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Identification and bioinformatics analysis of lactate dehydrogenase genes from *Echinococcus granulosus*

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ABSTRACT

Objective: To identify full length cDNA sequence of lactate dehydrogenase (LDH) from adult *Echinococcus granulosus (E. granulosus)* and to predict the structure and function of its encoding protein using bioinformatics methods. **Methods:** With the help of NCBI, EMBI, Expasy and other online sites, the open reading frame (ORF), conserved domain, physical and chemical parameters, signal peptide, epitope, topological structures of the protein sequences were predicted and a homology tertiary structure model was created; Vector NTI software was used for sequence alignment, phylogenetic tree construction and tertiary structure prediction. **Results:** The target sequence was 1 233 bp length with a 996 bp biggest ORF encoding 331 amino acids protein with typical L-LDH conserved domain. It was confirmed as full length cDNA of LDH from *E. granulosus* and named as EgLDH (GenBank accession number: HM748917). The predicted molecular weight and isoelectric point of the deduced protein were 3 5516.2Da and 6.32 respectively. Compared with LDHs from *Taenia solium*, *Taenia saginata asiatica*, *Spirometra erinaceieuropaei*, *Schistosoma japonicum*, *Clonorchis sinensis* and human, it showed similarity of 86%, 85%, 55%, 58%, 58% and 53%, respectively. EgLDH contained 3 putative transmembrane regions and 4 major epitopes (54aa-59aa, 81aa-87aa, 97aa-102aa, 307aa-313aa), the latter were significant different from the corresponding regions of human LDH. In addition, some NAD and substrate binding sites located on epitopes 54aa-59aa and 97aa-102aa, respectively. Tertiary structure prediction showed that 3 key catalytic residues 105R, 165D and 192H forming a catalytic center near the epitope 97aa-102aa, most NAD and substrate binding sites located around the center. **Conclusions:** The full length cDNA sequences of EgLDH were identified. It encoded a putative transmembrane protein which might be an ideal target molecule for vaccine and drugs.

1. Introduction

Cystic echinococcosis (CE) is a zoonotic parasitic disease caused by larval stage (echinococcus cyst or hydatid cyst) of *Echinococcus granulosus (E. granulosus)*, which is found in the small intestine of dogs. The echinococcus cyst can parasitize in various organs of human and cloven-hoofed livestock (sheep, cattle, horses, *etc.*), causing space-occupying lesions. CE is worldwide in distribution and has high prevalence in parts of Eurasia (for example, the Mediterranean region, the Russian Federation and adjacent independent states and China), Africa (northern and eastern regions), Australia, and South America[1]. In China, CE is endemic in pastoral areas of Northwest China with high disease incidence covering 44% of the total land areas. About 500 000-600 000 patients are suffering from this disease and approximately 50 million population are exposed to infection, which cause considerably socioeconomic problems on patients family and over 500 million RMB loss in livestock farming annually[2-5].

Based on construction of full-length cDNA library from adult *E. granulosus*[6], large-scale sequencing of 5′ end expressed sequence tags (EST) and Blastn/Blastx searching for the ESTs, an EST from clone Z009-003_C06 having high homologus ratio (60%) with lactate dehydrogenase (LDH) from *Schistosoma japonicum* was identified, which had a 5′ end but without 3′end or polyA, it was presumed as a

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cDNA fraction coding LDH of *E. granulosus*. Then the full-length cDNA sequence with polyA was obtained by the method of walking sequencing and gene splicing. This study was to identify the obtained cDNA sequence and to predict the structure and functions of its encoding protein using bioinformatics methods in the hope of getting valuable information for the further study.

2. Materials and methods

2.1. Materials

Construction of full-length cDNA plasmid library for adult *E. granulosus*[2], large-scale sequencing of 5′ end EST, Blastn/Blastx searching and analysis, walking sequencing and gene splicing had been done by the cooperation between our team and United Gene Group Limited, Shanghai.

