Comparative Proteomic Analysis of Taenia solium Cysticercus and Adult Stages

Lizhu Li (627454728@qq.com)
Zunyi Medical University
Biying Zhou
Zunyi Medical University
Wei Hi
Zunyi Medical University
Xianmin Fan
Zunyi Medical University
Meichen Liu
Zunyi Medical University
Bo Luo
Zunyi Medical University
Fengjiao Yang
Zunyi Medical University
Nan Jiang
Zunyi Medical University
Lingjun Wang
Zunyi Medical University

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Abstract

B ack ground : Cysticercosis is one of 20 tropical diseases classified as neglected by the World Health Organization (WHO). As the pathogenesis of cysticercosis is not yet clear, there is no vaccine that can completely cure the disease. This study attempts to identify the proteins of adult and larvae of Taenia solium by mass spectrometry, predict the function of differential proteins so as to further study the Pathogenic mechanism of larvae to host. This provides clues for the cysticercosis prevention and treatment. Methods: In our study, we separated proteins of adult and larvae of Taenia solium by High Performance Liquid Chromatography(HPLC) fractionation, and protein samples were also digested in liquid and identified by liquid chromatography tandem mass spectrometry(LC-MS/MS); the differentially expressed proteins were then processed by a bioinformatics and parallel reaction monitoring (PRM) analysis . Results: Our results showed that the 3658 proteins identified, 2481 contained quantitative information, according to the relative quantitative analysis of the protein expression level by label-free quantification, differentially expressed proteins between the two treatments were identified applying a P value < 0.05 and a twofold change threshold, of which 293 proteins were up-regulated whereas 265 proteins were down-regulated. Through the bioinformatics analysis, we found some proteins that were associated with the immune effector process in addition to diagnostic antigens and therapeutic targets. Conclusion: We conclude that the results facilitate a better understanding of the complexity of the cestode life cycle, mechanism to counter the host immune system, and host–parasite interactions in both developmental stages. Evading the host immune response may be key for the establishment of latent and persistent infections by cysticercus. Therefore, understanding the mechanisms through which the parasites manipulate the host immune response for their survival is important to our understanding of the intricate parasite-host interaction. Further studies are required to identify what function those proteins are, it may contribute to the development of novel strategies and vaccines against cysticercosis.

Background

Cysticercosis was classified as one of 20 neglected tropical diseases by the World Health Organization (WHO) [1]. The disease was caused by the larvae (cysticercus)of *Taenia solium*(*T. solium*). Neurocysticercosis(NCC) was the leading cause of epilepsy in the tropics, which could cause significant morbidity and mortality in humans. The life cycle of *T. solium* was complex, however, requiring the parasitism of two kinds of mammals: pigs and humans, which was served as intermediate host, whereas the latter was the only final host. The transmission of *T. solium* from a tapeworm carrier occured via the shedding of eggs in feces, followed by their ingestion by hosts (e.g., pigs and humans) and subsequent development into cysticercus [2].

In the past 10 years, vaccines and drugs have been jointly administered to pigs followed by strategic deworming treatments of infected humans [3]. But given its complex life cycle, the pathogenic mechanism of the tapeworm remained unclear. The development of the tapeworm has evolved a fitness-promoting strategy at each specific stage, thus providing favorable conditions for its survival. Metabolic enzyme activity was reportedly related to its growth strategy, and tapeworms generally have also evolved

special detoxification pathways, to absorb nutrients for their own metabolism as an energy source[4]. But it was not clear whether these key proteins were expressed throughout the life cycle of *T. solium* or only at a given developmental stage. Therefore, a proteomic analysis was essential to understand the survival strategy of this tapeworm and to develop novel vaccines and therapeutic approaches.

The life cycle of *T. solium* included egg, oncosphere, cysticercus, and adult. However, cysticercus remained asymptomatic for many years in its host, which might utilize effector proteins to suppress or evade host immune response [5]. But the immune response and the regulatory mechanism of cysticercus infection have not been fully elucidated. Hence, it was necessory for the development of cysticercosis vaccines [6].

Accordingly, In this study we sought to compare the proteomes of two consecutive developmental stages, finding both similarities and differences between *T. solium* cysticercus and adult. These differences in protein expression/activity might be crucial for influencing the parasite's invasion, survival, immune evasion, and worm development. The major goal of our study was to gain research insights that could be relevant to vaccines, new diagnostic methods, drug targets and immune mechanisms.

