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Short communication

Localization and expression profiling of a 31 kDa antigenic repetitive protein Sjp_0110390 in *Schistosoma japonicum* life stages

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ABSTRACT

Sj7TR is a 13 kDa repetitive region of a 31 kDa protein in *Schistosoma japonicum* known as Sjp_0110390 that showed high sensitivity and specificity in antibody detection against schistosomiasis patients. However, the current database for *S. japonicum* genes characterized it only as an expressed protein. A more thorough understanding of this antigenic protein is therefore necessary to possibly give more information about the nature of this protein and its role in the parasite. In this study, immunolocalization and expression profiling were done for Sjp_0110390 on the different stages of the parasite. Immunofluorescent assay showed that Sjp_0110390 was expressed in the young stages of the parasites including the schistosomula, eggs, aquatic and intra-molluscan stages. This was supported by the reverse-transcriptase PCR which confirmed the stage-specific expression of Sjp_0110390 and Western blot test which detected the protein in the extracted eggs proteins, but not in the adults. Furthermore, it was also highly expressed in infected *Oncomelania hupensis nosophora* snails suggesting that Sjp_0110390 might have a role in the development of the parasite inside the intermediate host. This result also suggests that Sj7TR might be used not only for human diagnosis but to detect snail infection as well.

Schistosoma japonicum is the major causative agent of schistosomiasis in South East Asia and China. Current available serological techniques for the diagnosis of schistosomiasis still depend on the use of crude egg antigen. However, the use of crude antigen has proven to produce false positive results, which can affect the disease surveillance in areas that already reached elimination level. Also, large-scale production of crude antigen is difficult which requires maintaining the parasite's whole life cycle inside the laboratory. The use of recombinant antigens therefore might be used to address these problems in schistosomiasis diagnosis. One recombinant antigen already tested for diagnostic purposes is a 13 kDa repetitive region of a 31 kDa protein Sjp_0110390 (GeneDB ID: Sjp_0110390). In a previous paper, the tandem repeat was labeled as Sj7TR and was tested against human sera [1], being a good candidate as a diagnostic antigen. However, Sjp_0110390 was not yet characterized and its expression in the life stages of *S. japonicum* has not yet been known. Available EST sequences (GenBank ID: CV42804.1, CV746246.1, CV750916.1, CV736328.1) corresponding to this gene were reported at the ones whose developmental stages were unknown [2].

Diagnostic antigens like Sj7TR need to be characterized to know their possible function in each stage of the parasite. This may help understand the principles behind their serological value in the mammalian hosts as well as determine what other possibilities this antigen might be capable of. Histidine-rich protein II of *Plasmodium falciparum* for example was found to be secreted through the surface membrane of the infected erythrocytes [3] making it a good antigen for the diagnostic tests developed years later [4,5]. Lactate dehydrogenases in *Taenia solium* were not specific antigens for immunodiagnosis but because of their characterization were suggested to be candidate targets for anti-*Taenia* drugs and vaccine development [6]. In this study, immunolocalization and expression

Abbreviations: ELISA, enzyme-linked immunosorbent assay; EST, expressed sequence tag; IFAT, indirect immunofluorescent antibody test; NSS, normal saline solution; ORF, open reading frame; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate buffered saline; PVDF, polyvinyliden difluoride; RT, room temperature; SDS, sodium dodecyl sulfate; T-PBS, phosphate buffered saline with Tween-20.

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profiling were done on Sjp_0110390 in both the intra-mammalian and intra-molluscan life stages to give some insights on this schistosome protein.

The cDNA sequence of Sjp_0110390 comprised a 822 bp ORF encoding 273 amino acid residues with the predicted molecular mass of 31.04 kDa and theoretical isoelectric point of pH 3.84 [7]. Based on an online tandem repeats finder (http://tandem.bu.edu/trf/trf.html), the sequence has 4.9 copies of the 165 bp-long repeat unit [8]. Sequence analysis reveals that it is highly hydrophilic, does not possess any transmembrane domain [9] and has no signal peptide [10]. The protein was also shown to not contain nuclear localization signal [11] and predicted to be cytoplasmic [12].

Yamanashi strain of *S. japonicum*, maintained using standard laboratory procedures in female ICR mice and their snail hosts, *Oncomelania hupensis nosophora*, was used for this study. Animal experiments done in this study were conducted according to ethical guidelines for the use of animal samples permitted by Animal Care and Use Committee, Dokkyo Medical University (Permit No. 0029) in accordance with the Guidelines for the Care and Use of Laboratory Animals, Dokkyo Medical University, The Law Concerning Kind Treatment and Management of Animals (Law No. 221) and Japanese Government Notification on Feeding and Safe-keeping of Laboratory Animals (No. 6), as well as by Obihiro University of Agriculture and Veterinary Medicine (Permit No. 23-153). Infected mice were anesthesized using ether before they were sacrificed. Perfusion method with normal saline solution was done to collect the intra-mammalian stages of the parasites. Schistosomules were taken from the perfused fluid of a 2 weeks post-infected mouse while the juvenile and fully developed adults were collected from the portal and mesenteric veins of 2 weeks and 10 weeks post-infected mice respectively. *S. japonicum* eggs were isolated from infected mouse intestines by a digestion method using actinase E (No. 122, Kaken Pharmaceutical Co., Ltd., Tokyo, Japan) and collagenase (C6885, Sigma–Aldrich, St. Louis, MO). Miracidia were collected after the eggs were hatched in fresh water. *O. h. nosophora* snails were infected with 5 miracidia and 6 months later were sacrificed to collect both the sporocysts and cercarial stages. The sporocysts were isolated by crushing the snails and separating them from the snail tissues whereas the cercariae were shed from the crushed snails. Another batch of *O. h. nosophora* snails were infected with 100 miracidia and were sacrificed 10 days later for histological sectioning. Tissues from non-infected snails were also taken as samples.

