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PROTEOMIC ANALYSIS OF *TAENIA SOLIUM* CYST FLUID BY SHOTGUN LC-MS/MS

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KEY WORDS

Taenia solium
Proteomics
Shotgun

ABSTRACT

Taenia solium cysts were collected from pig skeletal muscle and analyzed via a shotgun proteomic approach to identify known proteins in the cyst fluid and to explore host-parasite interactions. Cyst fluid was aseptically collected and analyzed with shotgun liquid chromatography-tandem mass spectrometry (LC-MS/MS). Gene alignment and annotation were performed using Blast2GO software followed by gene ontology analysis of the annotated proteins. The pathways were further analyzed with the Kyoto Encyclopedia of Genes and Genomes (KEGG), and a protein-protein interaction (PPI) network map was generated using STRING software. A total of 158 known proteins were identified, most of which were low-molecular-mass proteins. These proteins were mainly involved in cellular and metabolic processes, and their molecular functions were predominantly related to catalytic activity and binding functions. The pathway enrichment analysis revealed that the known proteins were mainly enriched in the PI3K-Akt and glycolysis/gluconeogenesis signaling pathways. The nodes in the PPI network mainly consisted of enzymes involved in sugar metabolism. The cyst fluid proteins screened in this study may play important roles in the interaction between the cysticerci and the host. The shotgun LC-MS/MS, gene ontology, KEGG, and PPI network map data will be used to identify and analyze the cyst fluid proteome of cysticerci, which will provide a basis for further exploration of the invasion and activities of *T. solium*.

Cysticercosis is a major parasitic zoonosis caused by the larvae of *Taenia solium*. Cysticercosis is most prevalent in Latin American countries, sub-Saharan Africa, and most of Asia, including China, India, and most of Southeast Asia (Ito et al., 2015). Humans or pigs can be infected by ingesting the feces of infected animals that contain fertilized eggs, at which point they become intermediate hosts. Upon ingestion, the embryos are released from eggs in the intestine and actively pass through the intestinal mucosa into the bloodstream, and then they flow to peripheral tissues, including the central nervous system, where they develop into cysticerci (Arriola et al., 2014; Garcia et al., 2014). The parasite colonizes host tissue, regulates the host immune response, and establishes long-term asymptomatic infection. When the central nervous system is affected, the symptoms of infection include headache, intracranial hypertension, and epilepsy (Terrazas, 2008; Davis, 2011). Absorption of host proteins during cysticercosis is essential for parasite survival in host tissues in processes such as osmotic regulation of worms, self-metabolic processes, and protection of innate immune responses; furthermore, host proteins have been confirmed to be contained in the cyst fluid, the excreted secretion products, and the tissue of metacestodes (Aldridge et al., 2006). In the process of parasitism, a large number of inflammatory type-1 cytokines, such as IFN-γ and IL-2, IL-12, IL-18, and TNF-α, appear around the cyst. Furthermore, fibrosis surrounds the cyst and produces mixed Th1 and Th2 immune responses, and the levels of interferon-γ, IL-18, IL-4, IL-10, IL-13, and transforming growth factor-β all increase; in the late stage of parasitism, inflammation and edema gradually disappear, and the vesicles collapse and eventually develop into calcified nodules (Nash et al., 2008). In this stage, the main response is Th2, and the expression levels of IL-4, IL-5, and IL-13 increase (Prodjinotho et al., 2020). Cyst fluid accounts for

most of the weight of the worm, participates in many metabolic and immune processes, and plays an important role throughout the parasitic process. Therefore, understanding the composition of cyst fluid protein is crucial for studying the interaction between *T. solium* and the host. In a recent study of cysticercosis proteomics, Navarrete-Perea et al. (2014) compared cysticercosis collected from the central nervous system and skeletal muscle and the sera from a cysticercosis patient with 2-dimensional electrophoresis. Quantitative analysis indicated that the host proteins accounted for 11–13% of the cystic protein content. Simultaneously, Navarrete-Perea et al. (2016) used 2-dimensional electrophoresis to analyze the tissue localization of cysts in different locations with PDQuest^{TM} (Bio-Rad, Hercules, California) and multivariate analysis and found that the protein patterns of cysts obtained from the central nervous system and skeletal muscle were significantly different. Diaz-Masmela et al. (2013) identified 7 specific antigens in cyst fluid compared with direct source homologs. Only annexin B1 and cAMP-dependent protein kinase are highly specific and may be developed as candidate diagnostic antigens for cysticercosis. As the primary method for separating and comparing protein mixtures, 2-dimensional electrophoresis (2-DE) combined with mass spectrometry (MS) has become a major tool for studying proteomics due to its high resolution, and it is often used to construct differential protein expression profiles (Dorri, 2019). The method separates proteins according to their isoelectric point in the first and second dimensions according to their molecular weight. However, 2-DE presents limitations, such as limited loading capacity, and it cannot separate insoluble proteins and extremely acidic and alkaline proteins, as well as low abundance proteins; the variation between protein gels is also a common problem with this method (Sá-Correia and Teixeira, 2010; Rabilloud, 2015). Studies in recent years have shown that the shotgun proteomics method requires only a single sample to identify hundreds of proteins compared to what is required for the 2-DE/MS proteomics method. After trypsin hydrolyses the protein, MS is used to assay each peptide with great efficiency (Hayes and Yates, 2002). The shotgun proteomic approach in the parasite field has been very successful in constructing differential expression profiles and screening for specific antigens such as *schistosomiasis*, *Eimeria maxima*, and *Fasciola hepatica* (Campos et al., 2017; Liu et al., 2017; Huang et al., 2018). Most of the recent studies have been single screenings of specific antigens; however, the interactions between the host and the parasite involve a large number of proteins, and these proteins are involved in an interconnected network. With the deepening of proteomics research in the field of parasites, some new methods should be used to comprehensively analyze cysticercosis (Burgess and Burchmore, 2012). In this study, the proteins from cysticerci cyst fluid were qualitatively analyzed with a shotgun proteomics method, and then bioinformatics methods were used to comprehensively analyze the selected proteins. The results of this study will provide researchers with a wealth of valuable information on the different developmental stages of the parasite, colonization invasion, and immune evasion.

