Tropical Biomedicine 32(1): 167–175 (2015)

Analysis of structure, function and epitopes of *Spirometra erinaceieuropaei* casein kinase I

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Received 27 March 2014; received in revised form 25 August 2014; accepted 26 August 2014

Abstract. *Spirometra erinaceieuropaei* casein kinase I (SeCKI) was analyzed using bioinformatical methods to predict its structure and function based on the deduced amino acid sequence from full length cDNA sequence of SeCKI gene with online sites and software programs. The longest open reading frame contains 448 amino acids, 50 kDa and theoretical pI of 4.73, with a complete tubulin domain, a SMART tubulin_C domain and a low complexity region. SeCKI has no signal sequence and no transmembrane domain, but is predicted to be located extracellularly. The secondary structure of SeCKI contains 12 α-helixes, 11 β-strands and 22 coils. SeCKI had 19 potential antigenic epitopes and 25 HLA-I restricted epitopes. Based on phylogenetic analysis of SeCKI sequence, *S. erinaceieuropaei* has the closest evolutionary status with *Hymenolepis microstoma*. Information from this study could provide important insights into the identification of diagnostic antigens and molecular targets of anti-sparganum drugs.

INTRODUCTION

One of the most important species of genus *Spirometra* tapeworms, which have with plerocercoids that can produce sparganosis in humans is *Spirometra erinaceieuropaei* (syn. *Spirometra erinacei* or *Spirometra mansoni*) and is most common in Asia whereas *Spirometra mansonoides* is mainly distributed in North America (Roberts *et al.*, 2009). Adults of intestinal parasites of some species of *Canidae* and *Felidae* do not generally infect humans; the first intermediate hosts are fresh water copepods (cyclops), whereas the second intermediate or paratenic hosts belong to a number of different species of vertebrates (viz. frog, snake and pigs) (Nithiuthai *et al.*, 2004, Shin *et al.*, 2008). Humans are accidental host, whose infection results mainly from drinking water contaminated with cyclops harboring procercoids, ingesting raw flesh of frogs and snakes infected with plerocercoids, or from placing frog or snake flesh on open wounds for treatment of skin ulcers or eye inflammations (Fukushima & Yamane, 1999, Magnino *et al.*, 2009). Sparganosis in human has been reported from many countries of the world but is most common in eastern Asia and the Far East (Shirakawa *et al.*, 2010).

Sparganosis poses a serious threat to human health; the plerocercoids usually lodge in subcutaneous tissues and muscles, but sometimes invade the abdominal cavity, eye, and central nervous system causing blindness, seizures, headache, epilepsy, paralysis, and even death (Shirakawa *et al.*, 2010). Ocular sparganosis is especially prevalent in China and Vietnam (Cui *et al.*, 2011b). Clinical diagnosis of sparganosis is rather difficult and is often misdiagnosed because the larvae have no predilection for particular sites in the human body and specific signs or symptoms are lacking. A definitive diagnosis of subcutaneous sparganosis can be achieved by detection of

the larvae in a biopsy specimen from the lesion, but confirmative diagnosis is difficult for visceral and cerebral sparganosis as the larvae can be found only by surgical removal (Murata *et al.*, 2007).

ELISA using the crude or excretory-secretory (ES) antigens of plerocercoids has high sensitivity for the detection of sparganum infection in humans, but the main disadvantage is the false negative results during the early stage of infection and the cross-reactions with sera from patients with other parasitic diseases (viz. cysticercosis, paragonimiosis and clonorchiosis) (Nishiyama *et al.*, 1994, Cui *et al.*, 2011a).

Burnett and Kennedy (Burnett & Kennedy, 1954) found in liver of rats a protein kinase, which was named casein kinase, for the reason that it phosphorylates artificial substrate casein. In fact, the casein kinase exists as a mixture of two kinds of protein kinases, one of which is the type I (casein kinase I, CKI) (Pinna *et al.*, 1969). CKI belongs to serine/threonine protein kinase family and is highly conserved in eukaryotes, mainly distributed in the cytoplasm, nucleus, cell membrane and cytoskeleton (Tuazon & Traugh, 1991, Graves & Roach, 1995, Knippschild *et al.*, 2005). CKI isoforms participate in many cellular processes, such as DNA repair, metabolic regulation, control regulation of signal transduction, vesicular trafficking, cell cycle progression, and cell development (Brookheart *et al.*, 2014, Choksi *et al.*, 2014, Gorietti *et al.*, 2014).

