -70°C deep freezer

3. Competitive RT-PCR competitor

HspA2 gene mRNA 가 primers
 cDNA (3106-3448) 가 343bp
hsp70 gene homology가 3' untranslated region (UTR) (Fig. 2. Schematic diagram). *HspA2* gene 343bp cDNA RNA RT-PCR
 RT-PCR pMOSBlue vector (Amersham, USA) cloning
pDMC1 plasmid . *pMOSBlue* vector 343bp sequence
 DNA sequencing . Competitor 343bp cDNA deletion mutant
 . *pDMC1* vector 가 343 bp PCR
 . *Mse* I . *Mse* I cDNA
 T4 DNA ligase ligation *pMOSBlue* vector cloning . Mutant cDNA
 bacterial clones plasmid DNA (*pDMC2*) PCR
HspA2 deletion mutant cDNA 가 200 bp . Internal control mRNA
 GAPDH sense primer sequence 5'-cggagtcaacggattggctgat-3'
 antisense primer 5'-agccttctccatgggtgaagac-3' . GAPDH RT-PCR
 306bp .

4. Reverse transcription- polymerase chain reaction (RT-PCR)

hspA2 gene antisense primer annealing
 . Total RNA 5ug 2 pmol mRNA antisense primer
 internal control GAPDH mRNA antisense primer 65°C 10
 denaturation . 4°C 7ul master mix [10X RT buffer, 1 ul
 dNTP mix (2.5mM each), 50mM MgCl₂, 200mM dithiothreitol] 가 42°C 5
 pre-warmed . Reverse transcriptase (AMVE, Promega) 가 42 °C
 50 incubated . 65 °C 15 incubation
 . Competitive PCR cDNA mutant PCR 가 . PCR
 sample 2ul 5ul 10X PCR buffer, 2.5ul dNTP mix (2.5mM each),
 0.5U Taq polymerase 10 pmol PCR primers 가 50ul
 mixture . PCR 94 °C 1 , 58°C 30 , 72°C 30

cycle 35 cycle 72°C 10
 sample 2 ul aliquots internal control GAPDH primers PCR
 tube . RT-PCR 2% agarose , EtBr
 UV gel video image band software program

Histological evaluation of testes

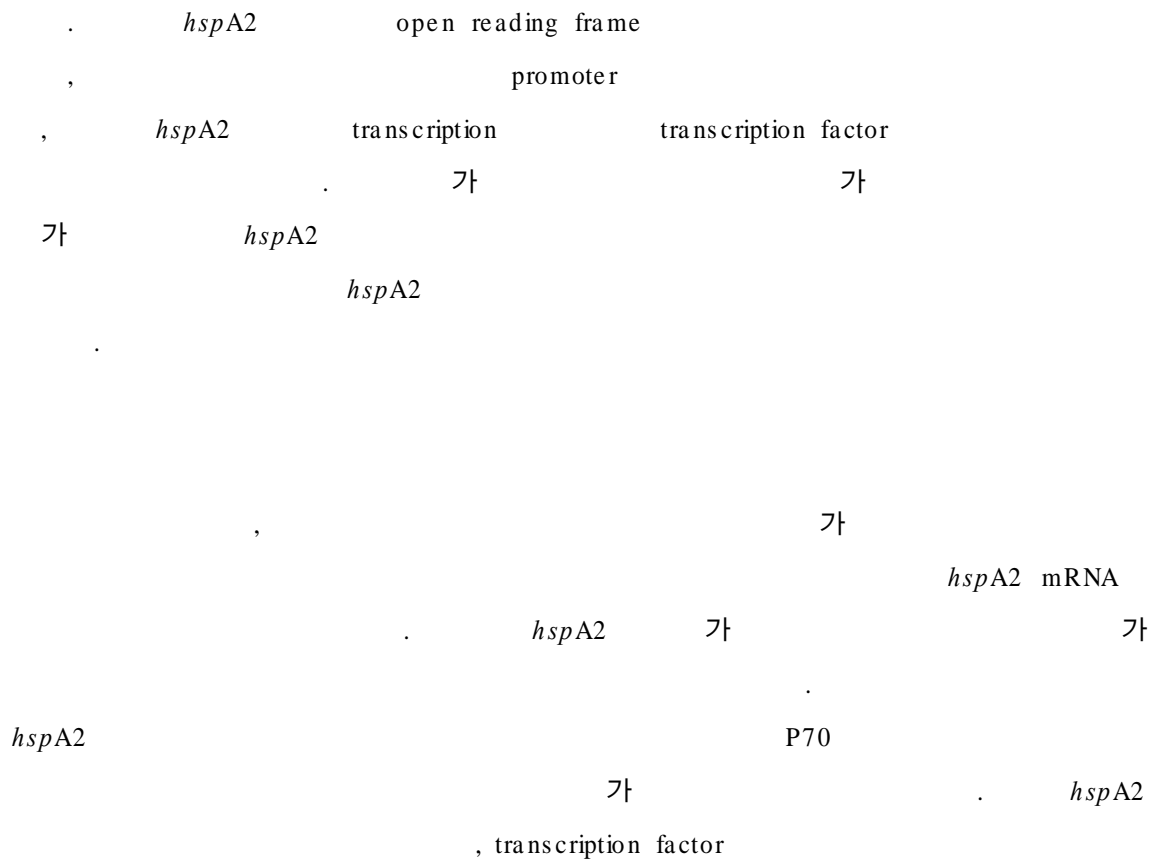
1 hematoxylin/eosin
 . (A)
 . (B) 1
 (C) 가

Competitive RT-PCR

2 competitive RT-PCR
 Internal control GAPDH RT-PCR 3 가
 (Fig 2, A). (Fig. 2, C) 가
 (Fig. 2, D) *hspA2* RT-PCR
 (Fig.2, B)
 (Fig 2, E).
 mRNA , 가 constitutive level
 (Fig 2, B).