MATERIALS AND METHODS

Ethics

Animals were treated according to the guidelines of the USA National Institutes of Health (publication No. 85-23, revised 1996). Animal protocols were reviewed and approved by the Ethical Committee of Jilin University affiliated with the Provincial Animal Health Committee, Jilin Province, China (ethical clearance number IZ-2009-08).

Cyst fluid and protein extracts

Pigs naturally infected with *T. solium* cysticerci were dissected. The cysts were collected from at least 3 different sites of 5 infected pigs under aseptic conditions. Inflammatory capsules surrounding the cysts were carefully removed to reduce host tissue contamination. The larvae were washed several times with ice-cold phosphate-buffered saline (pH 7.3). Harvests were performed within 90 min of tissue isolation. The cyst wall was cut with a scalpel in a dry Petri dish. Released cyst fluid was collected and pooled. The pooled cyst fluid was mixed 1:1 with HEPES buffer, homogenized in 8 M urea in 50 mM HEPES, pH 8.0, with protease inhibitors (Complete, Roche, Shanghai, China), vortexed and frozen and thawed 5 times, and centrifuged at 16,000 *g* for 15 min; finally, the supernatant was collected (Navarrete-Perea et al., 2017). The total protein concentration was determined with bicinchoninic acid, and the supernatant was stored at –80 C.

Trypsin digestion

A protein solution (30 μg) was dissolved in a 0.1% sodium dodecyl sulfate and 50 mM Tris-HCl (pH 8.0) solution and 1 M dithiothreitol (DTT) (Bio-Rad) to a final dithiothreitol concentration of 2–5 mM. The protein solution was heated at 60 C for 45–60 min and cooled to room temperature. Then 1 M indole-3-acetic acid (Sigma-Aldrich, St. Louis, Missouri) was added to a final concentration of 10–25 mM, and the solution was incubated for 45 min at room temperature in the dark. Next, 50 mM NH_4HCO_3 (pH 7.8)-diluted urea was added to a final concentration of no more than 1 M; the ratio of trypsin enzyme (Promega, Madison, Wisconsin) to substrate protein was 1:50. Then the enzyme solution was added, and the sample was incubated at 37 C for 20 hr. Finally, 2% formic acid (FA) was added, and the reaction was stopped at an FA concentration of 0.1%; subsequently, the sample was concentrated to a suitable volume (Sun et al., 2014).

Liquid chromatography and tandem mass spectrometry (LC-MS/MS)

Digested protein samples were assayed using liquid chromatography and tandem mass spectrometry (LC-MS/MS) on an Easy-nLC 1000 (Thermo Fisher) coupled Q Exactive (QE) (Thermo Fisher) mass spectrometer. Protein samples digested with trypsin were captured and desalted on a Zorbax 300SB-C18 peptide trap (Agilent Technologies, Palo Alto, California) and separated on a C18 reversed-phase column (0.15 × 150 mm; Column Technology Inc., Fremont, California). Mobile phases A (0.1% formic acid in HPLC-grade water) and B (0.4% FA in 84% acetonitrile) were collected using an Easy nLC system with a linear gradient of 4–50% B (50 min), 50–100% B (4 min), and 100% B (6 min) with a flow rate of 250 nl/min (Huang et al., 2018).

Protein identification and annotation

The mass spectrometry test raw file used MASCOT 2.2 (Matrix Science, London, U.K.) to search the corresponding database for

Table I. Some proteins from some cysticercus cellulosae screened with shotgun liquid chromatography-tandem mass spectrometry (LC-MS/MS). Abbreviations: MW, molecular weight; PI, isoelectric points.

No.	SwissProt/TrEMBL AC	Protein description	PepCount	UniquePepCount	CoverPercent	MW (kDa)	PI
1	X2D553	Fasciclin-1	50	36	53.66	86.95	5.84
2	D2U5C3	Phosphoenolpyruvate carboxykinase (fragment)	31	23	55.58	54.10	8.49
3	A0A1S5WII1	Enolase	21	18	45.62	46.61	6.48
4	E5LBB8	Trypsin-like protein	33	17	39.96	54.12	7.09
5	A0A291I2R3	Serpin	17	15	41.90	45.54	7.63
6	A0A0R3VUJ9	Aminopeptidase	13	12	11.57	114.10	6.88
7	K7SRY9	Actin	11	9	30.59	41.74	5.3
8	Q9U8G9	Cysticercosis 10 kDa antigen	88	8	43.53	9.65	8.89
9	Q8I0G1	Excretion-secretion antigen m13	52	8	50.59	9.82	9.75
10	A0A0R3VWJ0	Phosphoglycerate kinase	10	7	17.59	44.13	7.56
11	B6E4B3	8 kDa glycoprotein	31	5	48.24	9.66	9.78
12	A0A0R3VY11	Alpha-1,4 glucan phosphorylase	6	5	9.58	66.54	5.27
13	A0A0R3W350	Nucleoside diphosphate kinase	6	5	40.76	17.56	8.55
14	A0A0R3W9G0	Adenosylhomocysteinase	5	5	12.21	51.82	6.16
15	Q967Z2	Calcium binding protein	5	5	17.47	45.57	4.41
16	Q8MPB9	Small heat-shock protein	5	5	18.15	35.60	5.56
17	Q5GM20	GP50	5	4	23.51	29.57	8.68
18	A0A0R3WCA2	Amine oxidase	5	4	4.90	80.99	6.55
19	A0A0R3VV32	Tubulin beta chain	4	4	9.21	49.86	4.76
20	G8ZAG5	L-lactate dehydrogenase	4	4	14.50	35.46	7.09

protein identification results (Bollineni et al., 2018). The UniProt database was used to search for the protein sequences obtained from the European Institute of Bioinformatics (https://www.uniprot.org/), and the data were downloaded in FASTA format (Pundir et al., 2015). Blast2GO software was downloaded and installed (https://www.blast2go.com/). The downloaded protein sequences were compared with the *T. solium* genome database, annotated with the NCBI protein database (https://www.ncbi.nlm.nih.gov/protein/), and exported in the native format (Conesa and Gótz, 2008). The data in native format were subjected to gene ontology (GO) category analysis through the Web Gene Ontology Annotation Plot (WEGO) tool (http://wego.genomics.org.cn/). The 3 groups of datasets were simultaneously subjected to online analysis and were compared in 1 graph (Ye et al., 2018). The downloaded sequences then underwent KOBAS 3.0 (http://kobas.cbi.pku.edu.cn/annotate.php) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.kegg.jp/) online pathway enrichment analysis (Yang et al., 2018). A protein-protein interaction (PPI) network map was generated using STRING Version 10.5 (http://string-db.org), showing possible interactions with known proteins (Szklarczyk et al., 2017).

