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Zoological Research

Cloning and Functional Analysis of the Full Length cDNA Sequence of Eukaryotic Translation Initiation Factor 5 in *Schistosoma japonicum*

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Abstract: The expressed sequence tag of eukaryotic translation initiation factor 5 (*eIF*5) from the *Schistosoma japonicum* adult worm cDNA library through subtractive hybridization between male and female worms was analyzed by the bioinformatics method. The overlapping sequences were assembled into one that includes the complete open reading frame (GenBank accession number: AY686501). The full-length cDNA of *SjeIF*5 was cloned into a pET-28c(+) vector, which generated a prokaryotic expression plasmid, and a fusion protein of 18 kDa was induced in *Escherichia coli*. The recombinant expression of *eIF*5 protein of *Schistosoma japonicum* was purified. The immunoprotection test against schistosomiasis demonstrated that the recombinant protein worked to a certain extent, especially in the reduction of eggs in the liver of the host.

Key words: *Schistosoma japonicum*; *eIF*5; Expression; Vaccine

日本血吸虫真核翻译起始因子 5 基因的 cDNA 克隆和免疫保护功能分析

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摘要: 利用抑制削减杂交法筛选日本血吸虫雌雄虫差异基因, 从中获得真核翻译起始因子 5 基因(*eIF*5)的表达序列标。对该序列进行生物信息学分析: 对其 5′ 端进行电子延伸, 获得含完整开放阅读框的 cDNA 序列(AY686501)。将其亚克隆到表达载体 pET-28c, 进行重组表达。免疫保护效果实验表明, 重组表达蛋白具有显著抑制血吸虫卵在寄主肝脏中的沉积效果。

关键词: 日本血吸虫; 真核翻译起始因子 5(*eIF*5); 表达; 疫苗
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Schistosomiasis caused by *Schistosoma japonicum* is a major public health problem in China and Southeast Asia (Chen et al, 2002). Schistosomes possess a complex life cycles involving several distinct developmental stages in invertebrate and vertebrate hosts. At present, there has only been limited success in eradicating this parasitic disease despite intensive effects involving chemotherapy, vector elimination and health e-

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ducation. Moreover, chemotherapy mainly using praziquantel induces drug-resistance among parasites (Ross et al, 2001). Therefore, development of an effective vaccine may lessen the pathogenesis and dissemination of the parasites in the veterbrate host and thus potentially control schistosomiasis. An unique trait of schistosomes is their sexually dimorphic character. Previous studies have suggested the successful completetion of schistosomes development, which elicit the pathogenesis and epidemic of next cycle, is dependent on the appropriate signals between male and female worms (Basch, 1988; Cheng et al, 2005; Den & Erasmus, 1985; Gupta & Basch, 1987; LoVerdo & Chen, 1991; LoVerdo et al, 2000). Identification of the differentiated expression genes between male and female parasites may uncover molecular mechanisms on sexual maturation and development of schistosomes.

The expressed sequence tag (EST) sequence of eukaryotic translation initiation factor 5 in *Schistosoma japonicum* (*SjeIF*5) was obtained in the male library by suppression subtractive hybridization (SSH). Previous studies demonstrated eukaryotic translation initiation factor 5 (*eIF*5) plays an essential role in protein synthesis in eukaryotic cells (Katsura et al, 1999). Moreover, *Caenorhabditis elegans* showed sick, sterile, embryonic lethal and slow growth when the *eIF*5 gene was knocked down by RNA interference (Maeda et al, 2001). Other studies showed *eIF*5 in *Schistosoma mansoni* (*SmeIF*5) possible regulation of some gene's expression from schistosomulum in the adult worm (Oliver et al, 1998). Thus *eIF*5 has crucial functions in worm development and may be a potential vaccine candidate against schistosomasis. In present study, in order to further characterize the function of *SjeIF*5 for protective immunity we obtained the full-length cDNA sequences of *SjeIF*5 by bioinformatics analysis and investigated the primal effect of recombinant protein that expressed in *E. coli* against schistosomasis.

1 Materials and Methods

1.1 Materials

The life cycles of *Schistosoma japonicum* (Chinese strain) were maintained in Shanghai Institute of Animal Parasitology by New Zealand rabbits and *Oncomelania hapensis*. Adult schistosomes obtained from infected rabbits were infected with 1500 cercariaes after 42 days. Zealand rabbits (about 3.5 kg, male) and Balb/c mice (about 25 g, male) were purchased from Shanghai Experimental Animal Center.

