Molecular cloning and tissue distribution of a Schistosoma japonicum gene encoding AMY-1

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Abstract. It is well known that the mammalian associate of Myc-1 (AMY-1) plays a significant role in spermatogenesis or cellular differentiation. A full-length complementary DNA (cDNA) encoding AMY-1 of Schistosoma japonicum (SjAMY-1) was identified and isolated from a cDNA library. The gene contained an open reading frame of 315 nucleotides, encoding 105 amino acids. Sequence analysis showed that SjAMY-1 shares 65.7% homology with Homo sapiens AMY-1 amino acids and contains a conserved domain from the AMY-1 family. In this study, we cloned and expressed a recombinant SjAMY-1 (rSjAMY-1) with a molecular size of 14 kDa. The native SjAMY-1 in soluble worm antigen was identified by anti-rSjAMY-1 sera in the Western blot analysis, which demonstrated the presence of this protein in the parasite. Immunofluorescence studies indicated a localization of SjAMY-1 in various tissues and organs including the tegument and subtegumental muscles in adult worms, the ventral sucker and eggs. Given the key roles of mammalian AMY-1 in cell proliferation and differentiation, the characterization of SjAMY-1 may allow for a better understanding of the development of S. japonicum.

Introduction

Schistosoma japonicum (S. japonicum) is a blood-dwelling trematode of the genus Schistosoma, and is responsible for the serious but neglected human disease of schistosomiasis (1). Schistosoma have a complex life cycle involving distinct life cycle stages that are adapted for transmission between the aquatic snail and the mammalian hosts. Current attempts to control schistosomiasis depend solely on the repeated administration of the drug, praziquantel, which is effective against adult worms dwelling in the definitive hosts (2). However, with no replacements pending, a severe shortcoming of this drug is the developing resistance of the parasite (3). Therefore, efforts are now focused on identifying new targets against schistosomiasis.

Associate of Myc-1 (AMY-1) was originally identified as a c-Myc-binding protein that enhances c-Myc transcriptional activity (4). c-Myc was suggested to play key roles in cell proliferation, differentiation, transformation and apoptosis (5). AMY-1 mostly localizes in the cytoplasm of cells expressing c-myc at low levels, but translocates into the nucleus in the S phase of the cell cycle upon an increase of c-myc expression (4). In addition to a c-Myc-independent manner, AMY-1 plays other roles. Overexpression of AMY-1 alone triggers the differentiation of K562 cells to erythrocytes (5). AMY-1 is found in the mitochondria of somatic cells, and in sperm AMY-1 is found to play a role in spermatogenesis (7,8). AMY-1 is an essential modulator of protein kinase-A (PKA) (9), and PKA signaling in Schistosoma mansoni is required for cercariae viability and may play a role in the reproductive activity of adult worms (2). However, the characterization of the AMY-1 homolog from S. japonicum remains to be elucidated.

In the present study, we identified an AMY-1 homolog (SjAMY-1) from S. japonicum, cloned and expressed the SjAMY-1 gene and indicated the protein localization in various tissues of the schistosoma.

Materials and methods

Parasites and animals. The Chinese strain of S. japonicum maintained in Oncomelania hupensis snails was purchased from the Jiangsu Provincial Institute of Parasitic Diseases, Wuxi, China. Parasites in various developmental stages, including eggs, cercariae and adults were prepared as described (1). Briefly, cercariae were released from 10 infected snails in a beaker containing 20 ml of water under light for 2 h at room temperature. New Zealand white rabbits were percutaneously infected with 1000 S. japonicum cercariae. Adult worms were obtained by perfusion of the infected rabbits.
**Results**

**Isolation and sequence analysis of SjAMY-1.** The full length ORF of SjAMY-1 was amplified from the cDNA library of adults. Following sequencing, the ORF of 315 nucleotides encoding 105 amino acids was identified. Following the alignment ofAMY-1 homologs, the SjAMY-1 protein showed 56.2% identity with a homolog of *Ciona intestinalis* and 65.7% homology with a homolog of *Homo sapiens*. The homologs from a number of species showed a conserved domain from the AMY-1 family in the alignment of the sequences, with the a1a-96 domain conserved in SjAMY-1 (Fig. 1).

