Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Bioinformatic analysis for structure and function of TCTP from *Spirometra mansoni*

Ya-Jun Lu*, Gang Lu, Da-Zhong Shi, Li-Hua Li, Sai-Feng Zhong

Department of Pathogen Biology, Hainan Medical University, Haikou, P. R. China

ARTICLE INFO

Article history:
Received 10 May 2013
Received in revised form 15 June 2013
Accepted 15 July 2013
Available online 20 September 2013

Keywords:
Spirometra mansoni
Translationally controlled tumor protein
Bioinformatics analysis

ABSTRACT

Objective: To predict structure and function of translationally controlled tumor protein (TCTP) from *Spirometra mansoni* by bioinformatics technology, and to provide a theoretical basis for further study. **Methods:** Open reading frame (ORF) of EST sequence from *Spirometra mansoni* was obtained by ORF finder and was translated into amino acid residue by DNAclub. The structure domain was analyzed by Blast. By the method of online analysis tools: Protparam, InterProScan, protscale, SignalP-3.0, PSORT Ⅱ, BepiPred, TMHMM, VectorNTI Suite 9 packages and Phyre2, the structure and function of the protein were predicted and analyzed. **Results:** The results showed that the EST sequence was Sm TCTP with 173 amino acid residues, theoretical molecular weight was 19 872.0 Da. The protein has the closest evolutionary status with *Clonorchis sinensis*, *Schistosoma mansoni*, and *Schistosoma japonicum*. Then it had no signal peptide site and transmembrane domain. Secondary structure of TCTP contained two α-helices and eight β-strands. **Conclusions:** Sm TCTP was a variety of biological functions of protein that may be used as a vaccine candidate molecule and drug target.

1. Introduction

Spirometra mansoni is a kind of tapeworm that infects animals and human. Its adults less parasitize in human body. *Sparganum mansoni* that is plerocercoid of *Spirometra mansoni* causes *Sparganosis mansoni* in human[1]. Sparganosis is a worldwide parasitic disease[2]. This parasite is transmitted to humans in three different ways. First, human may be infected by drinking raw water that is contaminated with procercoid. Second, human may be infected by consuming raw flesh of the second intermediate hosts, such as frogs and snakes. Third, human may acquire infection by placing poultices frog or snake flesh on eyes or open wounds. The plerocercoid may intrude into human body and mainly invade the brain, eyes, abdominal cavity, spinal cord and subcutaneous tissues. The plerocercoid can damage tissues and cause blindness, paralysis, even death[3,4]. The sparganosis is a serious threat to human health[5].

Sparganosis mansoni is endemic in 48 countries. The cases have been described in Asia, Africa, Australia, South America, and the United States. The majority of cases occur in Southeast Asia and Eastern Africa. Ocular sparganosis is especially prevalent in China and Vietnam. The highest numbers of cases occur in Korea and Japan[6,7].

At present, the full-length cDNA library of *Spirometra mansoni* is constructed completely and expressed sequence tags (EST) are acquired. In this article, the coding gene of translationally controlled tumor protein was the research object. Its structure and function were predicted by the method of bioinformatics technology. These studies can supply orientation on further experiment and theoretical basis for vaccine research, drug development and prevention of

*Corresponding author: Ya-Jun Lu, Department of Pathogen Biology, Hainan Medical University, Haikou, P. R. China.
E-mail: luyajuncc@163.com
Foudation project: This work was supported by Natural Science Foundation of China (Foundation No. 81260254).

Spirometra mansoni.

2. Materials and methods

2.1. EST sequence

Spirometra mansoni was acquired from small intestinal in dog, then EST sequence was measured by the UNITED GENE GROUP LTD. in Shanghai, PRC.

2.2. Predicted methods

EST basic properties were analyzed online by ORF finder and Blast at NCBI. mRNA sequence was translated into amino acid sequence by DNA club. The structure domain and function domain were predicted by online analysis http://www.ebi.ac.uk/InterProScan/. The amino acid sequence was submitted to http://www.expasy.org/tools/protparam.html and its physical and chemical properties were predicted. Translationally controlled tumor protein was inquired from NCBI-PROTEIN. These proteins mainly came from the following species: *Arabidopsis thaliana*, *Mus musculus*, *Homo sapiens*, *Caenorhabditis elegans*, *Xenopus laevis*, *Danio rerio*, *Gallus gallus*, *Oryctolagus cuniculus*, *Drosophila melanogaster*, *Clonorchis sinensis*, *Schistosoma mansoni*, *Schistosoma japonicum*, *Plasmodium knowlesi* strain H, *Trichinella spiralis*, *Brugia malayi*, *Giardia lamblia* ATCC 50803, *Aedes aegypti* and *Plasmodium falciparum*. The target sequence and the above sequence were carried out multiple sequence alignment by ClustalX, then molecular evolutionary tree was constructed by MEGA4.1. Signal peptide was predicted by online analysis tools http://www.cbs.dtu.dk/servi-ces/SignalP-3.0/, and subcellular localization was predicted using http://psort.nibb.ac.jp/form2.html. Amino acid sequence was submitted on http://www.cbs.dtu.dk/ services/BepiPred/ in order to predict antigen epitope. Hydrophilic prediction was predicted at http://us.expasy.org/cgi-bin/protscale.pl. Secondary structure and tertiary structure were predicted by Phyre2[8,9].

