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Cloning, Expression and Immunization of The Hypoxanthine-guanine Phosphoribosyltransferase for *Schistosoma japonicum* Chinese Strain*

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Abstract A 1 270 bp full-length cDNA fragment was obtained from the *Schistosoma japonicum* (Chinese strain) adult cDNA library after the 3′ and 5′ ends of the incomplete expression sequence tag (EST) of hypoxanthine-guanine phosphoribosyltransferase of *Schistosoma japonicum* (*Sj*HGPRT) were amplified by the anchored PCR with 2 pairs of primer that were designed according to the published incomplete *Sj*HGPRT EST and the sequence of multiclone sites of library λgt11 vector. Sequence analysis indicated that this fragment, with an identity of 82% to hypoxanthine-guanine phosphoribosyltransferase of *Schistosoma mansoni* (*Sm*HGPRT), contained a complete open reading frame(ORF). The deduced amino acid sequence showed 83% identity to that of *Sm*HGPRT. This fragment was cloned into the prokaryotic expression vector pQE30, and subsequently sequenced and expressed in *Escherichia coli*. SDS-PAGE revealed that *M* of the recombinant protein was about 28 ku. Western-blot analysis showed that the recombinant protein was recognized by the polyclonal antisera from rabbits immunized with *Schistosoma japonicum* adult worm antigen. Mice vaccinated with recombinant protein revealed significant worm burden, liver eggs per gram (LEPG), fecal eggs per gram (FEPG) and intrauterine eggs of the female worms reduction percentage, compared with the controls. Taken together, the *Sj*HGPRT full-length cDNA can be cloned and expressed in *E.coli* as a recombinant protein that elicited immunity against the challenge infection with *Schistosoma japonicum*, indicating its potential as a partial protection vaccine candidate.

Key words *Schistosoma japonicum*, hypoxanthine-guanine phosphoribosyltransferase, cloning, expression

Schistosomiasis is a parasitic disease transmitted to man and other mammals *via* snails. Chitsulo *et al*.[1] reported that over 600 million people in 74 countries are at risk of infection, and 200 million people are infected worldwide, of whom 120 million are symptomatic and 20 million suffer from severe disease. In some countries, especially in China the incidence of schistosomiasis has been increasing, with the disease coverage of 12 provinces along the Yangtze River, and about 100 million people were at risk of the disease. Although schistosomiasis can be treated with Praziquantel, high recurrence rates require frequent treatment[2]. Today it is estimated that 860 000 people and several hundred thousand domestic animals are infected. New approaches for controling the disease are urgently needed. The WHO proposed that the development of vaccines for schistosomiasis be a priority strategy in the control of the disease[3].

As we know, the main pathologic lesion in patients or animals with schistosomiasis japonica is the granuloma formation around eggs deposited in the tissues of the host. Many scientists try to make an anti-schistosomiasis vaccine to weaken the pathologic lesions. Garcia *et al* [4]. reported the evidence of anti-embryonation immunity and egg destruction in mice vaccined with immature eggs of *Schistosoma japonicum*. Wang *et al*.[5~7] found that the 26～28 ku components extracted from the soluble immature egg antigen (SIEA) of *S. japonicum* are the major antigens responsible for both anti-fecundity and anti-egg immunity. It was confirmed by China National

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Schistosome Vaccine Unitive Test that this component, also known as the natural molecular vaccine (*Sj*SIEA26 ～28 ku) or vaccine No 1, demonstrated the best immunoprotective results in the experimental animals (worm reduction rate of 53.9% and pairing reduction rate of 89.3%)[8]. *Sj*SIEA26～28 ku was therefore regarded as a candidate vaccine to be used for the induction of anti-embryonation and anti-fecundity immunity. Since sufficient quantities of the 26～28 ku components used for a vaccine cannot be obtained from immature eggs, we sought to express the components one by one in bacteria. By means of two dimensional gel electrophoresis, we found that the *Sj*SIEA26～28 ku components consist of more than 10 different protein spots[9]. Peptide mass finger print maps and PeptIdent software analyses showed that one of the protein spots was significantly homologous to hypoxanthine-guanine phosphoribosyltransferase of *Schistosoma japonicum* (*Sj*HGPRT). Whether *Sj*HGPRT is a major target antigen responsible for the anti-embryonation and anti-fecundity immunity induced by *Sj*SIEA26～28 ku component remains an interesting topic[10]. Since the full-length cDNA of the *Sj*HGPRT was not isolated at present, we planned to clone the full-length cDNA of *Sj*HGPRT from adult worm cDNA library of *S. japonicum* and express *Sj*HGPRT in bacteria.