RESULTS

Shotgun proteomics of cyst fluid protein

After analysis of cysticercoid cyst fluid by QE shotgun LC-MS/MS, a total of 486 peptides were identified using UniProt and MASCOT 2.2, of which 158 proteins correlated with known proteins, and 328 proteins were previously uncharacterized. Uncharacterized protein refers to a protein presumed to exist based on the location and sequence of the chromosome in which the gene is encoded, though its structure and function have not yet been elucidated. Table I lists some of the activities and related metabolic processes of the proteins identified in cysticercosis, which may play an important role in the interaction between *T. solium* cysticerci and the host. MASCOT 2.2 was used to calculate the molecular weights (MWs) and isoelectric points (PIs) of known proteins identified in this study. The MW and PI distributions of the proteins were analyzed. Protein MWs ranged from 7.55 to 151.98 kDa. Among them, 111 (70%) proteins were less than 30 kDa. Additionally, 42 (26%) proteins were between 5 and 10 kDa. The protein PIs were distributed between 4.3 and 11.7, and 48 (30%) proteins were distributed in the range of 8 to 9. In addition, 3 known proteins with PI $>$ 10 were identified through shotgun analysis (Fig. 1).

Known proteins of cyst fluid annotated with gene ontology

To understand the functions of the proteins identified in this experiment, we performed GO analysis, which includes 3

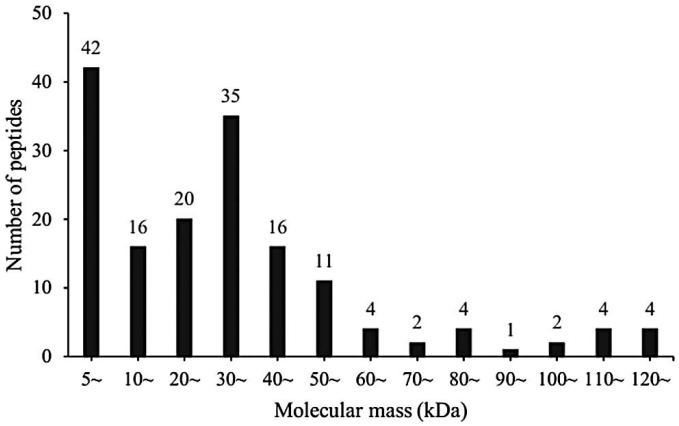

Figure 1. Screening and identification of molecular mass (MW) distribution of known proteins of cysticerci cyst fluid by shotgun LC-MS/MS, which ranged from 7.55 to 151.98 kDa.