**Expression and purification of rSjAMY-1.** The ORF of *sjamy-1* was cloned into the pET28a(+) vector and expressed with solubility in *E. coli* (DE3). The recombinant fusion protein was purified by nickel affinity chromatography under native conditions and analyzed with SDS-PAGE (Fig. 2). The rSjAMY-1 yield per 1 liter of culture amounts to 40 mg, as determined by the Bradford assay.

**Analysis of anti-SjAMY-1 antibody in S. japonicum-infected rabbit serum.** After 45 days, the sera were collected from rabbits infected with cercariae. The antibody titer of the sera was measured by ELISA (titer >3200) for immunoreactivity to SWAP (data not shown). With Western blot analysis, the rSjAMY-1 could not be probed by the infected sera of rabbits (Fig. 3). Accordingly, the sera of infected rabbits failed to recognize the native SjAMY-1 in SWAP. These results indicated that native SjAMY-1 may fail to induce circulating antibodies in infected rabbits.

**The recognition of native and rSjAMY-1 by anti-rSjAMY-1 sera.** The anti-rSjAMY-1 sera were collected from rats immunized with the recombinant protein, and these rats developed specific antibodies to titers >3.6x10^6, as determined by ELISA (data not shown). The native SjAMY-1 in SWAP and rSjAMY-1 were recognized by anti-rSjAMY-1 sera as shown.
in Fig. 3. Sera from pre-immune rats as a negative control did not identify the native SjAMY-1 or rSjAMY-1.

**Immunolocalization of SjAMY-1 in S. japonicum.** SjAMY-1 was probed by immunofluorescence in S. japonicum adults, cercariae and eggs. Fluorescence was observed in the tegument and subtegumental muscles of the adult worm (Fig. 4, panel A1). Immunofluorescence showed SjAMY-1 localized in the ventral sucker of the cercariae (Fig. 4, panel B1). The internal structures of the eggs were also stained with fluorescence (Fig. 4, panel C1). Corresponding fluorescence was not observed as the primary antibody in negative controls with serum from pre-immune rats (Fig. 4, panel A2, B2 and C2).

**Discussion**

AMY-1 may be a significant candidate as a key regulating molecule in S. japonicum, since it is known to stimulate the
transcriptional activity of c-myc, trigger cell differentiation, be involved in spermatogenesis and modulate the PKA signal pathway in vertebrates (4-9). In this study, the aim was to identify the AMY-1 homolog of *S. japonicum* and explore its specific characteristics in various developmental stages.

In this study, we have cloned a homologous gene of AMY-1 from *S. japonicum*, and named it SjAMY-1. Sequence analysis with alignment of amino acid sequences revealed a high degree of homology between SjAMY-1 and other homologs of a number of species. Since SjAMY-1 has been shown to share similarities with AMY-1 of *Homo sapiens*, including protein sequences with 65.7% homology and a conserved domain of the AMY-1 family, it is likely to be the homolog of *Homo sapiens* AMY-1 (Fig. 1). SjAMY-1 may be a specific protein for *S. japonicum* since no significant homolog was found in Platyhelminthes. The characteristics of AMY-1 in phylogenesis may provide a possible explanation for the parasite's escape from the immune system of the host.

Western blot analysis verified the presence of SjAMY-1 in the adult worms. The native SjAMY-1 detected by anti-rSjAMY-1 serum required a component of soluble worm extract. The fact that the recombinant SjAMY-1 and the native molecule failed to be recognized by the sera of infected rabbits indicates that the soluble protein in the schistosoma may be unable to stimulate the production of the circulating antibodies in rabbits during infection. The manner in which SjAMY-1 escapes from the immune surveillance should be determined. SjAMY-1 was mainly localized in the subtegmental muscles and tegument of adults, which is crucial in protecting the parasite against the host immune system (Fig. 4). Immunofluorescence was also found in the ventral suckers of cercariae, by which the cercariae are able to attach to and penetrate into hosts and in the internal structures of the eggs, which are a significant cause of hepatic fibrosis (Fig. 4).

Considering that *S. japonicum* is a parasite with activities of invasion and powerful egg production, SjAMY-1 may be a new potential target for vaccines or chemotherapeutic agents against schistosomiasis. With the available recombinant protein and antibodies, more studies should be conducted to elucidate the exact role of SjAMY-1 in *S. japonicum*.

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**References**