Recombinant Sj7TR protein (rSj7TR) was prepared as previously described [1,13]. Polyclonal antibody was produced using male ICR mice immunized intraperitoneally each with 100 μl of the recombinant rSj7TR coupled with complete Freund's adjuvant (Sigma–Aldrich, St. Louis, MO) for the first immunization and incomplete Freund's adjuvant (Sigma–Aldrich, St. Louis, MO) for the subsequent booster immunization. The rSj7TR was administered at 2 weeks intervals and blood samples were collected before and after the immunization. The presence of specific antibodies was detected via ELISA. Anti-rSj7TR IgG was purified using MAbTrap Kit (GE Healthcare, Waukesha, WI). Non-specific mouse IgG was also purified in the same manner from a non-immunized ICR mouse serum to serve as negative control.

Immunolocalization was done to know the distribution of Sjp_0110390 in different stages of *S. japonicum*. Two types of protocols for indirect immunofluorescent antibody test (IFAT) were used in this study namely: (1) for the intra-mammalian stages of *S. japonicum*, Qdot nanocrystals 655 (Quantum Dot Corp., Hayward, CA) conjugated anti-rSj7TR was used producing red fluorescence; and (2) for the aquatic/intra-molluscan stages, non-conjugated anti-rSj7TR and a secondary antibody Alexa Fluor 488 conjugated anti-mouse IgG (Invitrogen, Carlsbad, CA) producing green fluorescence was utilized. The negative controls used were Qdot 655 conjugated non-specific mouse IgG for the first protocol and just the secondary antibody for the latter. Conjugations of anti-rSj7TR and non-specific mouse IgG with QDot 655 were done according to the product instructions and confirmed by incubating and binding them with Protein G agarose (Boehringer Mannheim, Germany) producing fluorescence. Stages from the mice were treated with RPMI 1640 (Sigma–Aldrich, St. Louis, MO) and 10% fetal bovine serum (Filtron Pty Ltd., Victoria, Australia) overnight at 37 °C to remove the host antigens. For the IFAT samples, either the whole parasite (miracidium, sporocyst, cercaria, schistosomula, juvenile adult, eggs), frozen section (adult, eggs, infected snails, normal snails) or paraffin section (juvenile adult, adult, infected snail) were used. For staining whole parasite bodies, the samples were fixed in a mixture of 50% methanol and 50% acetone for 20 min at −20 °C. After washing with phosphate buffered saline (PBS) containing 0.05% Tween 20 (T-PBS), blocking was done with 1% bovine serum albumin in T-PBS for 30 min at room temperature (RT). The samples then were treated with anti-rSj7TR for 1 h at RT and washed three times with T-PBS. For the protocol using the non-conjugated anti-rSj7TR, additional incubation with the secondary antibody was done for 1 h at RT and then washed again three times with T-PBS. Anti-rSj7TR was diluted 1:100 with PBS for both protocols while 1:200 for the secondary antibody. For the snail samples and adult schistosomes, nuclear staining was done by incubating it with 2 μg/ml Hoechst 33342 (Molecular Probe, Eugene, OR) for 15 min at RT. IFAT smears were observed using a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan).

Fig. 1A shows the localization of Sjp_0110390 in the intra-mammalian stages of *S. japonicum*. Sjp_0110390 was seen localized on the ventral (VS) and oral sucker (OS) of the schistosomulum (SS) whereas it was not found in the juvenile adults (JA) or in the well-developed adults (DA). However, cut sections of the schistosome egg revealed the presence of Sjp_0110390 inside (EG). Sjp_0110390 has been proven to work against human sera for antibody detection against *S. japonicum* [1]. This mechanism might be explained by the expression of the protein on the schistosome eggs which are exposed to the host immune system once they are expelled by the gravid female and carried through the bloodstream.

In Fig. 1B, expression of Sjp_0110390 in the aquatic and intra-mammalian stages of the parasite were shown. Sjp_0110390 was seen concentrated in the apical gland/secretory duct (AG/SD) of the miracidium (MC). This might have contributed to the invasion processes of the parasite into the intermediate host snail. It will be interesting to know whether Sjp_0110390 has a function in the invasion of *S. japonicum* into the snail or in the development of the parasite inside the intermediate hosts.

Section of infected snails showed that Sjp_0110390 was highly concentrated on the parasite (Sj) inside the snail tissues (IOH) as compared to the normal snail section (NOH). Snail infection surveillance is one of the dim areas in schistosomiasis control program. Currently, snail infection was mainly determined by cercarial shedding or snail crushing method to reveal the presence of the cercaria in the intermediate hosts [14]. Recent technologies proved that polymerase chain reaction and loop-mediated isothermal amplification assay can be used to detect snail *S. japonicum* infection [15]. The strong expression of Sjp_0110390 in the infected snail may prove that this protein can be used as a target antigen for diagnosing snail infection. This protein can be a candidate antigen in the development of immunochromatographic test that can rapidly detect snail infection directly on endemic sites. Snail surveillance is a neglected integral part of schistosomiasis control program and improving this will therefore hasten the possible elimination of schistosomiasis. Accurate information on snail infection will definitely help in designing cost-effective control interventions other than mass drug administration.