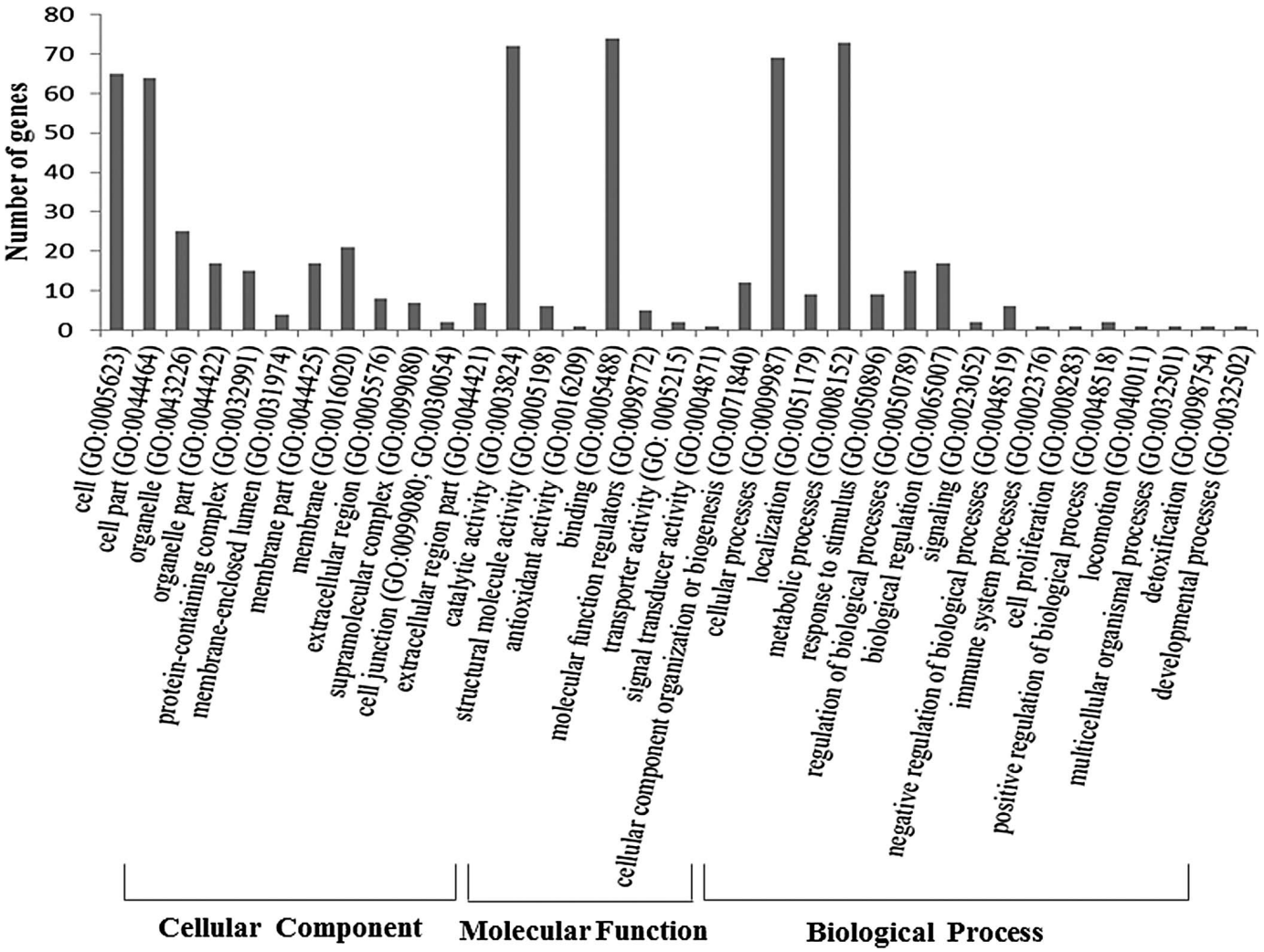

Figure 2. Gene ontology (GO) of cysticerci cyst fluid, WEGO divides the annotated proteins into 3 categories: cellular component, molecular function, and biological process. The number of genes is expressed as the number of annotation proteins.

categories: cellular composition, molecular function, and biological process categories (Fig. 2).

KEGG pathway enrichment analysis of known proteins in cyst fluid

KEGG pathway enrichment analysis showed that the known proteins identified are mainly involved in the PI3K-Akt signaling pathway, glycolysis/gluconeogenesis, pathways in cancer, the Hippo signaling pathway, and human papillomavirus infection (Fig. 3). KEGG pathway enrichment analysis indicated that the identified proteins are known to associate with the PI3K-Akt signaling pathway, including laminin, phosphoenolpyruvate carboxykinase, laminin gamma 1, laminin beta 1, 14-3-3 protein beta/theta/zeta, 14-3-3 protein epsilon, collagen type IV alpha, heat shock protein 90 kDa beta, serine/threonine-protein phosphatase 2A regulatory subunit B, collagen type IV alpha, guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1, guanine nucleotide-binding protein subunit beta-5, and serine/threonine-protein kinase mTOR (Fig. 4).

The KEGG pathway enrichment analysis indicated that the previously identified proteins are known to associate with the glycolytic pathway, including phosphoenolpyruvate carboxykinase, enolase, fructose-bisphosphate aldolase, phosphoglycerate kinase, glucose-6-phosphate isomerase, phosphoglucomutase, glyceraldehyde 3-phosphate dehydrogenase, 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase, L-lactate dehydrogenase, triosephosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, pyruvate kinase, and fructose-1,6-bisphosphatase I (Fig. 5). Table II lists some proteins associated with the PI3K-AKT/mTOR and glycolysis/gluconeogenesis pathways in the KEGG pathway map naming convention.

Generation of a protein interaction network from the known proteins in cyst fluid

A PPI network was generated, and functional relationships between known proteins were analyzed with STRING Version 10.5, as shown in Figure 6. Networks associated with known proteins were extracted from the STRING database, and the lines with different colors between proteins show various types of