In order to study the early specific diagnostic antigens, the structure and function of SeCKI from the amino acid sequence deduced from its cDNA (GenBank accession no. 392495120) were predicted using bioinformatics techniques.

MATERIALS AND METHODS

The analytical methods applied to full-length cDNA sequence of SeCKI (GenBank accession No. 392495120) were as previously reported (Liu *et al.*, 2013). In brief, information regarding open reading frame (ORF), conserved domain(s), physical and chemical parameters, signal peptide, transmemebrane sequence(s), epitope(s), and topological structure were obtained using programs available at NCBI, EMBI and Expasy on-line sites. Clustal X, BioEdit and MEGA4.1 softwares were used for multiple sequence alignment and phylogenetic tree construction.

RESULTS

Physical and chemical properties of SeCKI

SeCKI 1,504 bp cDNA of encoded a maximum ORF of 448 of amino acids, 50 kDa and theoretical isoelectric point (pI) of 4.73, with 3'UTR located at the positions 1348-1504 (Figure 1). Calculated extinction coefficients is 40,965M^{-1} cm^{-1} at 280 nm in water, assuming all pairs of Cys residues form cystines. Predicted half life is 30, > 20 and >10 hours in mammalian reticulocytes (*in vitro*), yeast (*in vivo*) and *Escherichia coli* (*in vivo*), respectively. The instability index (∏) was calculated to be 41.43 and was thus classified as unstable. Aliphatic index was 74.44 and average hydropathicity (GRAVY) -0.339.

Structural domain, hydrophobicity, signal peptide, subcellular localization and transmembrane domain

The predicted SeCKI structure domains contain a complete tubulin domain, a SMART tubulin_C domain and a low complexity region located at positions 47-244, 246-383 and 425-448 (Figure 2). Using the scale Hphob./Kyte & Doolittle, SeCKI has an obvious hydrophobic region at the N-terminal region (Figure 3). Based on SignalP-4.1 software SeCKI lacks a signal sequence (Figure 4). The D score is 0.104, lower than the cutoff score 0.45, and so SeCKI has no cleavable signal peptide. Prediction of TMHMM Server v. 2.0 suggests that SeCKI has no transmembrane domain. Results of k-NN prediction suggest that the possibility of SeCKI being located in the cytoplasm, mitochondrion, nucleus, vacuole or