In this study, we for the first time cloned the full-length cDNA of hypoxanthine-guanine phosphoribosyltransferase of *S. japonicum*(*Sj*HGPRT) from *S. japonicum* adult worm cDNA library by anchored PCR. In addition to sequence analysis, we constructed pQE30/*Sj*HGPRT prokaryotic expression plasmid and successfully expressed the protein in large quantity in *E.coli*. Western blot assay indicated that the gene cloned was of strong antigenicity. At the same time a large amount of recombinant protein was purified and applied to animal immunoprotection study for the purpose of evaluating its potentiality as a candidate vaccine against *S. japonicum*.

1 Materials and methods

1.1 cDNA library, plasmid, bacteria, biochemical and chemical reagents

The adult worm cDNA library for *S. japonicum* Chinese strain was a generous gift from Professor Chen Shuzhen of Nanjing Medical University. pQE30 prokaryotic expression plasmid, *E.coli* M15 and Y1090 were kindly donated by professor Wu, Medical College, Sun Yat-sen University. Restriction enzymes, T4 DNA ligase and ExTag DNA polymerase were purchased from TaKaRa Company Limited (Dalian, China). The HRP-conjugated goat anti-rabbit IgG was from Sino-American Biotechnology Company. All other regents were purchased from Sigma in the highest purity available.

1.2 Animals

Six-week-old Kunming female mice and domestic rabbits were used in the experiment. Animals were housed in a barrier environment in the Central of animal Health of Xiangya (CAHX). Mice and rabbits were fed with specific food and water. All animals were acclimatized to the conditions for a week prior to experiment. Animal experiments were performed according to the protocols approved by CAHX Animal Care and Use committee.

1.3 Parasites and antisera preparation

The snail used for schistosome infection were obtained from Hunan Provincial Institute of Schistosomiasis Research, Yueyang, Hunan China. The rabbits infected 2000 cercariaes for 42 day were anesthetized with halothane, and then *S. japonicum* adult worms were recovered by perfusion from the portal vein of infected rabbits and were homogenized on ice to prepare soluble adult worm antigen. Three domestic rabbits were vaccined by subcutaneous injection with 0.3 mg of soluble adult worm antigen prepared as above emulsified in FCA or FIA (Freund's complete or incomplete adjuvant) of the same volume for the primary and two boost vaccinations respectively. The polyclonal antiserum against *S. japonicum* adult worm antigen (*Sj*AWA) was collected by carotid artery two weeks after the third immunization. The sera were pre-absorbed with *E.coli* /pQE30/M15 cell lysate prior to test.

1.4 Preparation of template

Lambda phage DNA was prepared by plating the phage at 10^5 pfu per plate on 150 mm plates. Confluent lysis was achieved at 42℃ after approximately 12 h. The phage were recovered by rotating the plates at 4℃ for 12～16 h after the addition of 8～10 ml of SM buffer (0.1 mol/L NaCl, 8 mmol/L $MgSO_4$, 0.2% gelatin, 50 mmol/L Tris, pH 7.5). The buffer was then centrifuged at 2 000 *g* for 5 min at 4℃. The pellets were discarded and supernatants were extracted with 4% polyethylene glycol (PEG). After incubation at room temperature for 30 min the sample was recentrifuged at 12 000 *g* for 15 min at 4℃. The

supernatant was removed and the pellets were resuspended in a total volum of 100 μl of distilled water. After being boiled for 5 min, the sample was stored at −20℃.