Methods

Parasite

T. solium adult was obtained from naturally infected patients dewormed by the betel-pumpkin seeds method[6]. Normal pigs take 5 identical slices of gravid proglottids(about $2*10^5$ eggs per pig)by mouth. After three months, cysticerci were isolated from pig tissues, and these samples stored at −80°C in a freezer before their analysis.

Protein Extraction

Each sample was first ground with liquid nitrogen into cell powder and then transferred into a 5-mL centrifuge tube. Next, four volumes of lysis buffer (8 M urea, Sigma, China), 1% protease inhibitor(Calbiochem, Germany) was added to the cell powder, followed by sonication three times on ice using a high intensity ultrasonic processor (Scientz). The remaining debris was removed by centrifugation at 12,000 rpm for 10 min at 4°C. Finally, the supernatant was collected and the protein concentration determined with BCA kit(Beyotime, China) according to the manufacturer's instructions.

Trypsin Digestion

For the digestion, the protein solution was reduced with 5 mM dithiothreitol for 30 min at 56°C, and then alkylated with 11 mM iodoacetamide for 15 min at room temperature in darkness. The protein sample was then diluted, by adding 100 mM TEAB, to yield a urea concentration of less than 2M. Finally, trypsin was added in a 1:50 trypsin-to-protein mass ratio for the first digestion overnight, Then added pancreatin at a mass ratio of 1:100 (pancreatin: protein), and continue enzymatic hydrolysis for 4 hours.

HPLC Fractionation

The tryptic peptides were separated into fractions by high pH reverse-phase HPLC, using a Thermo Betasil C18 column (5-μm particles, inner diameter 10-mm, 250-mm length). Briefly, peptides were first separated by a gradient spanning 8–32% acetonitrile (pH 9.0) over 60 min, which generated sixty fractions. Then, these peptides were pooled into six fractions and dried by vacuum centrifuging.

LC-MS/MS Analysis

The tryptic peptides were dissolved in 0.1% formic acid (solvent A) and directly loaded onto a homemade reversed-phase analytical column (15-cm length, 75-μm inner diameter.). The gradient consisted of an increase from 6–23% solvent B (0.1% formic acid in 98% acetonitrile) over 26 min, followed by 23–35% over 8 min and climbing to 80% in 3 min, then holding it at 80% for the last 3 min, all done at a constant flow rate of 400 nL/min on an EASY-nLC 1000 UPLC system. The peptides were subjected to an Nanoelectrospray Ionization(NSI) source followed by tandem mass spectrometry (MS/MS) in a Q ExactiveTM Plus (Thermo) coupled online to the UPLC system. The electrospray voltage applied was 2.0 kV; the m/z scan range was 350 to 1800 for each full scan, and intact peptides were detected in the Orbitrap at a resolution of 70,000. Peptides were then selected for MS/MS by using NCE set to 28, with fragments detected in the Orbitrap at a resolution of 17,500. A data-dependent procedure was used, one that alternated between a single MS scan followed by 20 MS/MS scans with a 15.0-s dynamic exclusion. The automatic gain control (AGC) was set to 5E4. The fixed first mass was set to 100 m/z.

Database Search

The secondary mass spectrum data was searched using Maxquant (v1.5.2.8, http://www.maxquant.org/). Retrieve parameter settings: The database is Taenia_asiatica_60517_PR_20190708 (10328 sequences). Trypsin/P was specified as the cleavage enzyme, permitting up to four missing cleavages. The mass tolerance for precursor ions was set to 20 ppm in the ‘First search’ and 5 ppm in the ‘Main search’, while the mass tolerance for fragment ions was set to 0.02 Da. Carbamidomethyl on Cys was specified as the fixed modification and acetylation modification and oxidation on Met were specified as the variable modifications. The false discovery rate (FDR) was adjusted to < 1% and minimum score for modified peptides was set a priori to > 40.

Bioinformatics

GO Annotation

Gene Ontology (GO) annotation(http://www.maxquant.org/) of the parasite’s proteome was based on the UniProt-GOA database (http://www.ebi.ac.uk/GOA/). First, each identified protein ID was converted to a UniProt ID and then mapped to the existing GO IDs by protein ID. For those identified proteins that could not be annotated by the UniProt-GOA database, InterProScan software was used to annotate the proteins’ GO function based on the protein sequence alignment method. Then, all the proteins were respectively classified by GO annotation according to its three main categories: biological process, cellular component, and molecular function.