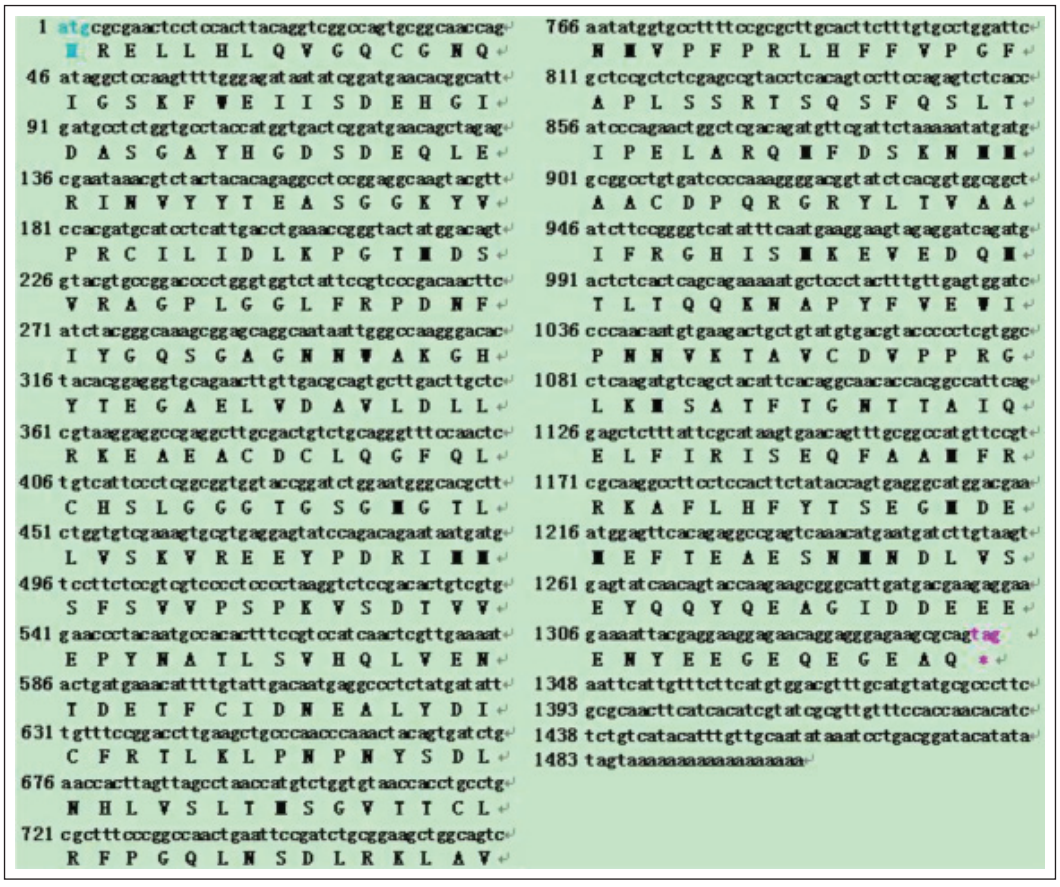

Figure 1. Sequences and amino acid residues of SeCKI

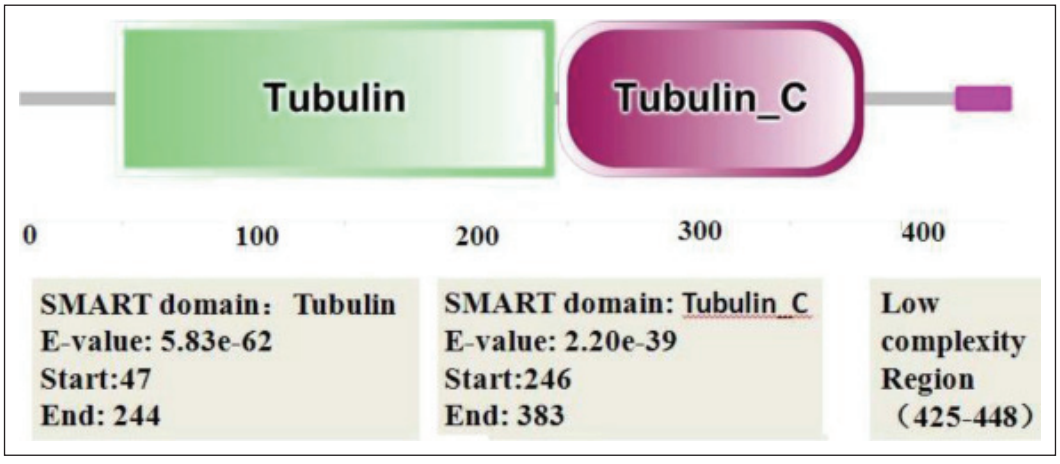

Figure 2. Prediction of structure domains of SeCKI by SMART servers

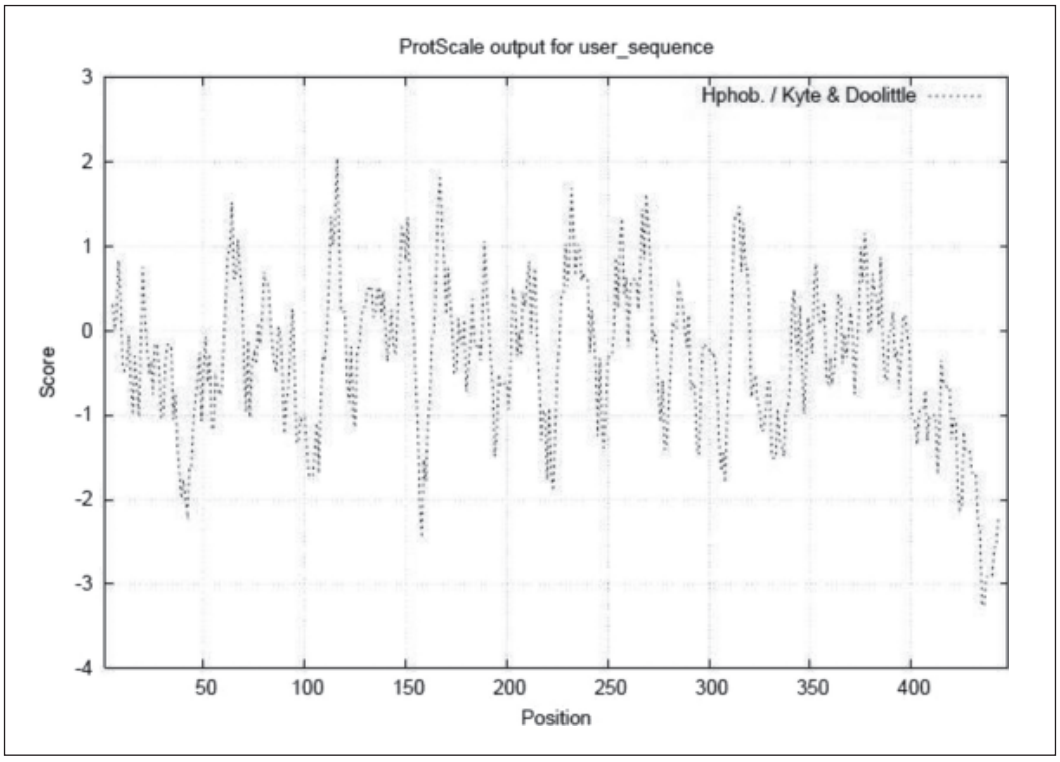