The full-length cDNA sequence of clone Z009-003_C06 was used in this study. Other LDH amino sequences of model organisms and other parasites used in this study were obtained from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index. html) and listed as follows: *Schistosoma japonicum* (SjLDH, FJ560911), *Clonorchis sinensis* (CsLDH, AY666121), *Plasmodium falciparum* (PfLDH, AAK12097), *Toxoplasma gondii* (TgLDH, Q27797), *Trichomonias vaginalis* (TvLDH, AAC72735), *Caenorhabditis elegans* (CeLDH, NP_496503), *Drosophila melanogaster* (DmLDH, Q95028), *Danio rerio* (DrLDH, NP_571321), *Xenopus laevis* (XlLDH, AAH45015), *Gallus gallus* (GgLDH, NP_990615), *Mus musculus* (MmLDH, NP_034829), and *human* (HsLDH-A, BAD96798). LDH sequences of *Taenia solium* (TsLDH) and *Taenia saginata* asiatica (TaLDH) were obtained from previous reports[7, 8].

2.2. Methods

Methods applied here were the same as reported in our previous work[9]. Briefly, with the help of NCBI, EMBI, Expasy and other online sites, the protein information was obtained including the open reading frame (ORF), conserved domain, physical and chemical parameters, signal peptide, epitope, topological structures of the protein sequences and a homology tertiary structure model was created; Vector NTI software was used for sequence alignment, phylogenetic tree construction and tertiary structure prediction.