1.5 Primer design

According to the contiguous nucleotide sequence of the multiclone sites of cDNA library vector λgt11, expression sequence tag(EST) of *Sj*HGPRT(accession number: BU803192) and the *S. japonicum* full-length cDNA obtained subsequently (accession number: AY841891), six primers P1, P2, P3, P4, P5, P6 were designed by means of Oligo Primer Analysis Softwere and synthesized by TaKaRa Company Limited. The following are the sequences of the six primers: P1, 5′ ggtggcgacgactcctggagcccg 3′, P2, 5′ ttgacaccaggcc-aactggtaatg 3′, P3, 5′ ccctcatacctaggagacgtgc 3′, P4, 5′ attctctgtaccttgtgtgccgaa 3′, P5, 5′ gcggatccatg-tctggtgttatgaaaag 3′, P6, 5′ acgtcgacttaaactgatttcgg-cacac 3′. (P1, P2 primers is the contiguous nucleotide sequence of the multiclone sites of cDNA library vector λgt11)

1.6 Production of DNA by PCR

The 5′ end of EST of *Sj*HGPRT was amplified using a Hema 480 thermal cycler in a total volume of 100 μl with 2 μmol/L of each primer P1 and P3, 0.2 μg cDNA library of *S.japonicum* adult worm as template, 0.2 mmol/L of dNTPs, 1 U of ExTaq polymerase and buffer. The PCR was performed firstly at 96℃ for 3 min, then 35 cycles of 94℃ 40 s, 56℃ 20 s and 72℃ 40 s, and 72℃ 8 min. The PCR amplification of the 3′ end of EST of *Sj*HGPRT was performed with the same thermal cycler and primer P4, P2. The PCR condition was 3 min at 96℃, followed by 35 cycles of 94℃ for 1 min, 61℃ for 1 min and 72℃ for 2 min, and finally at 72℃ for 8 min. The amplification products were analyzed by electrophoresis in a 1% TAE-agrose gel and purified using a kit (TaKaRa Co.), and finally sequenced from both ends by ABI PRISM™ 377XL DNA Sequencer. For all sequences derived from PCR-amplified DNA, at least five clones from separated reactions were sequenced in order to minimize potential errors. A 1 270 bp full-length cDNA of *Sj*HGPRT was obtained after spelling EST (BU803192), the 3′ end and 5′ end of EST of *Sj*HGPRT with electronic software.

1.7 Cloning and expression plasmid construction of the full-length cDNA

After a 1 270 bp full-length cDNA fragment of *Sj*HGPRT was obtained by spelling EST (BU803192), the 3′ end and 5′ end of EST of *Sj*HGPRT with electronic software, the primers P5 and P6 used for ORF amplification of the full-length cDNA could be designed and synthesized. The entire coding sequence (CDS) for the schistosomal HGPRTase was amplified with primer P5 and P6 which were flanked with restriction enzymes *Bam*H Ⅰ and *Sal* Ⅰ respectively, P5 as the sense primer, P6 as the anti-sense primer. The PCR condition was as the same as that of the 3′ end amplification of the EST of *Sj*HGPRT. The *Bam*H Ⅰ/*Sal* Ⅰ fragment (696 bp) was purified using agar gel DNA purification kit (TaKaRa). The target fragment was then ligated to the same restriction enzyme sites of pQE30 vector to obtain recombinant plasmid pQE30/*Sj*HGPRT by standard manipulation. The recombinant plasmid was transformed into *E.coli* M15 competent cells. The recombinants were confirmed by restriction enzymes digestion, agar gel electrophoresis, PCR and finally sequencing.

1.8 Protein expression and purification

The *E. coli* M15 with recombinant plasmid or pQE30 were grown at 37℃ with a final concentration of 100 g/L ampicillin, and isopropyl-β-D- thiogalactopyranoside (IPTG) was added to a final concentration of 1.0 mmol/L, when optical density in the culture was 0.6. After growing for another five hours at 28℃, the *E.coli* M15 induced with IPTG were harvested by centrifuge with 3 000 *g* for 10 min at 4℃. The pellets were resuspended in buffer A (50 mmol/L Tris-HCl, pH 8.0) and lysed by sonication on ice. After centrifuged at 10 000 *g* for 15 min at 4℃, the pellet was resolved in buffer A containing 8 mol/L urea and recentrifuged at 10 000 *g* for 15 min at 4℃, and finally the supernatant containing the recombinant protein was stored at −20℃. After SDS-PAGE, the gels were incubated in 0.1 mol/L KCl for 10～20 min at 4℃, and the targets were cut out from gels. The recombinant polypeptide was electroeluted out of the gels in the cup at negative electrode and into the cup at positive electrode with electrophoresis buffer of 12.5 mmol/L Tris-100 mmol/L glycine-0.05% SDS at 200 mV for 2 h. The eluted polypeptide was dialyzed against sodium phosphate buffer (0.01 mol/L, pH 7.2) overnight at 4℃ and freezed out at −70℃.