Figure 3. Hydrophobicity of SeCKI

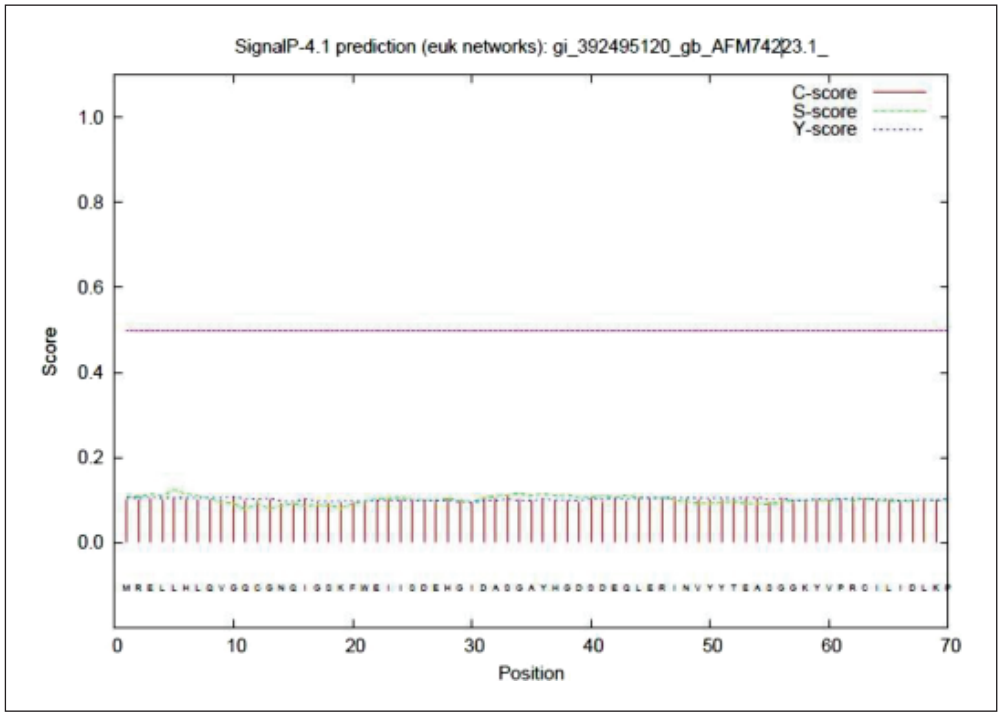

Figure 4. Prediction of SeCKI signal peptide

cytoskeletal network are 52.2%, 17.4%, 13.0%, 8.7% and 8.7%, respectively, suggesting a maximum possible likelihood of an extracellular location (k = 23).