3. Result

3.1. Gene identification

Target sequence was 1 233 bp length with a 996 bp biggest OFR starting from 20 bp (ATG) and ending at 1 015 bp (TGA), which encoded 331 amino acid protein with polyA locating at the positions 1 053 bp-1 071 bp. Nucleotide sequence and deduced amino acid sequence were shown in Figure 1. The deduced protein sequence contained a complete L-LDH-NAD conserved domain and an L-LDH active site VGEHGDS (189aa -195aa); other typical L-LDH domains and motif were also existed according to protein domains and motif prediction. These results suggested that the insert sequence of clone Z009-003_C06 was full-length cDNA of LDH from *E. granulosus*, named as EgLDH; its accession number of GenBank was HM748917.

```
   1 GGGTCACCCTTGTGGAGTCATGTCTGTGGAGGGGTTGTTGTTGCCTTTGGAGATGGAA
                     M  S  V  E  G  L  L  L  P  L  E  M  E
  59 CAGTGTTTTGGGCGTGAGCGGAAGGTTTCTGTTGTTGGTGCGGGAGCAGTAGGCACGGCA
      Q  C  F  G  R  E  R  K  V  S  V  V  G  A  G  A  V  G  T  A
 119 GCGGTGTTTGCTATTATGACTAAAGGTATTGCAAACACTGTCGCTCTCTACGATATTGAC
      A  V  F  A  I  M  T  K  G  I  A  N  T  V  A  L  Y  D  I  D
 179 GAAGATAGATGCAACGGTGAAGTGATGGACTTGGACCAAGGCTCACTGTTTCTGGAGTCT
      E  D  R  C  N  G  E  V  M  D  L  D  Q  G  S  L  F  L  E  S
 239 TGTAGAGTAATTGGTGGCAAAGATATAACGAAGACTGCGGACTCGGATATCGTTGTAGTA
      C  R  V  I  G  G  K  D  I  T  K  T  A  D  S  D  I  V  V  V
 299 ACAGCTGGGGCCCGGCAAGCTGTTGGCGAATCCAGATTGAACCTTGTTCAACGCAATGTT
      T  A  G  A  R  Q  A  V  G  E  S  R  L  N  L  V  Q  R  N  V
 359 GATATATTTAAAAAACTAATTCCTACTCTCGTTGAACAAAGCCCAAAGTGCATTCTCGTT
      D  I  F  K  K  L  I  P  T  L  V  E  Q  S  P  K  C  I  L  V
 419 ATCGTTACAAATCCAGTTGATATCATGACCTATGTCTCCTGGAAGTTAAGCGGCTTTCCA
      I  V  T  N  P  V  D  I  M  T  Y  V  S  W  K  L  S  G  F  P
 479 CAGCATCGTGTCTTAGGATCTGGAACCATGCTTGACACTGCTAGATTTCGGCACATTCTT
      Q  H  R  V  L  G  S  G  T  M  L  D  T  A  R  F  R  H  I  L
 539 GGCGAGAAGTTGAATGTGCATCCTAGTGCCATACACGGTTACGTAGTCGGCGAACATGGA
      G  E  K  L  N  V  H  P  S  A  I  H  G  Y  V  V  G  E  H  G
 599 GACTCGAGTGTGCCAGTATGGAGTAGGGTGACCGTTGGTGGAGCAAATCTCTGTGACATT
      D  S  S  V  P  V  W  S  R  V  T  V  G  G  A  N  L  C  D  I
 659 TATCCCAAGATCGGCCAAGCCGGCGATCCCGACGATTTTGCTTCCATTCACAAGGCTGTT
      Y  P  K  I  G  Q  A  G  D  P  D  D  F  A  S  I  H  K  A  V
 719 GTCGATAGCGCCTACGAAATCATTCGCATGAAGGGCTGCACTGCTTGGGCCATAGGTCTC
      V  D  S  A  Y  E  I  I  R  M  K  G  C  T  A  W  A  I  G  L
 779 TGTTGCGCCTCCCTGTGTAATGCGATTCTGCGAAACAAGAAAATTGTGATTCCAGTTTCC
      C  C  A  S  L  C  N  A  I  L  R  N  K  K  I  V  I  P  V  S
 839 ACCTCACTTAAGGGCAAACTTGGTATCAAAGAGGAGGTGTTCACGAGCGTGCCCTGTATA
      T  S  L  K  G  K  L  G  I  K  E  E  V  F  T  S  V  P  C  I
 899 GTCGACAGCAGCGGCGTGTCTGCAGTGATCAACCTCGAGTATTCGCCTAGTGAGAAACAA
      V  D  S  S  G  V  S  A  V  I  N  L  E  Y  S  P  S  E  K  Q
 959 AGCCTGTTGGCCAGTGTTGAGACTCTTCAGAAGATTATCGCAGGCATCAAGTGGTGAATG
      S  L  L  A  S  V  E  T  L  Q  K  I  I  A  G  I  K  W  *
1019 AAGTGGGAAATTTACATACGTTCCATAGTGTTATGTGGATTGCGAGGTTTAGAAACCTTG
1079 TGCTATATGAGTATAGTTGTTGGCAAAAAAAAAAAAAAAAAACATGTCGGCCGCCTCGGC
1139 CTATGTGCGGCCGCCACCGC
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Figure 1. Full length cDNA sequence and deduced amino acid sequence of EgLDH.

3.2. Comparison of amino acid sequences between EgLDH and LDHs from other species