1.9 Western blot

According to the conditions described by Zhu *et al.*[11] with the following modifications Western blot was conducted.12% SDS polyacrylamide gel was used to analyze the recombinant protein. SDS-PAGE was

performed in the Mini Protein System (Bio-Red). After electrophoresis, the gels were either Coomassie Brilliant Blue R250-stained to visualize the protein bands or transferred onto nitrocellulose member analyzed by Western blot. For Western blot, the membrane was blocked with 5% skimmed milk in PBS-0.5% Tween 20 (blocking solution). The nitrocellulose filter was probed with the first antisera, the rabbit anti-SjAWA diluted in blocking solution (1∶200). A peroxidase labeled goat anti-rabbit IgG was used as the secondary antibody (1∶5 000) and specific binding was detected with H_2O_2 and diaminobenzidine (DAB) as a chromogenic substrate.

1.10 Immunization and parasite challenge

Immunoprotections were performed using the methods described by Chen *et al*.[12] with the following modification. 20 Kunming female mice were randomly divided into 2 groups. The test groups were subcutaneously injected at three sites with 100 μg recombinant *Sj*HGPRT (r*Sj*HGPRT) emulsified in 0.1 ml Freund's complete adjuvant (FCA). The control group with same dosage of PBS plus FCA was set up at the same time. The initial injections were followed by two sets of boosters at two weeks intervals. Two weeks after the third immunization the titer of specific antibody against r*Sj*HGPRT in mouse sera was detected with dot enzyme-linked immunosorbent assay (Dot-ELISA), and mice were percutaneously infected with (40±2) *S. japonicum* cercariae on abdominal skin. All mice were sacrificed and perfused 6 weeks after the cercariae challenge infection. Immunoprotections were assessed with the percentage reduction in worm burden, liver eggs per gram (LEPG), fecal eggs per gram (FEPG) and intrauterine eggs of the female worms according to the formula: % protection= $(1-Ni/Nc)\times 100\%$, where *Ni* and *Nc* are the mean number of worm burden, LEPG, FEPG and intrauterine eggs recovered from the immunized and the control, respectively.

1.11 Statistical analysis

Data are expressed as ($\bar{x}\pm s$). Difference between groups were analyzed by Student's *t*-test, with a $P<0.05$ considered significant.

2 Results

2.1 Anchored PCR productions and full-ength cDNA

In the present report, two fragments of 150 bp and 600 bp were obtained after the 5′ and 3′ ends of EST of *Sj*HGPRT were amplified from *S.japonicum* adult worm cDNA library by the anchored PCR with primers P1/P3 and P4/P2 respectively (Figure 1). There were 78 bp and 84 bp nucleotide overlaps with the 5′ and 3′ terminal of EST of *Sj*HGPRT (BU803192) in 150 bp and 600 bp fragments respectively, which indicated that the two fragments amplified were needful and interesting. After the three fragments mentioned above were pieced together with electronical software, a 1 270 bp full-length cDNA was obtained and submitted to GenBank (accession number: AY841891). Subsequent database search revealed that the deduced coding sequence (CDS) exhibited indentities of 82% and 83% to that of *S. mansoni* hypoxanthine-guanine phosphoribosyltransferase (*Sm*HGPRT) (accession number X07883) at nucleotide and amino acid levels, respectively.

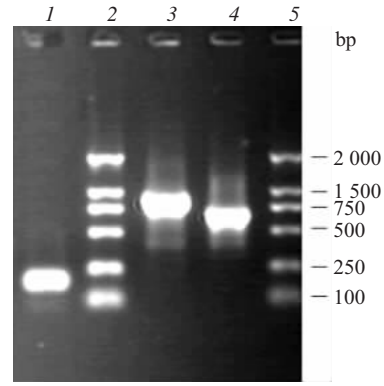

Fig. 1 Agarose gel electrophoresis of PCR amplified products

1: Anchored PCR amplified product of 5′ end of *Sj*HGPRT; *2*: DL2000 marker; *3*: PCR amplified product of ORF of full-length *Sj*HGPRT cDNA; *4*: anchored PCR amplified product of 3′ end of *Sj*HGPRT; *5*: DL2000 marker.