Construction of 3D model and enzyme activity prediction

First of all, PSIPRED v. 3.3 was used to predict the secondary structures of SeCKI, predicting 12 α-helixes, 11 β-strands and 22 coils (Figure 5). Then five models were set up as described by (Zhang, 2008). We selected the model with highest confidence C-score (Figure 6) calculated based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations. C-score is typically in the range of "5 - 2, and models with a C-score > 2 suggest high confidence. Enzyme homologs in PDB predicted by I-TASSER show the most reliable enzyme classification (EC) id 3.4.21.110, C5a peptidase, also known as streptococcal C5a peptidase.

Antigenic epitopes of SeCKI

Based on BepiPred 1.0b Server, epitope prediction algorithm consensus was used to predict SeCKI peptides recognized by the conserved HLA-restricted CD8+ T cells, which could induce effective and protective immune response in humans against *S. erinaceieuropaei*. SeCKI is predicted to have 17 potential antigen epitopes: aa 12-16, 27-44, 52-59, 70-80, 90-111, 140-146, 156-160, 170-182, 217-223, 245-248, 271-281, 303-308, 331-339, 354- 361, 366-372, 401-415, and 423-448. SeCKI has 25 conserved peptides based on a high HLA allele binding score (percentile rank < 0.6) (Table 1).