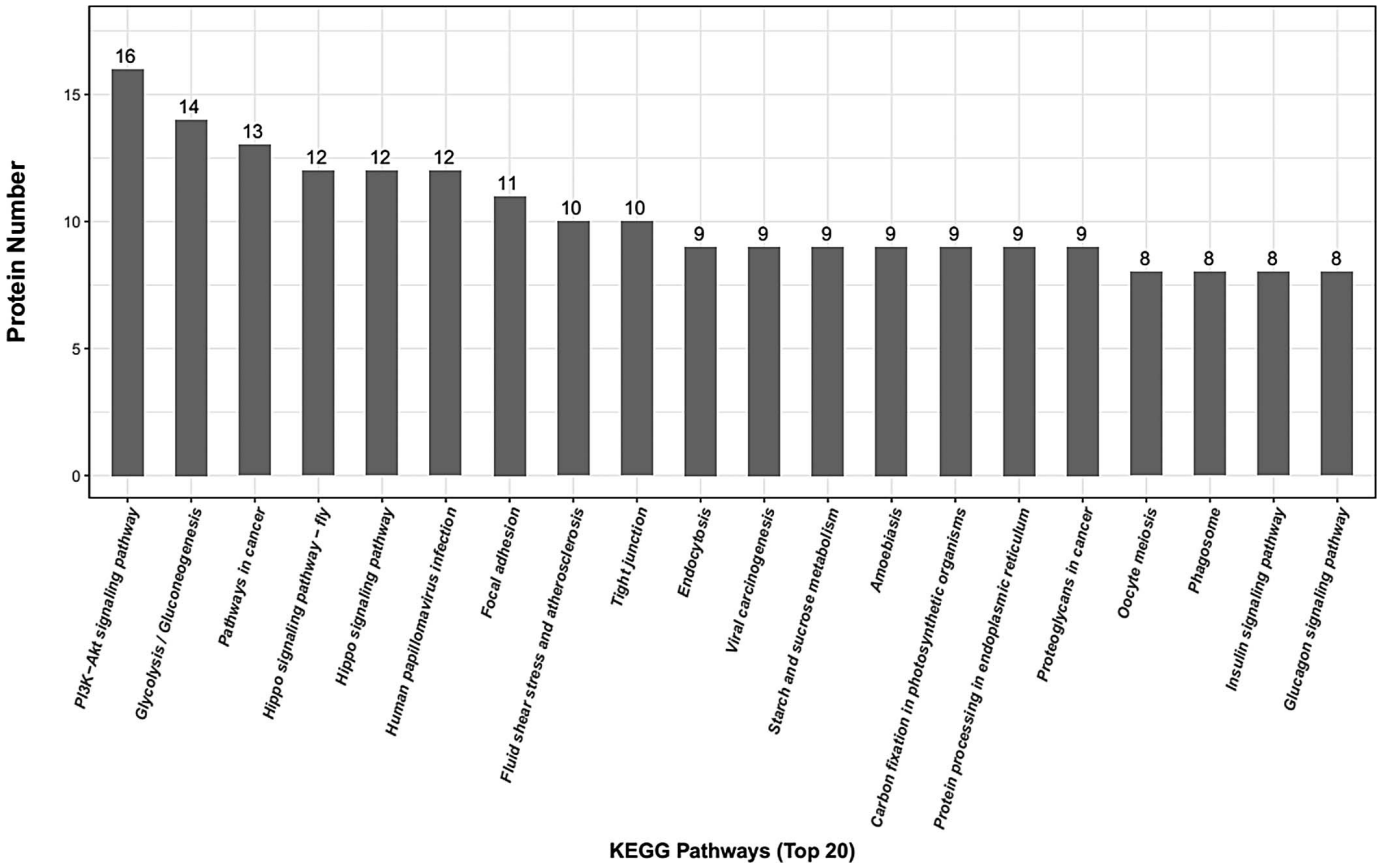

Figure 3. Pathway enrichment analysis of known proteins was performed with the Kyoto Gene and Genomics Encyclopedia (KEGG) (https://www.kegg.jp/). The figure shows the top 20 signaling pathways.

evidence of interaction. Actin-2 (Smp_161920), heat shock 70 kDa protein (Smp_106930), serine/threonine kinase (Smp_094760), annexin (Smp_074150), and other proteins were found in the junction nodes of the PPI network. However, the most concentrated proteins in nodes are those involved in metabolic processes, such as enolase (Smp_024110), pyruvate kinase (Smp_065610.1), L-lactate dehydrogenase (Smp_038950), and other proteins.

DISCUSSION

Cysticercosis is a disease caused by the larvae of the pork tapeworm (*Taenia solium*), which can cause neurological symptoms, such as seizures, when the larvae infect the brain parenchyma (Rodriguez et al., 2012). There are 2 reasons for the long-term survival of larvae in the host. First, the larval antigens constantly mutate to escape the host immune system. Second, the larvae secrete proteins that interfere with host immune recognition. Since cyst fluid contains parasites and host proteins, Santos et al. considered that there is an exchange of proteins between the host and the parasites (Santos et al., 2016). One of the host protein functions is immune evasion, which can be achieved by the parasite binding to host molecules or by reducing the exposure of the associated parasite antigen, thus helping the parasite achieve immune evasion while suppressing host immunity (Monteiro et al., 2010). Secreted proteins highly expressed in cyst fluid can cause host immunosuppression and alter host metabolism by affecting the cytokine network and signal transduction pathways of the host immune system or by inhibiting some important enzymes, such as glycolytic enzymes and neuron-specific enolases (Sciutto et al., 2007). Therefore, secreted proteins are the most important type of protein for studying the interaction between *T. solium* cysticerci and the host. The analysis and study of secreted proteins and their functions in cysticerci cyst fluid can also improve the understanding of the mechanism of cysticerci escape from the immune system. The disease is not simply a parasitic invasion; it also involves an immune interaction between the parasite and the human host (Schmid-Hempel, 2009). The study of specific interactions between parasites and hosts has given us greater insight into cysticercosis. At this stage, high-throughput proteomics can be used to identify proteins and analyze metabolic pathways that may be associated with disease progression to find specific antigens associated with disease progression and potential diagnostic biomarkers (Lee et al., 2007).

In this study, qualitative analysis was carried out on the protein components of cysticerci using a shotgun technique. A total of 486 proteins were identified, of which 158 were known. In cases where no protein sequence database is available, the genome sequence is employed as a search database (Yilmaz et al., 2016). Genomics assists us in understanding metabolic pathways, development, and reproduction, parasite-host interactions, parasite invasion, etc., and provides ideas for the prevention and control of cysticercosis (Lv et al., 2015). The gene number of *T. solium* is 12,490, the genome size is 122.3 Mb,