2.2 Determination and construction of the recombination expression plasmid

The entire coding sequence (CDS) of the *Sj*HGPRT amplified from cDNA adult worm library with primers P5/P6 was about 700 bp (Figure 1). The fragment was cloned into the expression vector pQE30 digested with the same restriction enzymes *Bam*HⅠ and *Sal*Ⅰ to generate pQE30/*Sj*HGPRT. Digested with restriction enzymes *Bam*HⅠ and *Sal*Ⅰ, an about 700 bp DNA fragment was cleaved off from the recombinant plasmid, designated as pQE30/*Sj*HGPRT. With the pQE30/*Sj*HGPRT DNA as a template and P5/P6 as primers, the product of PCR revealed similar size to the insert from the recombinant plasmid (Figure 2).

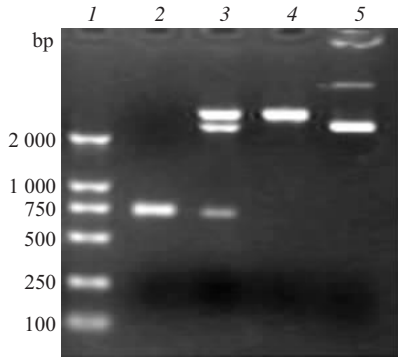

Fig. 2 Restriction and PCR identification of the recombinant plasmid pQE30/*Sj*HGPRT

1: DL2000 marker; *2*: PCR amplification using recombinant pQE30/*Sj*HGPRT as template and P5/P6 as primers; *3*: Recombinant pQE30/*Sj*HGPRT digested by restriction enzyme *Bam*HⅠ and *Sal*Ⅰ; *4*: pQE30 digested by restriction enzyme *Bam*HⅠ and *Sal*Ⅰ; *5*: pQE30 plasmid.

2.3 Protein expression and purification

The recombinant plasmid pQE30/*Sj*HGPRT and pQE30 were used for expression. After transformed into *E. coli* M15 competent cells, prokaryotic expression was induced with 1.0 mmol/L IPTG at 28℃. Induced expression in *E. coli* resulted in a constant level of recombinant protein production. The prokaryotic expression protein, designated as r*Sj*HGPRT, was about 28 ku recombinant protein in large quantity, which can be visualized in 12% SDS-PAGE (Figure 3) and recognized by polycloned antiserum against *Sj*AWA (Figure 4). In the present paper, the prokaryotic expression recombinant protein was purified as the following procedures: SDS-PAGE, 0.1 mol/L KCl-staining, gel band section, electroelution and lialyses.

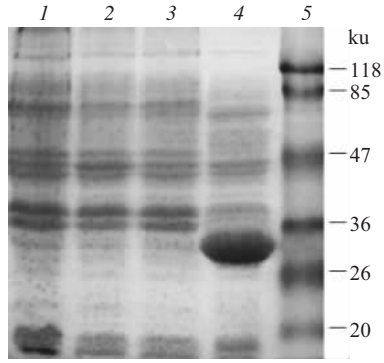

Fig. 3 SDS-PAGE analysis of expression of pQE30/*Sj*HGPRT and pQE30 in *E. coli* M15

1: pQE30 with IPTG induction; *2*: pQE30 without IPTG induction; *3*: pQE30/ *Sj*HGPRT without IPTG induction; *4*: pQE30/ *Sj*HGPRT with IPTG induction; *5*: Protein marker.

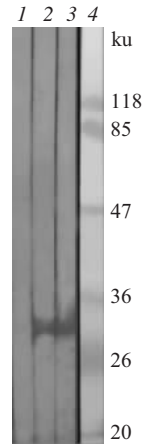

Fig. 4 Western blot analysis of the immunoreactivity of expression product of the recombinant plasmid pQE30/*Sj*HGPRT in *E. coli* M15

1: Incubated with normal rabbit sera; *2,3*: Incubated with sera from rabbit immunized with *S. japonicum* adult worm antigen; *4*: Molecular marker.