Using EgLDH protein sequence as a reference, conservative sites shared with LDHs of other species were as follows: 28 (G), 31 (G), 105 (R), 108 (L), 112 (N), 115 (I), 128 (P), 138 (N), 138 (P), 164 (L), 165 (D), 168 (R), 192 (H), 193 (G), 250 (A), 281 (G), 291 (P), 298 (G). Among these conserved sites, 105 (R), 165 (D) and 192 (H) are the key catalytic sites of LDH. Similarities of amino acid sequences between EgLDH and LDHs from other species were shown in Table 1. The highest homology was with TaLDH (86%) and TsLDH (85%), while the lowest was in TvLDH (17%); it should be mentioned that EgLDH had 53% homology with HsLDH.

Phylogenetic analysis implied that EgLDH, TaLDH and TsLDH originated from the same ancestor, all of which belong to the class of Taeniidae, Cyclophyllidea, Cestoidea, *Phylum Platyhelminthes*, while the evolutionary distance between EgLDH and SeLDH belong to Pseudophyllidea was far apart (Figure 2).

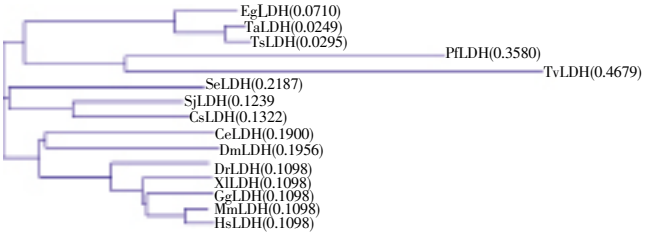

Figure 2. Phylogenetic relationship between EgLDH and LDHs from other species.

3.3. Physical and chemical parameters, signal peptide and subcellular localization prediction

The putative gene product of EgLDH had a predicted isoelectric point (PI) of 6.32 and a molecular weight of 35 516.20 Da; its extinction coefficient value for solution at 280 nm was 31 565 units/M/cm. The estimated half-life of protein predicted in mammals, yeast and *Escherichia coli* were 30 h, >20 h and >10 h, respectively; its instability coefficient was 27.96, thus EgLDH was predicted as a stable protein. A suspected signal peptide cleavage site was located between 17aa-18aa. No target signal of mitochondria, nucleus or peroxisome was observed. Subcellular localization prediction showed EgLDH to be a cytoplasmic protein (94.1% reliability) which located in the endoplasmic reticulum, mitochondria, cytoplasm, Golgi complex and nuclear potential with possibility of 44.4%, 22.2%, 11.1%, 11.1%, 11.1%, respectively.

3.4. Hydrophility and linear-B cell epitopes prediction

The results of hydrophilicity estimation and linear B-cell epitopes prediction were similar. Possible epitopes were located at residues 54aa-59aa, 81aa-87aa, 97aa-102aa, 190aa-198aa, 216aa-227aa and 307aa-313aa. Comparison was done between the major epitopes of EgLDH and the corresponding regions of TaLDH, TsLDH, SeLDH, SjLDH, CsLDH and HsLDH; the results revealed 54aa-59aa, 81aa-87aa, 97aa-102aa, 307aa-313aa were low similarity compared with the corresponding regions of human's and other parasite's LDH except TaLDH and TsLDH, which implied that these epitopes might be ideal diagnostic antigen epitopes (Table 2).

3.5. Analysis of secondary structure and topology of protein, substrate-binding site & NAD-binding site, post-translation modification site.

Figure 3 showed predicated results of secondary structure and topology of EgLDH. Three potential transmembrane regions (26aa-43aa, 127aa-144aa and 245aa-262aa) were existed, with N-terminal region inside membrane and C-terminal outside, which implied that EgLDH might be a transmembrane protein. 44aa-126aa and 263aa-311aa were located outside membrane. There were 15 predicated NAD-binding sites and 7 predicated pyruvate-binding sites, mainly locating around three key catalytic sites: 105 (R), 164 (D) and 192 (H). There were also different types of phosphorylation sites on EgLDH including one of cAMP/cGMP dependent protein kinase (22aa-23aa), six of protein kinase C (73aa-75aa, 127aa-129aa, 146aa-148aa, 166aa-168aa, 275aa-277aa, 310aa-312aa), four of casein kinase Ⅱ (122aa-125aa, 162aa-165aa, 236aa-239aa, 308aa-311aa) and one of tyrosine kinase (231aa-238aa). Besides, there were five N-myristoylation sites (28aa-33aa, 42aa-48aa, 96aa-101aa, 207aa-212aa, 252aa-257aa) and one active site of L-lactate Dehydrogenase (189aa-195aa) existing in the protein.