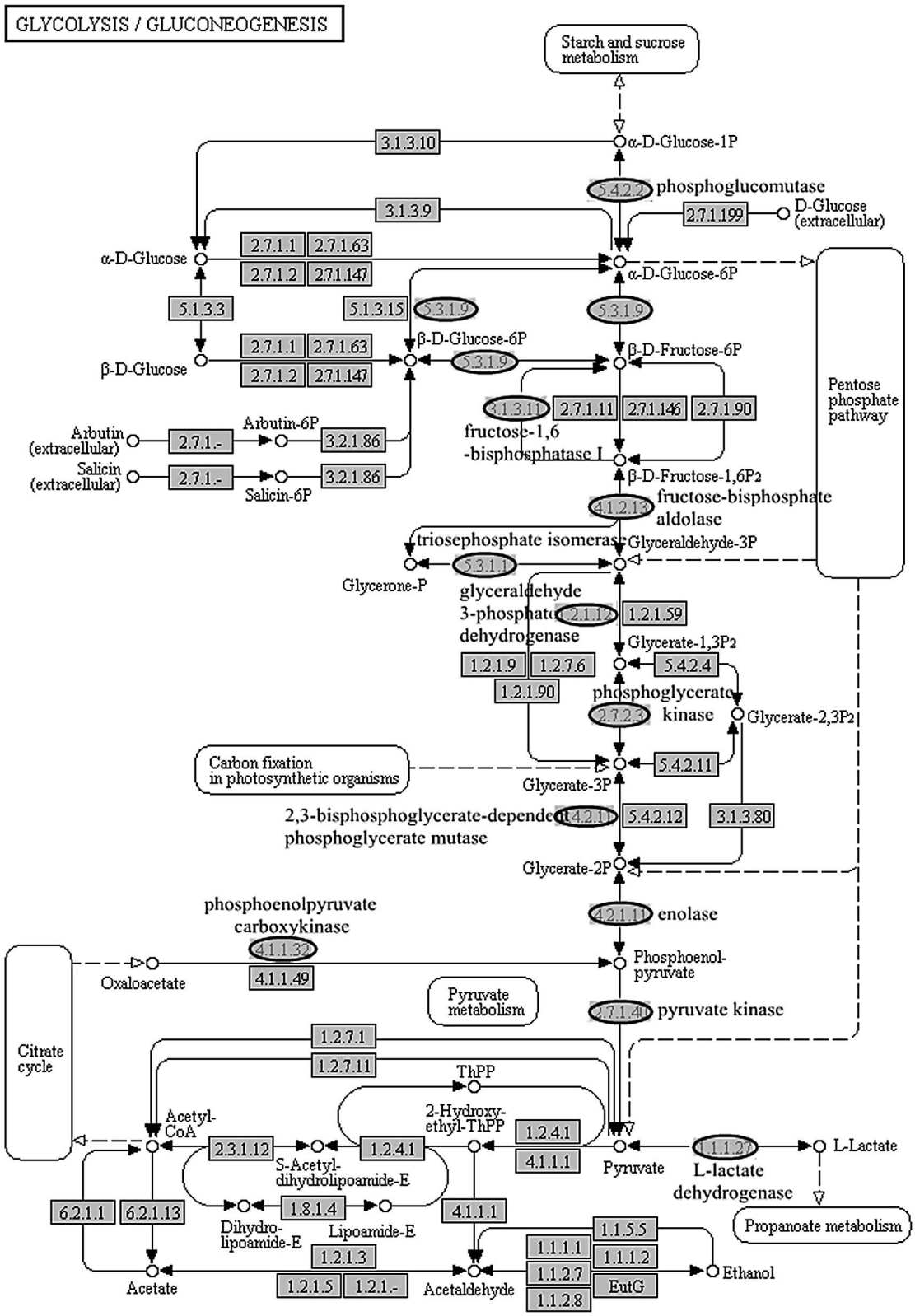

Figure 4. The glycolysis/gluconeogenesis signaling pathway constructed with the Kyoto Gene and Genomics Encyclopedia (KEGG) (https://www.kegg.jp/), which shows the identified proteins are overrepresented in the glycolytic pathway and were marked in an oval in this study.

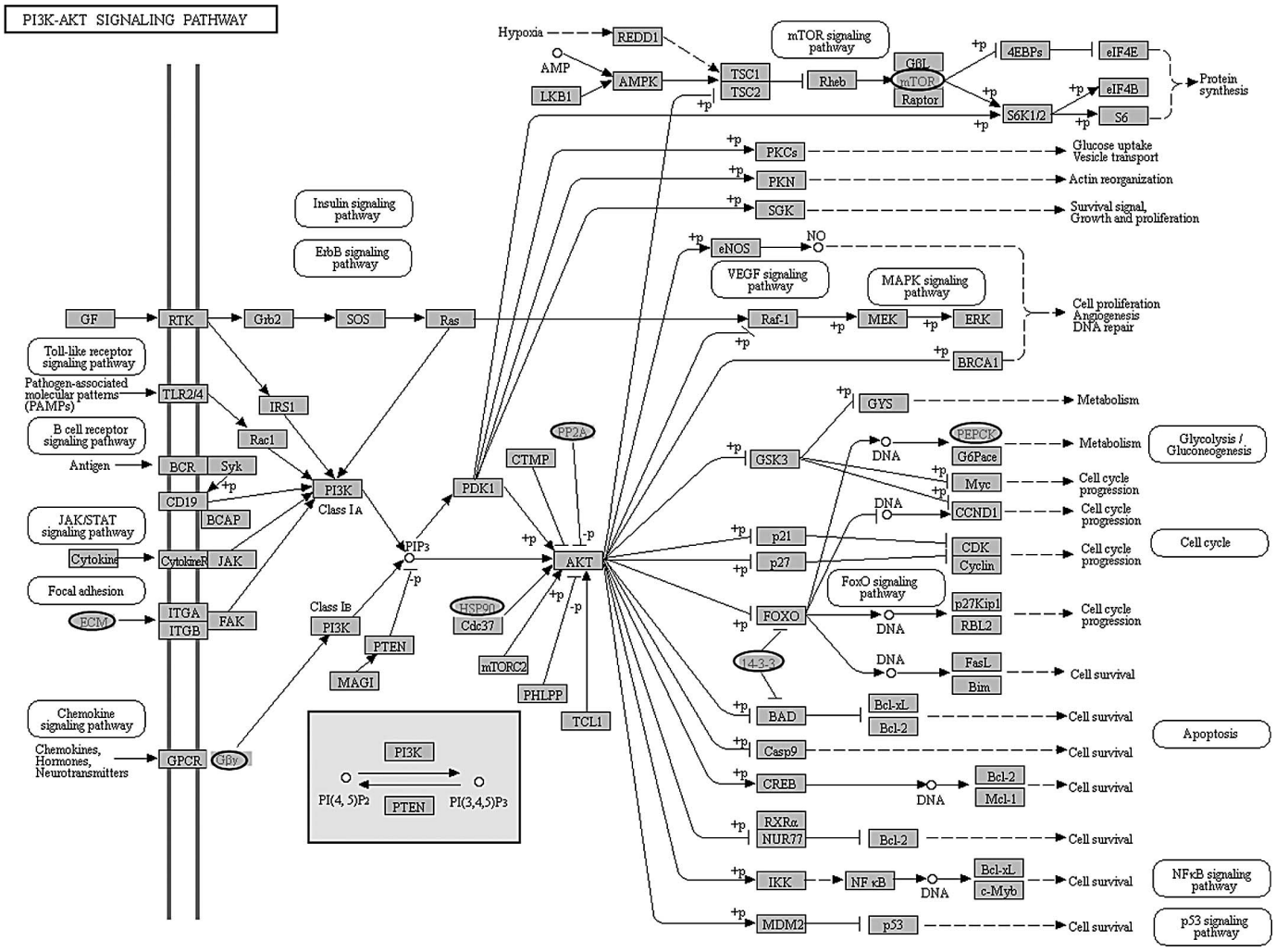

Figure 5. The PI3K-Akt signaling pathway constructed with the Kyoto Gene and Genomics Encyclopedia (KEGG) (https://www.kegg.jp/), which shows the identified proteins are overrepresented in the PI3K-Akt signaling pathway and were marked in an oval in this study.