Domain Annotation

For each identified protein, its domain functional description was annotated using InterProScan (a sequence analysis application) based on the protein sequence alignment method, for which the InterPro domain database was used. InterPro (http://www.ebi.ac.uk/interpro/) is a database that integrates diverse information about protein families, domains, and functional sites, and makes it freely available to the public via Web-based interfaces and services. Central to the database are diagnostic models, known as ‘signatures’, against which protein sequences can be searched to determine their potential function. InterPro has promising utility for the large-scale analysis of whole genomes and meta-genomes, as well as for characterizing individual protein sequences.

Functional Enrichment Analyses

Enrichment of Gene Ontology

For each category, a two-tailed Fisher’s exact test was used to test the enrichment of the differential protein expression against all the identified proteins. The GO term with a corrected P-value < 0.05 was deemed significant.

Enrichment of Protein Domains

Each category of protein was researched in the InterPro database (a resource that provides the functional analysis of protein sequences by classifying them into families and predicting the presence of domains and important sites, http://www.ebi.ac.uk/interpro/). Then a two-tailed Fisher’s exact test was employed to test the enrichment of each differentially expressed protein against all the identified proteins. Protein domains with a corrected P-value < 0.05 were designated as significant.

Enrichment of Pathways

The Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kaas-bin/kaas_main) database was used to identify enriched pathways, by applying a two-tailed Fisher’s exact test to test the enrichment of the differentially expressed protein against all the identified proteins. The pathway with a corrected P-value < 0.05 was considered significant. These pathways were classified into hierarchical categories, as recommended on the KEGG website.

PRM Analysis

Proteins were treated with dithiothreitol and iodoacetamide. Proteins were then digested overnight at 37°C by trypsin (trypsin: protein = 1:50). The peptides were dissolved in liquid chromatography [ow phase A (0.1% formic acid in water) and separated by an EASY-nLC 1000 ultra-high performance liquid phase system. Mobile phase A was 0.1% formic acid and 2% acetonitrile in water, while mobile phase B was 0.1% formic acid and 90% acetonitrile in water. Liquid phase gradient was: 0-16 min, 6-20% phase B; 16-22 min, 20-30% phase B; 22-26 min, 30-80% phase B; 26-30 min, 80% phase B, with [ow rate maintained at 500 nL/min. The peptides separated by ultra-high performance liquid phase system were injected into an NSI ion source for ionization, followed by analysis in a QExactive Plus mass spectrometer. The ion source voltage was set to 2.0 kV, and the precursor and product ions were detected and analyzed using high-

resolution Orbitrap. Full scans were collected at a resolution of 70,000 at 350 1150 m/z, and the PRM scan at a 17,500 resolution. Data Independent Acquisition (DIA) was used for data collection, and the fragmentation energy of the HCD was 27%. Full scan automatic gain control (AGC) was 3E6, and the maximum ion implantation time (Maximum IT) was set to 50 ms. The PRM scan automatic gain control (AGC) was 1E5, and the maximum ion implantation time (Maximum IT) was 170 ms. The isolation window was 1.6 m/z. Peptide parameters: Protease was Trypsin [KR/P], the maximum number of missed sites was 0, the length of the peptides was 7 25 amino acids, and cysteine alkylation was a Zxed modiZcation. Transition parameter: the precursor ion charge was 2, 3, the product ion charge was 1, and the ion type was b, y. The selection of ionic fragments started from the third to the last, and the mass tolerance of the ion matching was 0.02 Da.

Results

Protein Identification

According to MS/MS analysis, the total peptide identified was 942271, the specific peptide was 29761, a total of 3658 proteins were identified, of which 2481 contained quantitative information **(**Table 1, 2**)**. Relative standard deviation (RSD) is a statistical method to evaluate the quantitative repeatability of protein by using the quantitative value of protein between three repeated samples in six groups of samples **(**Figure 1**)**. A corrected P-value of 0.05 and minimum fold-change of 2 were set as threshold criteria for determining significant differential expression of a protein. Through comparing *T. solium* adult and cysticercus, we found 293 up-regulated proteins (adult) and 265 down-regulated proteins (cysticercus)**Table S2)**.

Table 1
MS/MS spectrum database search analysis summary.