3.6. Homology modeling and predication of tertiary structure

The homology tertiary structure was showed as Figure 4. Epitopes 54aa-59aa, 81aa-87aa, 97aa-102aa and 307aa-313aa were all on the protein surface, among which there were NAD and substrate binding sites locating on epitopes 54aa-59aa and 97aa-102aa respectively. Key catalytic residues 105R, 165D and 192H formed a catalytic center which near epitope 97aa-102aa. Most NAD and substrate binding sites were located around the epitope 97aa-102aa.

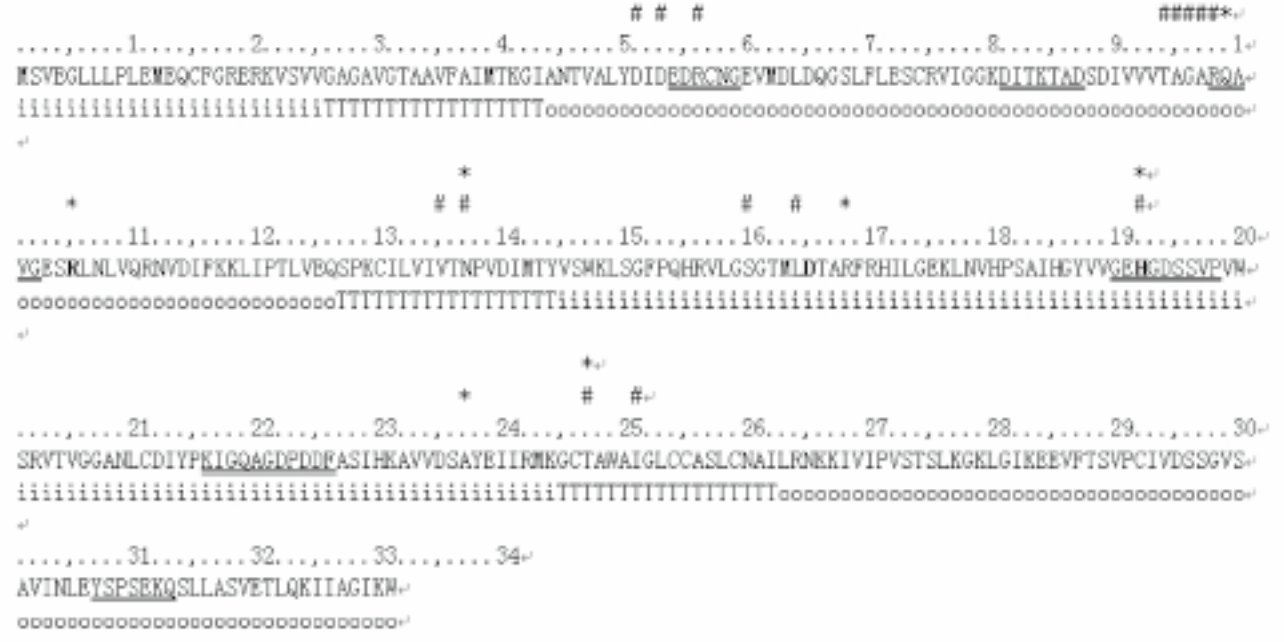

Figure 3. Predicted amino acid sequence and topology of EgLDH.
Underline: epitope; Bold: key catalytic residue; I: intramembrane; O: outramembrane; T: transmembrane; #: NAD binding site; *: substrate (pyruvate) binding site.

Table 1
Similarity of predicted amino acid sequence of EgLDH and LDHs from other species.

	EgLDH	TaLDH	TsLDH	SeLDH	SjLDH	CsLDH	PfLDH	TvLDH	CeLDH	DmLDH	DrLDH	XlLDH	GgLDH	MmLDH	HsLDH
EgLDH	100	86	85	55	58	58	29	17	49	50	54	52	54	53	53
TaLDH		100	95	52	54	55	31	16	48	50	52	52	55	52	52
TsLDH			100	52	53	56	30	17	48	50	52	52	54	51	51
SeLDH				100	59	59	27	14	55	55	55	55	53	55	55
SjLDH					100	75	28	16	58	57	59	61	58	61	60
CsLDH						100	30	16	58	56	57	57	57	58	57
PfLDH							100	22	28	27	28	27	28	28	28
TvLDH								100	16	14	15	14	16	15	15
CeLDH									100	64	63	62	64	61	61
DmLDH										100	64	62	63	64	63
DrLDH											100	80	77	79	77
XlLDH												100	84	86	86
GgLDH													100	86	85
MmLDH														100	94
HsLDH															100

Table 2
Similarity of major epitopes of EgLDH with the corresponding regions of TaLDH, TsLDH, SeLDH, SjLDH, CsLDH and HsLDH.

EgLDH	TaLDH(%)	TsLDH(%)	SeLDH(%)	SjLDH(%)	CsLDH(%)	HsLDH(%)
54aa–59aa	83.33	100.00	33.33	33.33	33.33	50.00
81aa–87aa	85.71	85.71	28.57	14.29	14.29	28.57
97aa–102aa	100.00	100.00	60.00	60.00	60.00	60.00
190aa–198aa	100.00	100.00	88.89	100.00	100.00	100.00
216aa–227aa	100.00	100.00	45.45	54.55	54.55	18.18
307aa–313aa	85.71	85.71	28.57	28.57	28.57	14.29

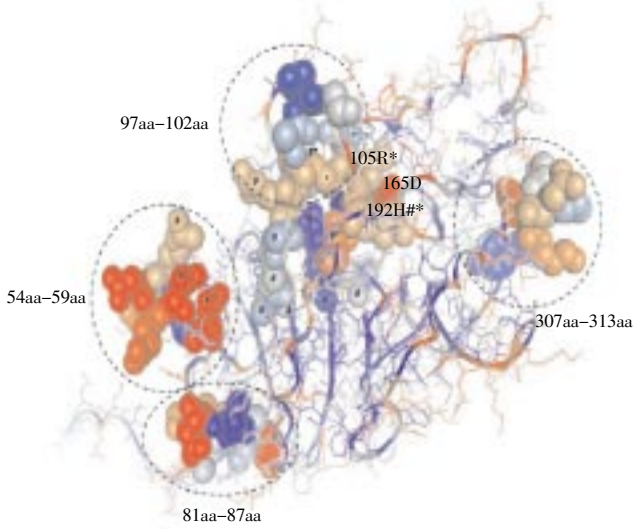

Figure 4. Tertiary structure model of EgLDH (Cycle: main epitope region, 105R, 165D, 197H: key catalysis sites; #: NAD binding site; *: substrate binding sites).

4. Discussion

China is one of the countries with high incidence of CE. This disease can not only impede livestock production and development but also can cause serious public health and socioeconomic problems in epidemic areas[2-5]. The main controlling strategies include cutting off the life cycle of the parasite, preventing intermediate host (human and livestock) and terminal host (dog) from infection, treating infected terminal host and blocking egg spreading[10]. Dogs are the main source of infection and their quantity are less than that of livestock. In addition, it is more effective, safe and economic to apply parasiticide or vaccine to dogs. Since they are easier to practice, these treatment and prevention methods are regarded as one of the fundamental approaches to control the prevalence of CE[11].