3. Results

3.1. EST basic properties

The results from ORF finder analysis at NCBI showed that this amino acid sequence had the longest ORF. Initiation codon was ATG, termination codon was TAA, and the full-length was 519 bp (Figure 1). The full-lengh of amino acid sequence translated by DNA club was 173 amino acid residues (Figure 2) containing a complete conservative domain of translationally controlled tumor protein (TCTP).

ATGATCATCTACCACGATTTGCTTAGCGATGGCGAGATGTTCAGCGAC
GGCTTCAAAGTTATTAAGGTGAACGATTTCATCTGGGAGGTTGAAGGG
AAATCGATCACCGTTAAAGAAGGTGTCGATGGTGCTCTTTTGGGGGCC
AACCCTTCGGCTGAAGAAGCGGAGGAGGTCGAAGATGGCGTCATAAC
CGTTATTGACTTGGTTTATGCCCACCAATTGCAAGAGTCTTCGCCTGGT
TCAAAGAAATATTACATGGACTTCCTGAAAGACTACCTTAAAAAGCTT
AAGACCAAAATGGAAGCTGACAAAGTCGATGAAACAACCGTAGCCAA
GTTTATGAAGGAGTCGCAGGCCTACGTCAAGGAGAAACTCCTGGCCGA
CTTCAACAACTTGACATTTTACCAACCCAAGACTTCCAGTGATGAATTC
TACTTCATCCCAATGAACTACCGCGACGATGAATCTACGCCTTACTTCG
TCTTTTTTGCAAATGGCCTTAAAGAAGAGAAGGTGTAA

Figure 1. Sequences of ORF.

MIIYHDLLSDGEMFSDGFKVIKVNDFIWEVEGKSITVKEGVDGALLGANP
SAEEAEEVEDGVITVIDLVYAHQLQESSPGSKKYYMDFLKDYLKKLKTK
MEADKVDETTVAKFMKESQAYVKEKLLADFNNLTFYQPKTSSDEFYFIP
MNYRDDESTPYFVFFANGLKEEKVZ

Figure 2. Amino acid residues.

3.2. Physical and chemical properties

Molecular weight of the protein was 19 872.0 Da. Theoretical isoelectric point (pI) was 4.59. Extinction coefficient in 280 nm aqueous solution was 20 400 $U \cdot M^{-1} \cdot cm^{-1}$. The half-life was 30 h, > 20 h, > 10 h, respectively in mammalian reticulocytes (*in vitro*), yeast (*in vivo*) and *Escherichia coli* (*in vivo*). The instability index (Ⅱ) was computed to be 26.23. Aliphatic index was 76.59.

3.3. Structural domain, hydrophobicity, signal peptide and subcellular localization

The structural domain was Mss4-like protein or translationally controlled tumor protein by InterProScan. The maximum was 2.311 at 66 residue position (Val) and the minimum was -2.544 at 154 residue position (Asp) by the method of Hphob. HPLC/Parker & al. The Sm TCTP was hydrophilic without obvious hydrophobic regions (Figure 3).

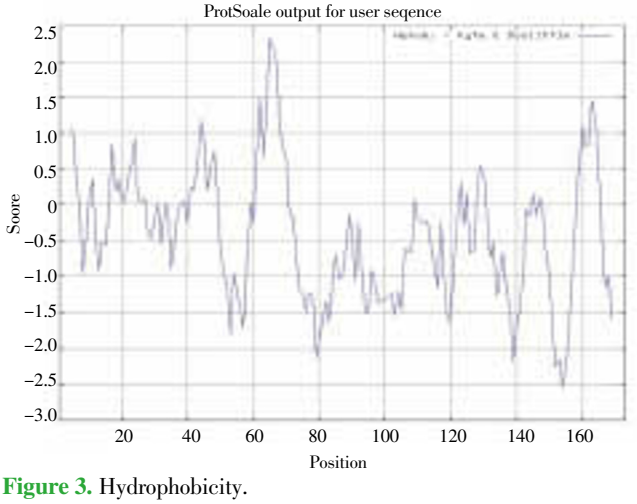

Figure 3. Hydrophobicity.

SignalP-NN result: there was a peak fraction at 33 residue position. The score was 0.426 that was too low without split site. So the protein had no signal peptide. The peptide chain was located in the cytoplasm, mitochondria, bubble body, peroxidase body, endoplasmic reticulum, cytoskeleton and nucleus with the possibility of 43.5%, 21.7%, 17.4%, 4.3%, 4.3%, 4.3%, 4.3%. The maximum possible location was in the cytoplasm inside (k = 23).