Total spectrum	Matched spectrum	Peptides	Unique peptides	Identified proteins	Quantifiable proteins
942271	223234 (23.7%)	30689	29761	3658	2481

Table 2
Differentially expressed protein summary (filtered according to a threshold value of expression fold-change with a P-value < 0.05); A = adult, L = larva.

Compared groups	Regulated type	Fold-change > 1.2	Fold-change > 1.3	Fold-change > 1.5	Fold-change > 2
A/L	up-regulated	888	798	619	293
	down-regulated	628	564	431	265

Protein Functional Classification Based on Gene Ontology in Cysticercus and Adult Stage

The identified proteins were classified according to their molecular function, cellular component and biological process. Among these differentially expressed proteins, many were involved in metabolic process, cellular process, response to stimulate, and biological regulation. In the stage of cysticercus and adult of *T. solium*, the predominant molecular functions were related to binding (e.g., protein binding) and catalytic activity (hydrolase and oxidoreductase activity). Regarding biological processes, a number of proteins were involved in metabolic and cellular processes. For cellular components, our GO results indicated the identified proteins were primarily associated with cell parts, organelles, and macromolecular complexes.

The fourteen down-regulated (cysticercus stage) proteins were most enriched in terms of regulation of biological process and anatomical structure development at biological function, such as protein kinase C, E3 ubiquitin protein ligase, SH2 domain-containing protein, annexin, cAMP dependent protein kinase regulatory, and fatty acid binding protein **(Table S2)**. Both annexin and human plasminogen protein were known for their strong antigenicity, which might be used as diagnostic antigens. Additionally, 25 other immune effector proteins were found **(Table S3)**.

The results revealed that the most of down-regulated proteins were enriched in regulation of protein localization to membrane, bicarbonate transport, neuroepithelial cell differentiation, and mechanoreceptor differentiation, all under the biological process (BP) category. Molecular function was enriched for cytoskeletal protein binding and actin binding. Cellular component was enriched in terms of intracellular vesicle, cytoplasmic vesicle, plasma membrane region, and secretory vesicle. Domain enrichments included LIM domain, calponin homology (CH) domain, EF-hand domain pair, dynein light-chain type 1 **(**Figure 2A**)**.

The up-regulated (adult stage) proteins were mainly enriched in terms of regulation of metabolic process in biological function **(**Figure 3A**)**, Most proteins were involved in metabolic process, of which nineteen of these proteins have been studied in other parasites, all of which were related to the growth, development and of parasites, Only 2 proteins related to immune function **(Table S4, S5)**.

KEGG Enrichment of Differentially Expressed Proteins in Cysticercus and Adult Stage

In cysticercus stage, the KEGG enrichments included lysosome, glutathione metabolism, porphyrin and chlorophyll metabolisms, and 2-oxocarboxylic acid metabolism **(**Figure 2B, 2C**)**. isocitrate dehydrogenase [NADP], NAD_binding_2 domain-containing protein, 6PGD domain-containing protein and Phospholipid

hydroperoxide glutathione were involved in glutathione metabolism; 5-aminolevulinate synthase, Porphobilinogen deaminase, Coproporphyrinogen III oxidase and Protoporphyrinogen oxidase were involved in porphyrin and chlorophyll metabolisms; Isocitrate dehydrogenase [NADP], Aconitate hydratase and Citrate synthase were involved in 2-oxocarboxylic acid metabolism; ADP ribosylation factor binding protein GGA1, Beta-galactosidase, Prosaposin a preproprotein and LITAF domain-containing protein were involved in lysosome.

In adult stage, the KEGG enrichments included glutathione metabolism, pyrimidine metabolism and Ribosome biogenesis in eukaryotes. Microsomal glutathione S transferase 3, Glutathione S-transferase and CYTOSOL_AP domain-containing protein were involved in glutathione metabolism, CTP synthase, PNP_UDP_1 domain-containing protein and Thymidylat_synt domain-containing protein were involved in pyrimidine metabolism; Ribonucleoside diphosphate reductase subunit and Ribonucleoside-diphosphate reductase were involved in glutathione metabolism and pyrimidine metabolism. 39S ribosomal protein L3, 60S ribosomal protein L3, Ribosomal_L2_C domain-containing protein, 40S ribosomal protein S4, Ribosomal protein L11, Ribosomal S15a protein, Ribosomal protein l5, Large subunit ribosomal protein l7e and 39S ribosomal protein L12 were associated with the ribosome biogenesis in eukaryotes (Figure 3B, 3C).