and the GC% is 42.9% (Tsai et al., 2013). Most of these known proteins are low-molecular-weight proteins, and their isoelectric points were mainly between 8 and 9. Low-molecular-mass cyst fluid antigens have good specificity in immunodiagnosis and have been considered the preferred antigens for immunodiagnosis (Sako et al., 2006). The most abundant secreted proteins in the initial screening of cyst fluid are enolase, trypsin-like protein, serpin, 8 kDa glycoprotein, calcium-binding protein, GP50, etc. (Hancock et al., 2003, 2006; Zurabian et al., 2005; Rueda et al., 2011; Maldonado-Aguayo et al., 2014; Pajuelo et al., 2015; Ayón-Núñez et al., 2018). These proteins are closely related to the interaction between the host, pathogen energy metabolism, and related signaling pathways, and they are important for maintaining the normal life activities of worms. To fully understand the role of known proteins in the cyst fluid of cysticercosis, the identified proteins were functionally classified by GO annotation. The results revealed 104 annotated proteins with 71 molecular functions involved in 68 biological processes. Most of the identified proteins are involved in cellular and metabolic processes, and molecular functions also play major roles in catalytic activity and binding. Most of the catalytic activity and ionic binding functions are related to metabolic processes. The host provides a living environment for the parasite but also responds to the pathogen by innate or adaptive defense mechanisms. To maintain parasitic activity in the host and homeostasis of their metabolism, most parasites have a unique redox system (Salinas, 2013). Therefore, the study of key catalytically active enzymes and pathways can be used to understand the transformation of metabolic processes caused by environmental changes during the parasitic complex life cycle.

KEGG pathway enrichment analysis of known proteins in cyst fluid revealed that the known proteins were mainly enriched in the PI3K-Akt and glycolysis/gluconeogenesis signaling pathways, and the involved glycolysis/gluconeogenesis signaling pathways were relatively complete, suggesting that the pathway may be active. Many known proteins were found in the PPI results, but the main nodes consisted of enzymes involved in glucose metabolism. The catabolism of sugar can be completed during parasitism, which can directly provide energy for the growth and

Table II. Proteins associated with PI3K-AKT/mTOR and glycolysis/gluconeogenesis pathways in the KEGG pathway map name.

Map_Name	Map_ID	KEGG_id/KO	Protein_ID	Protein description
PI3K-Akt signaling pathway	ko04151	K05637	A0A158RAE9	Laminin, alpha ½
		K01596	A0A0R3VWM5	Phosphoenolpyruvate carboxykinase (GTP) [EC:4.1.1.32]
		K05635	A0A158R6L4	Laminin, gamma 1
		K05636	A0A0R3W2P7	Laminin, beta 1
		K16197	V5T6E6	14-3-3 protein beta/theta/zeta
		K16197	A0A0R3WE77	14-3-3 protein beta/theta/zeta
		K06630	A0A0R3WF90	14-3-3 protein epsilon
		K06630	A0A0R3WAA1	14-3-3 protein epsilon
		K06237	A0A158R7A0	Collagen, type IV, alpha
		K16197	A0A0R3WEZ7	14-3-3 protein beta/theta/zeta
		K09487	A0A0R3WCF8	Heat shock protein 90 kDa beta
		K04354	A0A0R3VVP8	Serine/threonine-protein phosphatase 2A regulatory subunit B
		K06237	A0A0R3W133	Collagen, type IV, alpha
		K04536	A0A0R3W6I1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1
		K04539	A0A0R3W5V2	Guanine nucleotide-binding protein subunit beta-5
		K07203	A0A158R8B3	Serine/threonine-protein kinase mTOR [EC:2.7.11.1]
mTOR signaling pathway	ko04150	K08272	A0A0R3W2E7	Calcium binding protein 39
		K07203	A0A158R8B3	Serine/threonine-protein kinase mTOR [EC:2.7.11.1]
Glycolysis/ gluconeogenesis	ko00010	K01596	A0A0R3VWM5	Heat shock 70 kDa protein 1/2/6/8
		K01689	A0A1S5WII1	Enolase [EC:4.2.1.11]
		K01689	V5T827	Enolase [EC:4.2.1.11]
		K01623	A0A0R3VSW8	Fructose-bisphosphate aldolase, class I [EC:4.1.2.13]
		K00927	A0A0R3VWJ0	Phosphoglycerate kinase [EC:2.7.2.3]
		K01810	A0A158R8S6	Glucose-6-phosphate isomerase [EC:5.3.1.9]
		K01835	A0A158R7F5	Phosphoglucomutase [EC:5.4.2.2]
		K00134	A0A0R3W211	Glyceraldehyde 3-phosphate dehydrogenase [EC:1.2.1.12]
		K01834	A0A0R3W3E9	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase [EC:5.4.2.11]
		K00016	G8ZAG5	L-lactate dehydrogenase [EC:1.1.1.27]
		K01803	Q9GTX8	Triosephosphate isomerase (TIM) [EC:5.3.1.1]
		K00134	A0A0R3WEJ0	Glyceraldehyde 3-phosphate dehydrogenase [EC:1.2.1.12]
		K00873	A0A0R3VT40	Pyruvate kinase [EC:2.7.1.40]
		K03841	A0A0R3VXG9	Fructose-1,6-bisphosphatase I [EC:3.1.3.11]

development of *T. solium*. Studies of the glycolytic pathway in *Toxoplasma gondii* (Shukla, 2018) and *Plasmodium* (van Niekerk et al., 2016) have confirmed the importance and role of the glycolytic pathway in different developmental stages of the parasite, and the enzymes in the pathway are also important under the growth conditions for *T. solium*. Studies of the glycolytic pathway will help to reveal the persistence of parasites in the host and the growth and metabolic requirements during different periods.