3.4. Antigen epitope and transmembrane domain

The sequence was compared with the host's homologous sequences corresponding regional sequence by BepiPred 1.0b Server. The potential antigen epitopes may be 33(aa)~41(aa), 46(aa)~60(aa), 74(aa)~83(aa), 100(aa)~108(aa), 136(aa)~142(aa), 51(aa)~157(aa), and 168(aa)~173(aa). The value of transmembrane domain was less than 1. Sm TCTP had no transmembrane domain, located outside the membrane.

3.5. Multiple sequence alignment and molecular evolution

Multiple sequence alignment displayed the amino acid residues were identical and highly conserved between species on sites 72(H) and 74(L). Sm TCTP had the closest evolutionary status with *Clonorchis sinensis*, *Schistosoma mansoni*, and *Schistosoma japonicum* by phylogenetic analysis (Figure 4).

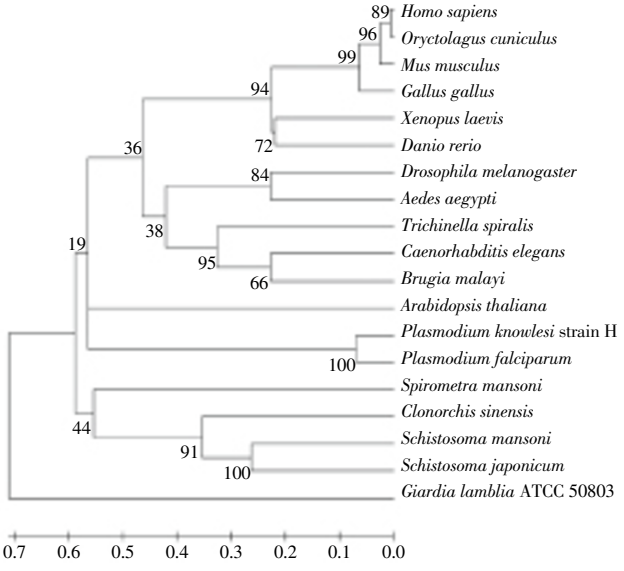

Figure 4. Phylogenetic analysis.

3.6. Secondary structure and tertiary structure

This protein has two α-helices (82aa~102aa, 107aa~125aa) and 8 β-strands (2aa~6aa, 12aa~15aa, 21aa~38aa, 63aa~71aa, 74aa~76aa, 132aa~136aa, 145aa~150aa, 158aa~171aa) (Figure 5).

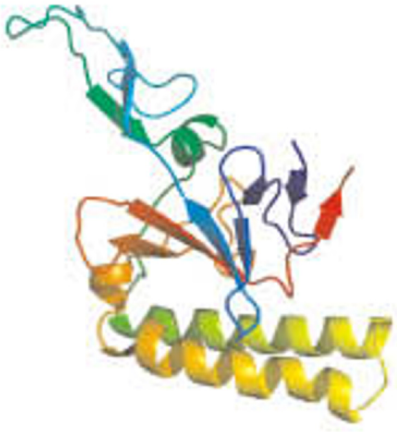

Figure 5. Tertiary structure.

4. Discussion

Translationally controlled tumor protein widely exists in eukaryotic organisms and is highly conservative and homologous[10]. TCTP is originally found in sarcoma cell line and erythroleukemia cell line of rats and considered to be the growth associated protein[11]. TCTP is found that it also exists in other tumor cells and normal cells except renal cell[12].

TCTP has a variety of biological functions: (1) promote basophilic granulocyte to release histamine factor; (2) combine with microtubules by cell cycle; (3) has activity binding with calcium, adjust absorption of calcium, participate in calcium transportion[13,14]; (4) resistance to apoptosis[15]; (5) the lower expression of TCTP can cause the expression changes about intracellular ubiquitin-proteasome system, tumor metastasis related genes, cytoskeleton-associated proteins and ion binding proteins of 27 kinds of proteins; (6) is receptor protein of artemisinin-based drug[16].

The basic properties, structure and function of Sm TCTP were predicted and analyzed by bioinformatics technology. The protein is stable protein, characterized by hydrophilic, located in the cytoplasm, no signal peptides and transmembrane region. It has seven potential antigen epitopes, two α-helices located in 82aa~102aa and 107aa~125aa that is main structural components, and 8 β-strands scattered in the whole protein. Sm TCTP has the closest evolutionary status with *Clonorchis sinensis*, *Schistosoma mansoni* and *Schistosoma japonicum*, the farthest evolutionary status with its hosts (*Homo sapiens* and *Mus musculus*) by phylogenetic analysis. The information suggests the protein may be a potential vaccine candidate. Sm TCTP is one of artemisinin-based drug receptor, and

it's a target of artemisinin-based drug. So artemisinin-based drug may become a new drug for the treatment of *Sparganosis mansoni*.

The research on Sm TCTP had not yet been reported. Therefore, the conclusion may provide important biology clues and theoretical basis for prevention and control of *Spirometra mansoni*.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This work was supported by Natural Science Foundation of China (Foundation No. 81260254).

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