Differentially Expressed Protein Quantification by PRM Analysis

In this study, we analyzed 9 of 20 differentially expressed proteins with PRM instead of traditional western blotting. The quantitative information of PRM protein was calculated according to the ion peak area of the peptide. Five proteins were identified in adult(Figure 4a). S1 motif domain-containing protein, Cullin-1, Protein kinase domain-containing protein, Proliferating cell nuclear antigen. Four proteins were identified in cysticercus(Figure 4b). Citrate synthase, Glutamate synthase, Synaptotagmin protein 4, Neurogenic locus notch protein.

Discussion

In this study, we performed sequence alignments between the proteins of *T. solium* cysticercus and adult by comparing them to the *T. asiatica* database, and these proteins were analyzed by bioinformatics. We found the proteins differed starkly between the two developmental stages, Although the survival of cysticercus and adult depended on the energy metabolism in their hosts. Their main energy source, carbohydrates, might be catabolized by aerobic respiration or by two complementary anaerobic pathways, those of lactate fermentation and malate dismutation [3]. Additionally, both cysticercus and adult relied upon glutathione metabolism to grow and develop in their host. The difference between the two stages was that adult could also survive through pyrimidine metabolism.

Glutathione played a role in antioxidative defense and in maintaining the reducing environment of the cytosol, and many of the known glutathione-dependent processes were directly related to the specific lifestyle of the parasite. For *Plasmodium falciparum*, proteins involved in GSH-dependent processes were studied not only as factors in the pathophysiology of malaria but also as potential drug targets [8]. According to KEGG, pyrimidine and purine were the raw materials for the synthesis of DNA and RNA. The parasite itself synthesized pyrimidine and formed its own set of survival mechanism. Accordingly, it was plausible then, to generating new leading compounds to treat malaria and schistosomes by targeting the purine and pyrimidine pathways [9, 10]. In our study, glutathione-related enzymes were identified in proteomics of both cysticercus and adult, whereas proteins involved in pyrimidine metabolism were identified only in adult. Hence, the pyrimidine metabolic pathway might be used as a starting basis for the novel treatment of taeniasis, which also showed the differences in growth and development between cysticercus and adult.

Comparative analysis of mitochondrial genomes is often been used in phylogenetic studies. The complete nucleotide sequence of *T. solium* mitochondrial DNA (mtDNA) has been determined [11]. The sequence is 13,709 base pairs in length and contains 36 genes: 12 for proteins involved in oxidative phosphorylation, namely adenosine triphosphatase subunits 6 and 8 (ATP6 and 8), 2 for ribosomal RNAs, and 22 for transfer RNAs. The mitochondria of most metazoan animals contained their own circular mitochondrial DNA (mtDNA), which usually contains the genes for large- and small-subunit ribosomal RNAs (LrRNA and SrRNA, respectively), 22 transfer RNAs (tRNA), and 11–12 proteins involved in oxidative phosphorylation: adenosine triphosphatase sub units 6 and 8 (ATP6 and 8), cytochrome b, cytochrome c oxidase subunits 1-3(Cox 1-3), and NADH dehydrogenase subunits 1-6 and 4 L (ND 1-6 and 4 L). The arrangement of these genes within the molecule is useful for deducing evolutionary relationships among distantly related taxa. Our obtained results for the proteomic identification of ribosomal proteins and cytochrome proteins were consistent with the above, as well as for the enzymes involved in pyrimidine metabolism in adult,

which indicated that *T. solium* had a strong ability to synthesize DNA during its growth and development. Yet other researchers, by analyzing the genomes of four species of tapeworms, found many genes lost from the genome in course of adapting to a parasitic life history, albeit some genes that could improve the survival rate of parasites were nonetheless retained and amplified at the same time. For example, the tapeworm genome lacked genes related to the synthesis of fatty acids and cholesterol de novo [12, 13]. Over time, tapeworms have lost their ability to synthesize essential fats and cholesterol essential for larval development, which they instead obtained from their hosts. Nevertheless, there were numerous lipid elongating enzymes and fatty acid transporter genes, and many fatty acid-binding proteins and apolipoprotein B antigens were expressed, which might be related to the parasite consuming a substantial amount of nutrients in the process of its growth and development. These proteins were identified here in the proteomics of both cysticercus and adult. This showed that the tapeworm was constantly evolving to be adapt to its environment, and it was precise because these proteins played a corresponding function that the tapeworm could survive and became a target for disease treatment.