Trizol and Silver Bead DNA gel isolation kits were supplied by Shanghai Sangon Biological Engineering Technology & Service Company (Sangon, Shanghai, China). Access RT-PCR system kits were obtained from Promega Corporation (Promega, Madison, USA). T4DNA ligase was supplied by TaKaRa Bioengineer Company (TaKaRa, Dalian, China). His·Bind kits were purchased from Novagen Company (Novagen, Darmstadt, Germany). A DNA Thermal Cycler 480 was purchased from the PERKIN ELMER Company. Bio-Rad electrophoresis was obtained from Bio-Rad Labatories Company. GeneTools software (V1.0) was supplied by BIOTools Company (BIOTools, Alberta, Canada). A computer workstation (Pentium 4 2.00 Ghz, 384Mb RAM) was supplied by the Legend Company (Legend, Beijing, China). The primers were synthesized by Shanghai Genecore Company (Genecore, Shanghai, China).

1.2 Methods

1.2.1 Analysis of *SjeIF*5 by bioinformatics Sequence homology searches in databases (NCBI and EBI) were performed with the BLAST (http://www.ncbi.nih.gov/BLAST/) and FASTA (http://www.ebi.ac.uk/fasta33). The searches revealed the overlapping sequences, and formed a complete sequence that includes the open reading frame by GeneTools. The amino acid sequence of *SjeIF*5 was deduced by InterPro (http://www.ebi.ac.uk/Interpro) and aligned with *SmeIF*5 by GeneQuiz (http://www.ebi.ac.uk/GeneQuiz).

1.2.2 Cloning and expressing the recombinant protein in *E. coli* *SjeIF*5 gene was cloned from the total RNA of *Schistosoma japonicum* using RT-PCR amplification with sense primer 5′ ACGAATTCATAAGACTTGAAGCTGATCGT 3′, and antisense 5′ CCTCTCGAGAGAGACAACAGATAGGCGTT 3′ (*Eco*RⅠ and *Xho*Ⅰ restriction sites were underlined respectively) and was cloned into the expression vector pET28c which contains the same restriction sites. RT-PCR amplification conditions were as following: 48 ℃/4 – 5 min, 94 ℃/2 min; followed by 40 cycles of 94 ℃/45 s, 60 ℃/1 min and 68 ℃ for 2 min, and finally 68 ℃/8 min. The resulting recombinant vector containing *SjeIF*5 was named pET28c-*SjeIF*5.

pET28c-*SjeIF*5 was used to transform the competent cells of *E. coli* BL21; a single colony was injected into 20 mL LB liquid medium with 100 mg/L ampicillin; then incubated in a rotary shaker at 37 ℃ and 150 rpm. After the A_{600} of cell density reached 0.6, isopropylthio-β-D-galactoside (IPTG) was added to a final concentration of 1.0 mmol/L to induce gene ex-

pression. 1.5 mL culture broth was harvested and centrifuged for 2 hours. The pellets were dissolved into 2 × SDS-PAGE loading buffer and boiled for 10 min, centrifuged at 10 000 rpm for 2 min, and 5 μL supernatants were used to run the SDS-PAGE.

1.2.3 Purification of the recombinant protein The *E. coli* cells transformed by the plasmid of pET28c-*SjeIF*5 were induced to express the recombinant protein in rotary shaker at 37 ℃ for 2 hours and centrifuged at 10 000 rpm for 10 min. The pellet was resuspended using the binding buffer containing 5mM imidazole, 0.5 M NaCl and 20 mM Tris-HCl, and then the mixture was broken by sonication. After centrifugation, the supernatant was applied to His·Bind column following the manufacture's instruction. The purified protein was analyzed by SDS-PAGE.

1.2.4 Immunoprotection assessment Recombinant *SjeIF*5 (*rSjeIF*5) was used to immunize Balb/c mice. Before being injected into the mice, the fusion protein was dialysed to discard Tris followed by PBS (0.2 mol/L, pH 7.2). Mice in the test group were subcutaneously injected with 50 μg fusion protein emulsified in the same volume of Freund's Complete Adjuvant (FCA), while control groups with PBS in the same volume of FCA and challenged with (40 ± 1) cercariaes of *Schistosoma japonicum* after two weeks in the third vaccination, then sacrificed to perfuse the worms after 42 days. The immunoprotection was assessed by the reduction percentage of worms and eggs in the vaccinated animals compared with controls. Statistical analysis employed a one-way ANOVA.

2 Results

2.1 Analysis of *SjeIF*5 by bioinformatics

As shown in Fig. 1, a 1 328 bp cDNA sequence was assembled, which included initial codon ATG and 3′ploy(A) tail. It contained a 375 bp ORF, encoding a protein of 119 amino acids, with the initial codon ATG at position 853 and stop codon TGA at position 1209. The sequence of the cDNA can be obtained from GenBank (GenBank accession number: AY686501).