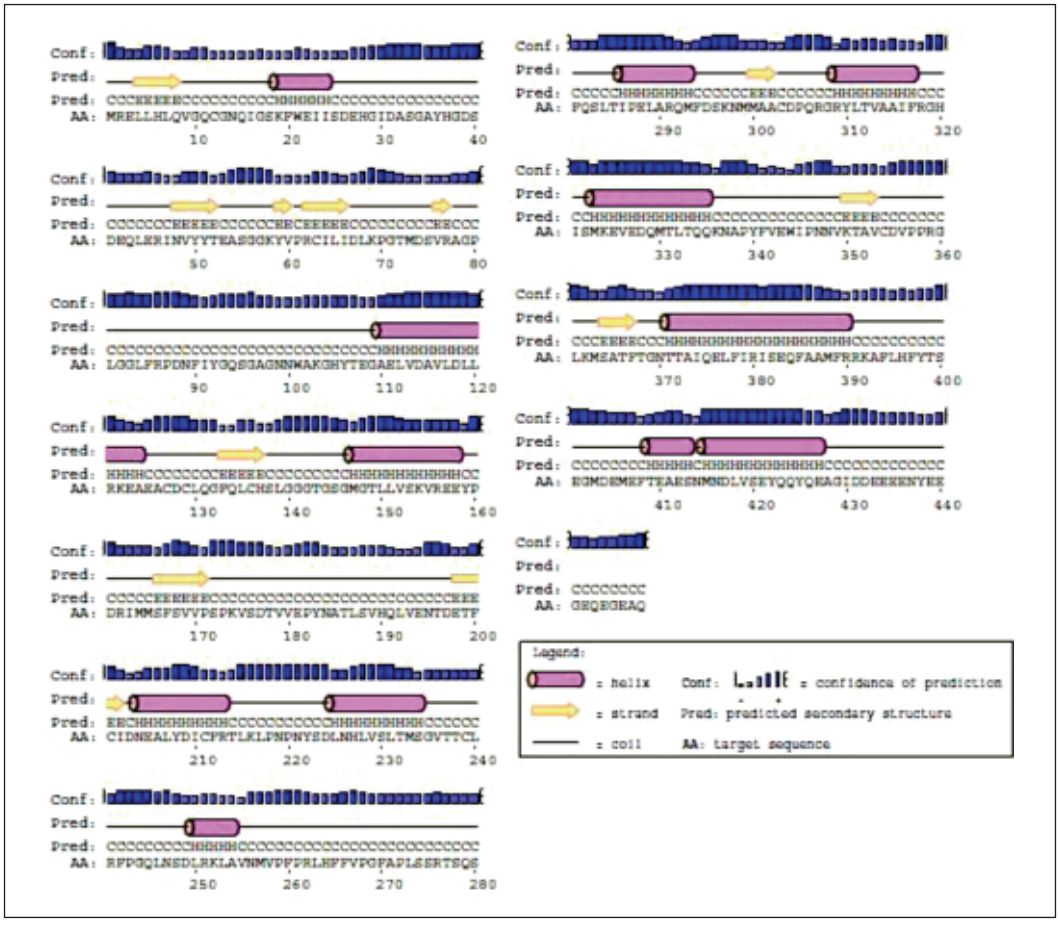

Figure 5. The predicted secondary structure of SeCKI by using PSIPRED

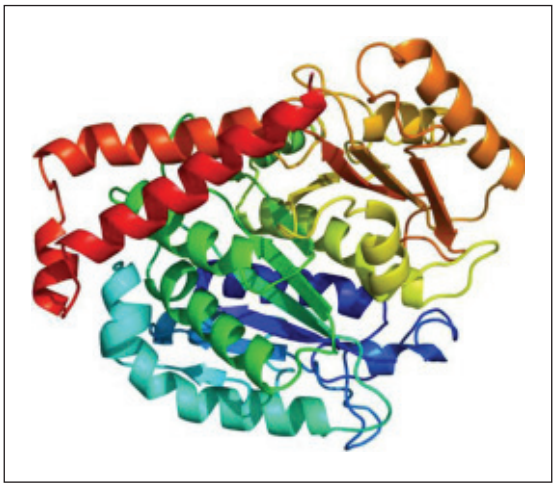

Figure 6. The 3D model of SeCKI

Table 1. The predicted HLA restricted $CD8^+$ T cell epitopes for SeCKI

Allele	Start	End	Peptide	Method	Percentile_rank
HLA-A*02:01	163	175	IMMSFSVVPSPKV	Ann	0.2
HLA-A*02:01	164	175	MMSFSVVPSPKV	Ann	0.3
HLA-A*02:01	64	76	ILIDLKPGTMDSV	Ann	0.3
HLA-A*02:01	206	217	ALYDICFRTLKL	Ann	0.4
HLA-A*11:01	163	174	IMMSFSVVPSPK	Ann	0.1
HLA-A*11:01	162	174	RIMMSFSVVPSPK	Ann	0.1
HLA-A*11:01	312	324	TVAAIFRGHISMK	Ann	0.3
HLA-A*11:01	165	174	MSFSVVPSPK	Consensus (ann/smm)	0.3
HLA-A*11:01	254	262	AVNMVPFPR	Consensus (ann/smm)	0.35
HLA-A*11:01	314	324	AAIFRGHISMK	Consensus (ann/smm)	0.45
HLA-A*11:01	387	398	AMFRRKAFLHFY	Ann	0.5
HLA-A*11:01	284	297	LTIPELARQMFDSK	Ann	0.5
HLA-A*11:01	311	324	LTVAAIFRGHISMK	Ann	0.5
HLA-B*07:02	69	81	KPGTMDSVRAGPL	Ann	0.2
HLA-B*07:02	60	73	VPRCILIDLKPGTM	Ann	0.2
HLA-B*07:02	260	273	FPRLHFFVPGFAPL	Ann	0.2
HLA-B*07:02	271	284	APLSSRTSQSFQSL	Ann	0.2
HLA-B*07:02	159	169	YPDRIMMSFSV	Consensus (ann/smm)	0.4
HLA-B*07:02	357	367	PPRGLKMSATF	Consensus (ann/smm)	0.4
HLA-B*07:02	219	231	NPNYSDLNHLVSL	Ann	0.4
HLA-B*07:02	159	170	YPDRIMMSFSVV	Ann	0.5
HLA-B*07:02	260	271	FPRLHFFVPGFA	Ann	0.5
HLA-B*07:02	242	255	FPGQLNSDLRKLAV	Ann	0.5
HLA-B*07:02	172	179	SPKVSDTV	Consensus (ann/smm)	0.5
HLA-B*07:02	60	68	VPRCILIDL	Consensus (ann/smm/comblib_sidney2008)	0.5

