

## Heat Shock Protein A2 (HspA2)

<sup>1</sup>, <sup>2</sup>, <sup>3</sup>, <sup>4</sup>.

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### Expression of Heat Shock Protein HspA2 in Human Tissues

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In mouse, the heat shock protein 70-2 (hsp70-2) is found to have special function in spermatogenesis. Based on the observation, the hypothesis that human hspA2 (human gene; 98.2% amino acid homology with hsp70-2) might have important function in spermatogenesis in human testes was proposed. To test the hypothesis, we examined the expression of hspA2 in human tissues.

Expression vector pDMC4 for expression of the human hspA2 protein using pTricHisB (invitrogen, USA) was constructed and the expressed hspA2 protein was cross-reacted with antiserum 2A raised against mouse hsp70-2 protein. Based on the cross-reactivity, we determined the expression level of hspA2 protein in human tissues by western blot analysis using the antiserum 2A.

We demonstrated that antiserum 2A antibodies detected human hspA2 protein with specificity which was produced in the *E.coli* expression system. On Western blot analyses, significant hspA2 expression was observed in testes with normal

spermatogenesis, whereas a low level of hspA2 was expressed in testis with Sertoli-cell only syndrome. Also, a small amount of hspA2 was detected in breast, stomach, prostate, colon, liver, ovary, and epididymis. These results demonstrate that the hspA2 protein is highly expressed in male specific germ cells, which in turn suggests that hspA2 protein might play a specific role during meiosis in human testes as suggested in the murine model. However, further studies should be attempted to determine the function of hspA2 protein in human spermatogenesis.

**Key Words :** Spermatogenesis, hspA2 protein, Sertoli-cell only syndrome, and western blot analysis.

Heat shock protein (hsp) 가 (Miller , 1991).

hsp . Hsp

degradation, folding, assembly, transport (Georgopoulos , 1993). Hsp germ cell

kDa (hsp70) . hsp 가 70

hsp70

hsp70-2 (Allen , 1988) 가 ,

hsp70-2 hsp70-2 (Rosario , 1992), hspA2 98.2%

hspA2 hsp70-2 (Bonnycastle , 1994)

hspA2

hsp70-2 antiserum 2A , 가

hspA2 cross-reactivity가 ,

hs pA2

hs pA2

**1. Hs pA2 expression vector**

*HspA2* gene open reading frame (ORF) primers  
, plasmid cloning primer *EcoRI* site  
*HindIII* site . sense primer  
sequence 5'-gctcgggaattcagtcaggatgtctg-3' antisense primer 5'-agtgcaagcttagtccact  
tcttcgat-3' . Genomic DNA .  
1.5ml microcentrifuge tube , 60-65°C protease  
K (0.5% SDS, 0.1M NaCl, 0.05M Tris [pH8.0], 2mM EDTA) protease  
K 가 100ug/ml가 200ul 가 6  
60-65°C . 8M potassium acetate 37.5ul  
chloroform 250ul 가 30 , 10,000 rpm 8  
. RNase  
RNA DNA . HspA2 open reading frame (ORF)  
. 94 °C 1 , 62°C 30 , 72°C 2  
cycle 35 cycle 72°C 10  
. ORF 1.93kbp *EcoRVHindIII*  
agarose gel electroelution pBS plasmid cloning, *E.coli* strain Top10+  
transformation sequencing . (Fig. 1.  
Schematic diagram). Plasmid pDMC3 *E.coli* *EcoRVHindIII*  
electroelution ORF . ORF expression vector  
pTrcHis B (Invitrogen, USA) cloning hspA2 expression vector pDMC4 .  
Hs pA2 IPTG 1 mM 2, 4  
hs p70-2 antiserum 2A hspA2  
cross-re activity .

2.

가

가

3.

lysis buffer (50mM Tris pH7.5, 150 mM NaCl, 0.1% NP-40, 50mM NaF, 1mM DTT, 1mM PMSF, 10 g/ml soybean trypsin inhibitor, 10 g/ml leupeptine, 10 g/ml aprotinin)

4 , 10,000 rpm 10

SDS-PAGE western blot micro BCA assay

#### 4. Western blot

20  $\mu$ g SDS sample buffer (62.5 mM Tris pH6.8, 40 mM DTT, 2% SDS, 0.025% bromophenol blue, 10% glycerol) 10  $\mu$ l resuspension 5 boiling 12% SDS-PAGE nitrocellulose membrane electrotransfer blots 5% non-fat dried milk가 가 TBS-T (TBS buffer + 0.1% Tween 20) 1 blocking TBS-T 3 antiserum 2A (1:2,000 ) 가 1 . TBS-T 3 anti-rabbit 2 , 4-chloro-1-naphtol detection .



Allen (1988) heat shock stress P70 .

P70 가 (Allen , 1988).

Rosario (1992) P70 *hsp70-2* gene P70

hsp70-2 heat shock

hsp70-2 antiserum 2A western blot .

Hsp70-2

Dix (1996) gene targeting

*hsp70-2*

1 .

hsp70-2 가 hspA2

(Bonnycastle , 1994). hsp70-2 98.2%

가 (Bonnycastle , 1994). hsp70-2 antiserum

2A가 hspA2 cross-reactivity가

antiserum 2A peptide sequence hspA2

가 . Hsp70-2 611

628 antiserum 2A

NH<sub>2</sub>-SKLYQGGPGGGGSSGGPT-COOH hspA2 hspA2

611 634 NH<sub>2</sub>-SKLYQGGPG

GGSGGGGSGGASGGPT-COOH . hsp70-2

hspA2 622 627 deletion hsp70-2 623 serine

hspA2 adenine 가 . antiserum 2A

가 hspA2 cross-reactivity가 .

hspA2 1 cloning antiserum 2A ,

western blot

73kDa fusion detect antiserum 2A가 hspA2 cross-reactivity

가 hspA2 hsp70-2

Bonnycastle (1994) *hspA2* mRNA

constitutive .

mRNA 가 mRNA (Fig.3). mRNA hs pA2 mRNA hs pA2

가 hs pA2 hs pA2

가 hs pA2 hs p70-2

hs pA2 가

가 cross-reactivity 가 , hs pA2

antiserum 2A가 hs pA2

가 hs pA2

가

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**Fig. 1.** Schematic diagram for construction of expression vector, pDMC4.



**A.**

**B.**

**Fig. 2.** Expression of hsp A2 protein in *E.coli*; **(A)** induction of hspA2 protein by IPTG 1 mM following 0, 2, and 4 hours; **(B)** For western blot; Lanes; **1**, IPTG non-induced control; **2**, hspA2 induced by 1 mM IPTG for 2 hours; **3**, hspA2 in human testis extract; **4**, hsp70-2 in mouse testis extract.



**A.**

**B.**

**Fig. 3.** Expression of the HspA2 protein in human tissues. (A) Lanes; 1, testis with normal spermatogenesis ; 2, testis with Sertoli-cell only syndrome, (B) Lanes; 1, testis with normal spermatogenesis; 2, breast; 3, stomach; 4, prostate; 5, colon; 6, liver; 7, ovary ; 8, epididymis .