Adult *E. granulosus* lives in the small intestine of dogs, in which anaerobic glycolysis is the main source of energy of the parasite. Lactate dehydrogenase, as the terminase of glycolysis, can catalyze pyruvic acid into lactic acid and plays an important role in energy metabolism of the parasite. In theory, LDH and other glycolysis enzymes might be the potential drug and vaccine target candidates.

In this study, the complete cDNA sequence of EgLDH was obtained from full-length cDNA library of adult *E. granulosus*. EgLDH had 53% similarity to HsLDH and shared the same conservative sites and key catalytic sites with LDHs from other species. Phylogenetic tree implied that LDH is an ideal molecule for phylogenetic analysis.

It was predicted that EgLDH might be a membrane protein because it contained one putative signal peptide and three potential transmembrane regions. These characters were also existed in LDHs from different parasites, including *Taenia solium, Taenia asiatica, Schistosoma japonicum, Clonorchiasis sinensis, Plasmodium vivax*, and had been primary confirmed by experiments on *Clonorchiasis sinensis* and *Taenia asiatica*[12-15]. Sabine Bork *et al.*

had found LDH of *Babesia bovis* located not only on the surface of the parasite but also on the membrane of infected erythrocytes[16]. Kee-Hoon Kwak *et al* found that LDH had the highest activity in the tegument and subtegumental muscle layers of adult *Spirometra mansoni* and Sparganum[17]. All these studies implied that EgLDH possess characteristics as a membrane protein.

Six potential antigen epitops existed in EgLDH by linear B-cell epitope prediction. Four of them, 54aa-59aa, 81aa-87aa, 97aa-102aa and 307aa-313aa, were located outside membrane, which were high similarity compared with the corresponding regions of TsLDH and TaLDH while were low similarity with that of HsLDH, SeLDH, SjLDH and CsLDH. Especially, some NAD and substrate binding sites located on 54aa-59aa and 97aa-102aa, and the latter was close to the catalytic center formed by three key catalytic residues 105R, 165D and 192H; other binding sites were near this region by tertiary structure analysis. These indicated, when EgLDH reacted with corresponding antibody, it could not only mediate immune attack but also specifically inhibit the enzyme activity and hamper the binding of NAD and pyruvic acid, which could result in death of the parasite for accumulation of pyruvic acid in cell. Therefore, antibody of EgLDH could be regarded as a highly specific molecule drug. Analogues of NAD and pyruvic acid aimed directly at the epitope sites could also block the enzyme activity and killed parasites. So, these epitops are ideal targets for vaccine and drugs, and could be used in screening for new drugs against parasites. Although praziquantel, an important anti-parasite drug, has remarkable effect on some species of Platyhelminthes such as trematode and tapeworm, the exact mechanism of action is unknown. The author has found that praziquantel mediated inhibition of recombinant SjLDH, indicating LDH might be a molecular target for praziquantel[18]. Further investigation should be conducted on understanding of the possible association between EgLDH and praziquantel.

Conflict of interest statement

We declare that we have no conflict of interest.

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