The putative protein of *SjeIF*5 contains the W2 domain, which performs functions of conserved protein-protein interaction domain in translation factors. Sequence alignment of *eIF*5 between *S. japonicum* and *S. mansoni* showed they exhibited 67% identity at the protein level (Fig. 2).

2.2 Expression of the recombinant *SjeIF*5 in *E. coli*

The cDNA fragment encoding *SjeiF*5 was correctly cloned into the pET28c$^{(+)}$ vector, which was confirmed with both double enzyme digestion and sequence analysis. Expression of the fusion protein *rSjeiF*5 in *E. coli* was a 18 kDa molecular in 10% SDS-PAGE (Fig. 3).

2.3 Purification of *rSjeIF*5

*rSjeIF*5 purified was showed by SDS-PAGE in Fig. 4.

2.4 Immunoprotection assessment

The experimental and control group mice were still living on the 42th day after the cercariaes challenge. The reduction rate of worm and egg was counted. Significant egg reduction rate was observed in the test group compared with the control group (Tab. 1).

3 Discussion

In the present study, the EST of *SjeIF*5 generated by SSH in the male worm library was cloned to obtain full-length cDNA sequences. We also employed RT-PCR and Western blotting to confirm the differentially expressed *SjeIF*5 between male and female worms. The result showed there is no significant difference between male and female worms in *SjeIF*5 expression at both transcript and protein level (data not shown). Because *eIF*5 plays an important role in protein synthesis we investigated its effect on protective immunity. The results showed the reduction rates of worms and eggs in final host were 20.12% and 21.17%, respectively. Moreover, significant egg reduction rate was observed in the test group as compared with the control group. The results suggested that the antibodies directed at the cloned *SjeIF*5 cDNA sequence expressed antigenic epitopes targeted the reproductive organ of female worms, and then decreased the production of eggs. However the development of worms was not significantly suppressed in the test group possibly due to the parasitic characters of schistosomes, i.e., they can adjust themselves to survive in the final host.

The analysis of *SjeIF*5 motifs showed it has some tyrosine kinase, some casein kinase Ⅱ and some protein kinase C phosphorylation sites. The result confirmed *eIF*5 in conjunction with GTP in other species act as a classical GTPase-activator protein (Das et al, 2001). Previous study in *S. mansoni* revealed *SmeIF*5 altered the transcript of volumes at the different stages (Oliver et al, 1998). It is known that schistosomes have a complex life in which a number of well characterized morphologic, behavioral and metabolic changes take place during the development of the parasite. These changes may be associated with changes at the transcriptional activity of many specific genes, and ulti-

mately at the level of their proteins.

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1     GTTTCGGATA TTGCGAAAGC CTTGTACCGG AAACCCATTT ATCTCACGAA
51    GTTCTTTGGT TGTGAACTGG GAGCACAAAT TCATGTAGAC GAAAAGAATG
101   AGCGTTATAT CGTTAATGGG GCTCATCAGG CGGATAAGTT ACAAGAGTTA
151   TTGGACGGTT TCATCAAAAA ATTTGTATTG TGTCAAAGTT GTGGGAATCC
201   GGAGACCACC ATGCACGTTA ATAAAAAGGG TGGCACAGTC ACCACGATTT
251   GCAAAGCATG CGGCAGCCAA GGACAGCTCG ACGTCACTCA TAGACTTACC
301   CAGTATATTG TTAAAAACCC ACCTGAACCA GAAGCTACCC CGGCAAAATC
351   AAAGCAAAAG AAAGGCAAGA AGTCAAAAGG TGAGAATGAA GATGATGACA
401   ATGACGCAGC CACTAATGGT GATGAGGGTA GTCGCATCAG TCCTGTTAAT
451   ACCGATGAGG TTGATGATGA CGAGGACTGG CTGGAGGACA CCACAGAGGA
501   GGACACCACA GAGGAGGCTC GCCGACAGCG GATTAATGCA TTGTCAGCTA
551   TGGCGAAGTC CCTAGCTTTG TGCGACGATG TAGAGCGATC TGAAAATGAC
601   CGGGCAGATA TATTCTACAA GCATTTGCTG CAAATACATC AATCTGACAA
651   AGACTAGTCA AGTCGTAAAG ACATAAGACT TGAAGCTGAT CGTCTCAACC
701   TTGGTCCGCG CGCTGTATTG GTTGTCGCAG AGGTGTTATT TAACAATCCT
751   TCTACTATCC TTGCTGACAT TAGAAAATAC TCCCCATTAC TACTTCTGTT
801   CACTCGGTCA GGTGAGGACC AAAAGCGAGC TCAAATATAC ACATGGGGTG
851   CTATGGCGAA GCTTATTGAA AGATACTCAG ATCATGACTT GTTGAGTAAG
        M  A  K   L  I  E   R  Y  S   D  H  D  L   L  S  K
901   GCCTGTCACA TCCTCAAGAC TTTGTACGAT TGCGATATCG TCGAGGAAGA
        A  C  H   I  L  K  T   L  Y  D   C  D  I   V  E  E  D
951   TGTTATTTTA GGGTGGTACG ATAGAGGACC GTCCAAAAAA TTTGTTTCGC
        V  I  L   G  W  Y   D  R  G  P   S  K  K   F  V  S
1001  GGGAATTAAG TACGAAAATA CTAGCCCGCT GTGCTCCTAT GGTGACGTGG
      R  E  L  S   T  K  I   L  A  R   C  A  P  M   V  T  W
1051  TTGCGACAAG CCGAAGAGGA AGAGTCTGAA AGTTCTGAAG GAGAATCTGA
      L  R  Q   A  E  E  E   E  S  E   S  S  E   G  E  S  E
1111  GAATAACAGT CCTAAGACTA CTCATCCTGT TAATGGCTCA AATGGTACAG
        N  N  S   P  K  T   T  H  P  V   N  G  S   N  G  T
1151  AGGCTGTTGA CGCCAACGGA ACAGGTGATG ATGATGACGA TGTGGACATT
      E  A  V  D   A  N  G   T  G  D   D  D  D  D   V  D  I
1201  GACGCTATAT GAATTATTCA CTCGACGTGT TTAAAGTTAT CGTAACGCCT
        D  A  I   *
1251  ATCTGTTGTC TCTAATTCCT CATATTGTCG ATTTAAAGTT ATTATATTCT
1301  TGTTTGCCAA AAAAAAAAAA AAAAAAAAAA
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Fig. 1 cDNA and putative protein sequences of *SjeIF5*