2.4 Immunoprotection of the r*Sj*HGPRT

Recombinant protein (r*Sj*HGPRT) was used to immunize Kunming female mice. Two weeks after the third time immunization, dot-ELISA showed the titers of specific IgG in sera of the vaccination group before challenge reached 1∶6 400, which were significantly higher than that of the control group (Figure 5). Mice were sacrificed and perfused six weeks after infection. The recover worms , LEPG, FEPG and intrauterine

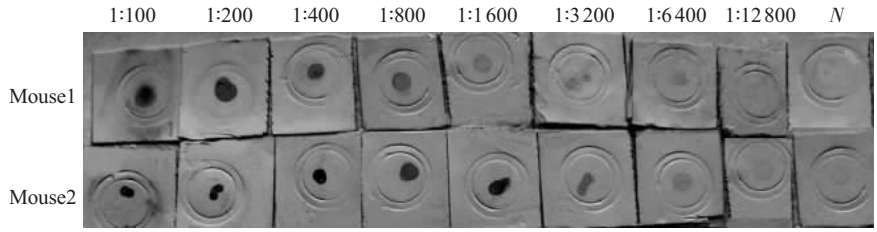

Fig. 5 Detection of specific antibody in sera from mouse1 and mouse2 immunized with r*Sj*HGPRT by dot-ELISA

N: 1:100 sera from mouse immunized with FCA.

eggs were counted. Significant worm burden reduction rates (22.28%, 21.23%, $P < 0.05$), LEPG reduction rates (42.90%, 45.70%, $P < 0.01$), FEPG reduction rate in Experiment Ⅱ (44.18%, $P < 0.01$) and intrauterine eggs reduction rate in Experiment Ⅱ (17.45%, $P < 0.05$) were observed in the test group, compared with the control group, as shown in Table 1.

Table 1 Effect of immunization with r*Sj*HGPRT on *S. japonicum* in Kunming mice

r*Sj* HGPRT	Immunized group	Control	% Reduction	P-Value
Experiment Ⅰ				
No.of mice	10	9		
LEPG/×10^3	17.09±1.48	29.94±2.60	42.90	<0.01
Worm burden	22.88±4.84	29.44±7.94	22.28	<0.05
Experiment Ⅱ				
No.of mice	10	10		
LEPG/×10^3	15.42±1.23	28.40±3.52	45.70	<0.01
Worm burden	23.63±3.54	30.00±2.98	21.23	<0.05
FEPG	1 578.81±239.78	2 828.28±466.18	44.18	<0.01
Intrauterine eggs	159.00±73.67	192.60±67.44	17.45	<0.05

Reductions of LEPG, worm burden, FEPG and intrauterine eggs in the vaccination group were significant ($P < 0.05$), compared with the control group.

3 Discussion

HGPRT catalyzes the phosphoribosylation of hypoxanthine and guanine to form inosine monophosphate (IMP) and guanosine monophosphate (GMP) in the presence of 5′ -phosphoribosyl-1-pyrophosphate (PRPP). Many parasites are known to lack enzymes for the de novo biosynthesis of purines[13, 14]. They need to obtain hypoxanthine from host cells and utilize it as a purine precursor for nucleic acid synthesis. This pathway is important for the salvage of purine nucleic acid biosynthesis. In addition to the lack of interconversions between adenine and guanine nucleotides in parasite, HGPRT is an essential enzyme in the human parasite *S.mansoni* for supplying guanine nucleotides and is proposed as a potential target for antiparasitic chemotherapy [15]. However, no investigation in its immunoprotection as a kind of candidate vaccine for schistosomiasis has been found.