The PI3K-Akt signaling pathway is known for its three major driving molecules, P13 kinase (P13K), AKT, and mammalian rifampicin target protein (mTOR), and it is thought to play important roles in proliferation, migration, invasion, and drug resistance (Gasparri et al., 2018). mTOR is a serine/threonine kinase and a downstream effector of the PI3K/Akt signaling pathway, and the components of this pathway interact with many other pathways, including the Ras/MAPK pathway (Ras family of small GTPase proteins/mitogen-activated protein kinases) (Saxton and Sabatini, 2017). Studies using *Echinococcus multilocularis* as a model organism have shown that host insulin, epithelial growth factor, and transforming growth factor can interact directly with the parasite through the conserved RTK signaling pathway and the serine/threonine pathway (Beall and Pearce, 2002; Brehm and Spiliotis, 2008). In addition, insulin stimulation was found to activate the parasite PI3K/Akt signaling pathway, and inhibition of the insulin receptor and closure of the PI3K signaling pathway in *Echinococcus* would result in reduced survival and developmental levels of parasite larvae. Currently, in the field of parasite research, how mTOR is activated and what secreted proteins promote mTOR signaling and regulate host cells have not been extensively studied. The P13k-Akt pathway and the glycolysis pathway of cysticerci are largely unstudied, and AKT also plays a very important role in glucose metabolism. Moreover, secreted proteins are mainly enriched in these 2 pathways and should be further studied to determine their roles in cysticercosis.

CONCLUSION

Cyst fluid from *T. solium* cysticerci dissected from the skeletal muscle of pigs was aseptically extracted and qualitatively analyzed with shotgun LC-MS/MS followed by bioinformatics analysis of the 158 identified proteins. The main pathways enriched were the PI3K-Akt signaling pathway and the glycolysis/gluconeogenesis signaling pathway. PPI network analysis showed that most of the interacting nodes were glycolysis-related enzymes, and gene ontology analysis showed that most proteins had catalytic and binding activities. These activities may play important roles in maintaining the parasitism and metabolism of cysticerci. The

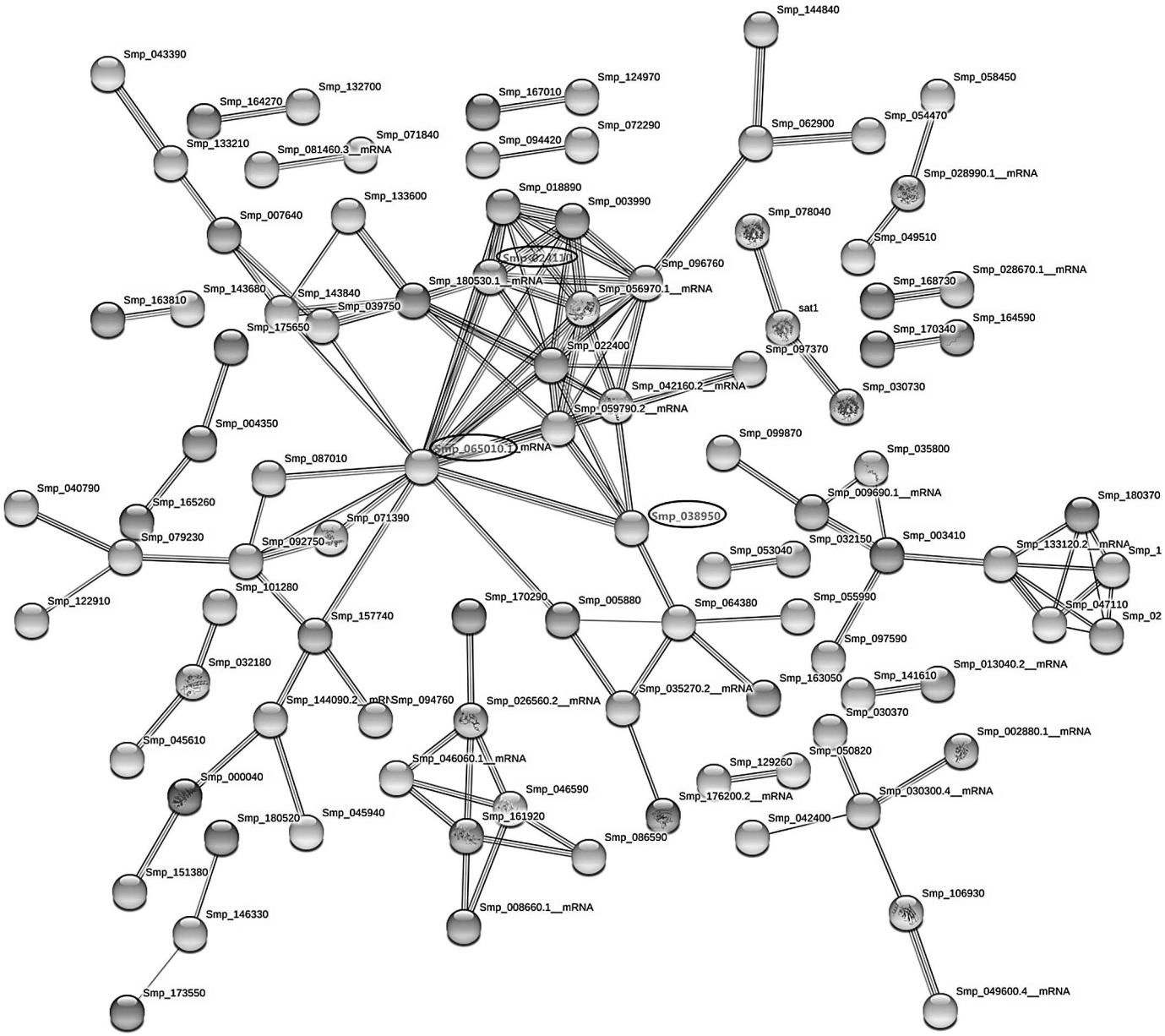

Figure 6. Protein-protein interaction network of known proteins in cystic fluid constructed with STRING version.

interaction between the host and the parasite is a dynamic and complex process. The parasite has a unique life history specific to the host and pathogenicity, which is related to the large amounts of proteins expressed or secreted by the worm at each stage. Therefore, the systematic analysis of cyst fluid proteins has demonstrable reference value for the characterization of parasitic mechanisms.

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