However, the more puzzling thing was that cysticerci reached their final location in the parenchyma of the brain, usually followed by a period of several months or years until the onset of disease symptoms. During this period, affected host individuals possibly remained asymptomatic through active evasion and suppression of host immunity, as the development of symptoms was known to strongly depend on the degree and intensity of the host response [14], Another main, related point was the oxidative stress response of ROS, and the activity of GST (glutathione S-transferase), a group of enzymes that could protect cells from ROS damage. Tapeworms typically had only one cytochrome P450 gene, suggesting their ability to oxidize many xenobiotics and steroids was substantially lower than that of their hosts. Uniquely, tapeworms and flukes have merged two key enzymatic functions for redox homeostasis in one single enzyme: thioredoxin glutathione reductase (TGR). The gene encoding TGR was essential for parasite survival, and TGR was a validated drug target in flukes.For downstream of TGR's gene, there were thioredoxins, glutaredoxins, and mu-class glutathione S-transferases (GSTs). Moreover, GSTs had immunogenicity and could be served as a specific target for the treatment of cysticercosis. Functional annotation of differentially expressed proteins between the two stages of *T. solium* revealed that the number of proteins identified as involved in the immune process was greater in cysticercus than in adult, of which 25 proteins were involved in the former's elicited immune response. This indicated that the immunosuppression caused by the cysticercus was more severe than that of adult. Accordingly, these findings provided the necessary data for better supporting the experimental verification of the immune-influencing mechanism of cysticercus, and could point to new possible targets for devising vaccines and therapeutants. Therefore, in summary, cysticercus and adult relied on different ways to resist the immune response of the host during their growth and development, and likewise severity of the ensuing disease that manifests also differed between them. Some important proteins in cysticercus and adult were detailed in the next section below, most of which were promising targets for specific antigens and candidate vaccines in the field of parasites. In follow-up work, we would plan to further screen the related proteins that have not been verified by immunity to elucidate and confirm their function.

The transcriptome is used to study the expression and regulation level of functional genes' mRNA, an approach that is highly sensitive to the detection of differentially expressed genes and can more accurately convey the entire complex process of expression-driven regulation of the transcriptome. Here, through the transcriptome analysis of *T. solium* cysticercus, we found that paramyosin, a major egg antigen, in addition to cathepsin L-like cysteine proteinase, 70-kDa heat shock protein, and the H17g or TEG-Tsol surface antigen had potential for developing worm antigens for immunodiagnostics or vaccines [15]. Similar to the results of our proteomic analysis, most of the proteins we identified in the parasite were also involved in cell processes, catalytic activity, and protein binding. Proteomics is the identification of the whole protein content present in a given sample, but when combined with a transcriptome-based perspective, it can yield a more comprehensive understanding of the pathogenic mechanism and treatment of Cysticercus cellulosae. However, proteomics can only detect the static proteins in a sample's preparation, whereas investigating the transcriptome can reveal the expression and regulation level of mRNA at different time points, letting us observe the related pathways more intuitively.

Identified Proteins of Cysticercus (Down-regulated)

Several reports that investigated the proteome of *T. solium* across its developmental stages have discovered that cytoskeleton, actin, and paramyosin could be used as targets for applied research on cysticercosis vaccines and potential diagnostic antigens (e.g., enolase, calcium binding protein, small molecule shock protein, 14-3-3 protein, tropomyosin α, α-1 tubulin, β tubulin, annexin B1 and cAMP cyclic adenylate protein dependent kinase) [16–19]. Calcium-binding protein was involved in various regulatory functions of host invasion by parasites, these mainly including members of the calmodulin family (CAM), the calcineurin B-like (CBL) family, and the calcium-dependent protein kinase (CDPK) family: all these harbor a highly conserved helix–loop–helix structure, namely the EF-hand domain. For *Plasmodium* and *Toxoplasma*, phosphatidylinositol could also be stimulated by ligands binding to surface receptors (such as G-protein binding receptors [GPCR]), to produce calcium signals, thereby stimulating multiple cellular pathways [20]. A cyclic GMP (cGMP)-regulated kinase of *Plasmodium*, the protein kinase G (PKG) might regulate the Ca^{2+} signaling pathway with ookinetes' movement being controlled, as well as the initiation of gametocytes, and a formation of a fissure in the schizont [21]. Fatty acid-binding proteins (FABP) were a family of proteins with isoforms in parenchymal and tegument cells. They were known to be involved in the uptake and transport of cholesterol and long-chain fatty acids, as well as the binding of triclabendazole [22], anti-oxidant activity, and immunomodulation [23]. Notably, FABP produced a high level of specific antibodies during *Schistosoma* infection, which confered a strong immune-protective effect [24], thus increasing the value of FABP in the design of vaccines. Similarly, for *Echinococcus granulosus*, FABP was under investigation as a potential drug target [25].