Although there were many reports about HGPRT of *S. mansoni*[13, 15], the nucleic acid sequence of the *Sj*HGPRT, especially the full-length cDNA for *S. japonicum*, was unknown. This study focused on amplification the 3′ end and 5′ end of incomplete EST of *Sj*HGPRT from adult worm cDNA library in an effort to obtain the full-length cDNA of *Sj*HGPRT for expression in bacteria. After anchored PCR, a 1 270 bp full-engthcDNA was obtained. Sequence analysis of the full-length cDNA for *S. japonicum* HGPRT indicated that it contains the biggest ORF beginning with the initiation codon ATG at position 17 to 19 and ending with TAA termination codon at position 710 to 712, followed by a strong termination codon TAA at position 737 to 739. The ORF is preceded by a 16 bp 5′ untranslated region and followed by a 558 bp 3′ untranslated region in addition to the polyA tail. The nucleotides around the start codon (ACGACATGTC) fulfill Kozak's criteria[16] for G in the −3 position and two polyadenylation addition signals ATTAA and AATAA are positioned at 1 216 and 1 236 respectively. Sequence analysis revealed that the full-length cDNA of *Sj*HGPRT shows 82% identity with *S. mansoni* HGPRT at the nucleotide level and contains a 693 bp open reading frame, which predicts a protein of 231 amino acid residues that is the same in number as that of *S. mansoni*. The deduced amino acid sequence exhibits 83% identity with *S. mansoni* HGPRT and only 46% identity with human HGPRT.

By cloning into pQE30, the full-length coding sequence was effectively expressed with proven antigenicity by Western blot using anti-adult worm sera. We vaccinated mice with r*Sj*HGPRT, PBS plus

FCA as control group. Significant immunoprotections against *S. japonicum* challenge were induced in r*Sj*HGPRT group comparing with the control group (Table 1). As *Sj*HGPRT is just one of the 10-plus different molecules in *Sj*SIEA26 ～28 ku, its immunoprotection against *S. japonicum* challenge was not as effective as that of the *Sj*SIEA26～28 ku whole molecules. However, the results of the single-molecule immunoprotection were encouraging and hopeful. In r*Sj*HGPRT-immunized mice, the degree of worm burden reduction was less than that of eggs reduction, suggesting that the single molecular vaccine effect may be primarily on anti-embryonation or anti-fecundity immunity, which resembled our previous works[6, 7].

In short, the results mentioned above indicate that the full-length cDNA of *Sj*HGPRT has been successfully cloned and *Sj*HGPRT is a promising candidate vaccine molecule, which also sheds light on gene recombinant approaches to the development of *S. japonicum* vaccine and lays a basis for our next investigation on the enzymological studies, X-ray crystallographic analysis, especially the combined immunoprotection with other molecules in the *Sj*SIEA 26～28 ku components.

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日本血吸虫中国株次黄嘌呤鸟嘌呤磷酸核糖转移酶的克隆、表达及其免疫保护性研究 *

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摘要 根据基因库中日本血吸虫次黄嘌呤鸟嘌呤磷酸核糖转移酶 (HGPRT) EST (BU803192) 以及日本血吸虫成虫 cDNA 文库载体 λgt11 多克隆位点邻近核苷酸序列设计引物，以日本血吸虫成虫 cDNA 文库为模板，采用锚式 PCR 对 *Sj*HGPRT 基因不完整的 3′端和 5′端进行扩增、测序，用电子软件拼接，获得 *Sj*HGPRT 全长 cDNA (1 270 bp)，经序列分析，推断该片段含有编码 *Sj*HGPRT 基因的完整阅读框，其编码基因与曼氏血吸虫次黄嘌呤鸟嘌呤磷酸核糖转移酶 (*Sm*HGPRT) 全长编码基因碱基一致性为 82%，其理论推导的氨基酸组成与曼氏血吸虫次黄嘌呤鸟嘌呤磷酸核糖转移酶的一致性约为 83%. 将其编码基因克隆到表达载体 pQE30 上，在大肠杆菌 M15 中获得准确、高效表达，表达产物分子质量约为 28 ku. 用日本血吸虫成虫抗原免疫血清对表达产物进行蛋白质印迹检测，在预测位置上出现明显的识别条带. 重组蛋白动物免疫保护性结果显示：在虫荷、每克肝卵、每克粪卵和雌子宫内卵数方面，疫苗组与对照组比较差异均具有显著性 ($P < 0.05$，$P < 0.01$). 结果表明，日本血吸虫次黄嘌呤鸟嘌呤磷酸核糖转移酶 (*Sj*HGPRT) 全长 cDNA 成功克隆并在大肠菌中得到表达，表达产物具有良好的抗原性和动物免疫保护效果，是一种潜在的具有部分免疫保护性的抗血吸虫病疫苗候选分子.

关键词 日本血吸虫，次黄嘌呤鸟嘌呤磷酸核糖转移酶，基因克隆，基因表达
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