Initial codon sequence was underlined and in bold, extension of the 5' flank sequence was shaded, stop codon was indicated by an asterisk and underlined.

Fig. 2 Alignment of *eIF5* between *S. mansoni* and *S. japonicum*

Amino acids in different colors were highly homogeneous sequences, the shadowed sequences were the domain that plays translation initiation function.

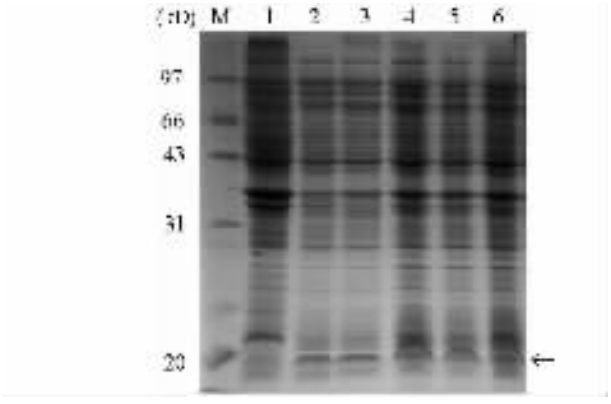

Fig. 3　SDS-PAGE analysis of the recombinant proteins in *E. coli*

M: marker; 2, 3, 4, 5, 6: pet28(c)-*SjeIF*5 induce by IPTG for 2, 4, 6, 8, and 10 h; 1: pET28(c) induced by IPTG for control. Arrow showed the *rSjeIF*5.

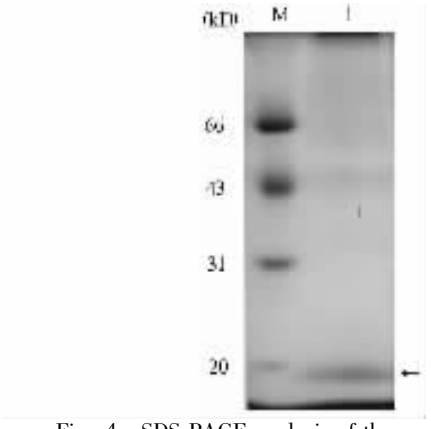

Fig. 4　SDS-PAGE analysis of the purified *rSjeIF*5

M: Marker; 1: *rSjeIF*5 showed by arrow.

Tab. 1　Worm and egg reduction rates induced by *rSjeIF*5 in mice

Groups	Average worm burden	Worm reduction rates(%)	EPG	Egg reduction rates(%)
Experimental group	20.17 ± 4.50	20.12 ± 1.50	6 834.56 ± 1 246.76	21.17 ± 3.43*
Control group	25.32 ± 5.30		8 730.13 ± 1 899.43	

Values were represented as $X \pm s$ ($n = 30$). *$P < 0.05$ vs. control group.
EPG means the number of eggs per one-gram liver.

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