Glycolytic enzymes were usually used for the development of drug and vaccine. Fructose-1-6-bisphosphate aldolase (FBPA) was a crucial glycolytic enzyme and a plasminogen-binding protein, it was be related to certain non-glycolysis functions, such as host cell adhesion, plasminogen-binding, and parasite invasion of the host. Research has demonstrated that FBPA was expressed on the surface of parasites and involved in parasitic motion and invasion by connecting surface adhesion proteins to the actin-myosin of parasites [26]. Specifically, FBPA could bind to the surface of the cell membrane, and then bind to plasminogen, using the activity of hydrolyzing surface-related proteins to help invade host cells. Hence, it was also considered as a potential vaccine candidate or chemotherapy target for *Trichinella spiralis* and Giardia infection [27–29].

The damage to the host caused by cysticercosis was more serious than that of adult, and mechanism by which the host immune system was evaded or manipulated remained unclear. But through a functional enrichment analysis, we uncovered 25 proteins in cysticercus that were involved in the immune process but only two such proteins in adult. Some of these proteins have been studied for immunity in other parasitic worms. For example, the *Trichinella spiralis* ES product thioredoxin peroxidase-2 could induce macrophages towards an M2-like phenotype, both *in vivo* and *in vitro*, and $CD4^+$ T cells increased in number after immunization of mice with rTsTPX2 and this mediated the expulsion of worm from the host to protect them, thus suggesting TsTPX2 was a potential vaccine candidate against trichinosis [30]. Also,

thioredoxin peroxidase was significantly recognized by melioidosis-positive sera in cysticercus stage, for which strong immunogenic properties rendered it an anticipated vaccine target[31]. Pyruvate kinase was a crucial glycolytic enzyme characterized in *Clonorchis sinensis*[32] that could promote the development of Th1 and inhibition of dendritic cells. Much like FBPA, it was also viewed as a promising target for drug therapy and vaccine development.

Furthermore, antigens and immunomodulatory proteins observed in cysticercus could engage in and foster a special mechanism of adaptation at this developmental stage, one that has evolved to evade the host immune system, which was a prerequisite for establishing a successful infection. In follow-up work, we would screen for some proteins and use them in related immune function experiments to further understand the immune escape mechanism of cysticercus.

Identified Proteins of Adult (Up-regulated)

Lacking a digestive tract, *T. solium* instead absorbs nutrients from its host across the body wall. The cortex absorbs all kinds of nutrients by means of diffusion and active transport, and also has the function of secreting and resisting the destruction of host digestive juice. Crucially, *T. solium* obtains its energy mainly via sugar metabolism. Adults rely mostly on glycolysis, but a few can also obtain energy from the tricarboxylic acid cycle and electron transfer system. Here we uncovered adult up-regulated proteins, most of which were related to metabolism, with just 2 proteins related to immunity. Given that adenosine 5'-triphosphate (ATP) was the most direct energy source used by this parasite, the adenylate energy charge (AEC) reflected its energy balance, which played a fundamental role in regulating the metabolism of the body. However, adenosine monophosphate deaminase (AMPD) could indirectly affect AEC. AMPD belonged to the amide hydrolase superfamily. It was not only part of the purine nucleotide cycle but also independently capable of regulating the dynamic equilibrium of the adenylate kinase reaction. In malaria parasites, the existence of AMPD did not affect its growth and development at any parasitic stage. However, when AMPD was overexpressed, it would lead to the death of the malarial parasite [33]. It was known that cytidine 5-triphosphate (CTP) was the basis for the construction of nucleotides and nucleic acids, and protein glycosylation depends on it [34]. Moreover, it worked as a high-energy molecule shown to be responsible for lipid biogenesis [35, 36] and participated in various cellular communication processes. But CTP was not self-synthesized in *Toxoplasma* spp., and, when it was absent, the growth and development of these parasites was adversely affected, making it a potential target for disease treatment [37]. Cullin protein was the most typical ubiquitin ligase family and a key tumor-associated protein. It could promote the proliferation of tumor cells and be used as a marker and therapeutic target for tumor prognosis. According to the GO analysis, Cullin protein was also involved in cell cycle progression, signal transduction and transcription, intracellular metabolism, and the corresponding response to stimuli. Yet, surprisingly, this protein has not been studied in parasites, we suggested that it could provide clues for the follow-up research [38–40]. Topoisomerase was a critical type of enzyme for overcoming the problem of chromosome topology that arose during DNA replication, transcription, recombination, and mitosis. Thus it was involved in cell growth, tissue development, and cell differentiation [41–43]. There were some enzymes which participated in a variety of DNA metabolic