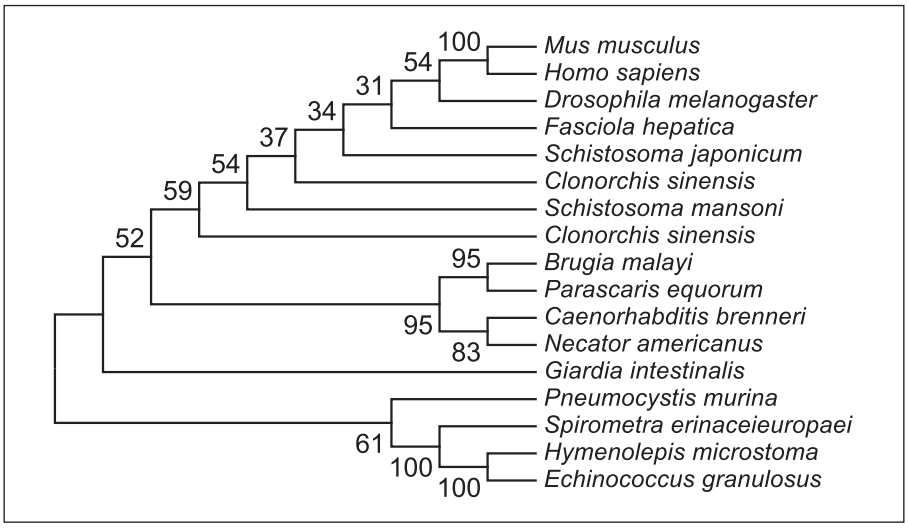

Figure 7. Neighbor-joining phylogenetic tree referred from SeCKI amino acid sequence of *Spirometra erinacei*. Bootstrap values are indicated on branches

Multiple sequence alignment and molecular evolution of SeCKI

Multiple sequence alignment and phylogenetic analysis of SeCKI compared with casein kinase I of other species, *S. erinaceieuropaei* has the closest evolutionary status with *Hymenolepis microstoma* (Figure 7).

DISCUSSION

Based on construction of full-length cDNA library of SeCKI, the sequence of SeCKI gene was 1 504 bp length with a 1347bp biggest ORF encoding 448 amino acids protein contained a complete tubulin domain, a SMART tubulin_C domain and a low complexity region. The predicted molecular weight and pI of the deduced SeCKI protein were 50 kDa and 4.73, respectively. Based on the phylogenetic analysis of SeCKI, *S. erinaceieuropaei* has the closest evolutionary status with *H. microstoma*, based on the sequences that had reported. The secondary structure of SeCKI contained 12 α-helixes, 11 β-strands, and 22 coils. The SeCKI had 19 potential antigenic epitopes and 25 HLA-I restricted epitopes.

SeCKI is predicted to contain a complete tubulin domain, a SMART tubulin_C domain and a number of low complexity regions. This tubulin domain is found in tubulin alpha, beta and gamma chains, as well as in the bacterial FtsZ family of proteins. These latter proteins are GTPases and are involved in polymer formation in bacterial cell division, forming a ring in the middle of the dividing cell that is required for constriction of cell membrane and cell envelope to yield two daughter cells. FtsZ can polymerise into tubes, sheets, and rings in vitro and is ubiquitous in bacteria and archaea.

The significances of the predicted C5a peptidase domain are as follows: surface-associated subtilisin-like serine peptidase with very specific substrate specificity; virulent strains of streptococci, including *Streptococcus pyogenes*, can evade human detection and phagocytosis by destroying complement chemotaxin C5a; cleavage of human C5a by this enzyme reduces the ability of C5a to bind receptors on the surface of polymorphonuclear neutrophil leukocytes and thereby abolishes its chemotactic properties; belongs to peptidase family S8A. (http: //enzyme.expasy. org/EC/3.4.22.43).

Finally, the predicted antigenic epitopes could provide important insights into the identification of diagnostic antigens for sparganosis and target molecules of anti-sparganum drugs.

Acknowledgements. This work was supported by the National Natural Science Foundation of China (grant no. 81172612).

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