가
hspA2
hspA2 가
 Allen (1988) heat shock stress
 P70
 P70
 가 (Allen , 1988).

Rosario (1992) P70 *hsp70.2* gene ,
heat shock P70 antiserum 2A
western blot . P70
Dix (1996)
gene targeting *hsp70.2*
1
가 가 . gene
disruption .
hsp70.2
hspA2
hspA2 2 1917bp 1 open reading frame
가 70,030Da (Bonnycastle , 1994).
hsp70.2 98.2% , heat shock *hsp70-1*
83.3% , *hsc70* 86.1% 가 .
heat shock 3'
untranslated RT-PCR primers .
vector cloning ,
. *hsp70.2* 100% .
Bonnycastle (1994) *hspA2*
constitutive .
가 *hsp70.2* . *hspA2*
가 , 가
가 가 level *hspA2*
가 . *hspA2* 가 ,
hspA2
hspA2 가
hspA2 3가



1. Meyer JM, Roos M, Rumpler Y. Statistical study of a semiquantitative evaluation of testicular biopsies. Arch Androl 1988;10:71-9.
2. Kretser DM de, Burger HG, Hudson B. The relationship between germinal cells and serum FSH levels in males with infertility. J Clin Endocrinol Metab 1974;38:787-93.
3. Girardo A, Silva E, Martinez I, Campos C, Guzman J. Pericentric inversion of chromosome 1 in three sterile brothers. Hum Genet 1981;58:226-7.
4. Leonard C, Bisson JP, David G. Male sterility associated with familial translocation heterozygosity: t(8;15) (q22;p11). Arch Andol 1979;2:269-75.
5. Johnson SG. Testicular biopsy score count a method for registration of spermatogenesis in human testis: normal values and results in 335 hypogonadal males. Hormones 1970;1:2-15.
6. Georgopoulos C, Welch WJ. Role of the major heat shock protein as molecular chaperones. Annu Rev Cell Biol 1993;9:601-34.

7. Allen RL, O'Brien DA, Eddy EM. A novel hsp70-like protein (P70) is present in mouse spermatogenic cells. *Mol Cell Biol* 1988;8:828-32.
8. Matsumoto M, Fujimoto H. Cloning of a hsp70-related gene expressed in mouse spermatids. *Biochem Biophys Res Commun* 1990;166:43-9.
9. Bonnycastile LLC, Yu CE, Hunt CR, Trask BJ, Clancy KP, Weber JL, et al. Cloning, sequencing, and mapping of the human chromosome 14 heat shock protein gene (HSP A2). *Genomics* 1994;23:85-93.
10. Dix DJ, Allen JW, Collins BW, Mori C, Nakamura N, Poorman-Allen P, et al. Targeted gene disruption of HSP70-2 results in failed meiosis, germ cell apoptosis, and male infertility. *PNAS* 1996;93:3264-8.
11. Rosario MO, Perkins SL, O'Brien DA, Allen RL, Eddy EM. Identification of the gene for the developmentally expressed 70 kDa heat-shock protein (P70) of mouse spermatogenic cells. *Devel Biol* 1992;150:1-11.

Figure 1. **A;** Schematic diagram of *hspA2* gene, and its primer sequences. Open box represents open reading frame (ORF) and straight lines represent untranslated regions (UTR). **B:** Schematic diagram for competitor plasmid construction, pDMC2.

Figure 2. Hematoxylin/eosin stains; (A) normal spermatogenesis, group 1; (B) spermatogenic arrest, group 2; (C) Sertoli-cell only syndrome, group 3. (X200).

A

B

Testes total RNA (500 ng)

competitor: $(1/4)^6(1/4)^5(1/4)^4(1/4)^3(1/4)^2(1/4)$ 1 Pg

C

Testes total RNA (500 ng)

competitor: $(1/4)^7(1/4)^6(1/4)^5(1/4)^4(1/4)^3(1/4)^2(1/4)$ Pg

D

Testes total RNA (500 ng)

competitor: $(1/4)^8(1/4)^7(1/4)^6(1/4)^5(1/4)^4(1/4)^3(1/4)^2(1/4)$ Pg

E

Figure 3. Competitive RT-PCR analysis for *hspA2* transcripts; For internal control GAPDH (A) lane 1; normal spermatogenesis, lane 2; spermatogenic arrest, lane 3; Sertoli-cell only syndrome, lane 4: no RT control. For competitive RT-PCR; (B) normal spermatogenesis, group 1; (C) spermatogenic arrest, group 2; (D) Sertoli-cell only syndrome, group 3; (E) Relative level of mRNA.