processes. In parasitic infections, the inhibition of topoisomerase II by different ketone benzene and furan derivatives in cells hindered the basic metabolic process of cells and eventually led to apoptosis. In view of the parasite DNA topoisomerase II, these compounds could be used as potential anti-parasite drugs. *Schistosome* parasite miRNA could mediate the activity of the frizzled protein (frizzled related protein1 [FRZB2]), which increased liver fibrosis. FRZB2 was a secreted frizzled protein-related protein, which could competitively bind to specific frizzled protein receptors to suppress the signal transduction of Wnt, thereby influencing the severity of liver fibrosis. The FRZB2 gene knockdown would affect *S. japonicum* morphology, development, and survival, as well as its reproductive capacity [44, 45]. Major vault protein was a highly conserved ribonucleoprotein in lower and higher eukaryotes, whose function remained unknown [46]. Some studies did find that *Schistosoma mansoni* had an adaptation mechanism to parasitize its hosts in the process of infection. *Plasmodium* glyceraldehyde-3-phosphate dehydrogenase acted as a glycolytic enzyme and also as a host plasminogen-binding protein, thereby interacting with the host's fibrinolytic system to establish an important mechanism for that parasite invasion, growth, and development. At the same time, it could be used as a potential diagnostic biomarker for a variety of parasites, such as *Plasmodium*, *T. solium*, *E. granulosus*, and those causing filariasis, as well as *S. mansoni*, and *Babesia microti* [47–51]. Proliferating cell nuclear antigen (PCNA)was a protein that acted as a processivity factor for DNA polymerase δ in eukaryotic cells. it was originally identified as an antigen expressed in the nuclei of cells during the DNA synthesis phase of the cell cycle[52, 53]. The distinct mechanism of cell cycle arrest was associated with the up-regulation or down-regulation of TbPCNA. Deregulating the intra-parasite levels of TbPCNAwas a potential strategy for therapeutically exploiting this target in the bloodstream form of *Trypanosoma brucei*, which showed that PCNA was related to the growth and development of the parasite [54].

T. solium adult was able to reproduce and survive in the host intestines for escaping the immune response without causing excessive damage to the host. In our study, we found that there were few proteins in adult related to immunity, and most of them were related to growth and development, which also reflected the difference in immune response between adult and cysticercus to the host.

Conclusions

We profiled the proteome of *T. solium* cysticercus and adult stages. Our results would facilitate a better understanding of the complexity of the cestode life cycle, mechanism to counter the host immune system, and host–parasite interactions in two developmental stages. Evading the host immune response might be the key for the establishment of latent and persistent cysticercus infection. Therefore, understanding the mechanisms through which the parasites manipulate the host immune response for their survival was important to our understanding of the intricate parasite-host interaction. Further studies would be required to identify the function of the proteins, which might contribute to the development of novel strategies and vaccines against cysticercosis. But because the database of comparison and method were single, and the data was only from *T. asiatica*. however, most of the proteins were uncharacterized proteins which were identified by mass spectrometry, and there was no further verification. In addition, few proteins have been studied related to the immune mechanism of *T. solium*

cysticercus and adult, our results showed the differential proteins were only different in expression. The functional differences need to be further studied. a single study of a protein could not fully explain the problem. In combination with metabonomics, genomics and transcriptome, we believe that we would throw light on the mechanism of immune escape caused by cysticercus from the pathway, which would be more beneficial to the follow-up treatment and vaccine research.

Abbreviations

HPLC
High Performance Liquid Chromatography
LC-MS/MS
liquid chromatography tandem mass spectrometry
PRM
parallel reaction monitoring

Declarations

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Availability of data and materials

Support the findings of this study are available from the corresponding author upon request.

Authors' contributions

LLZ drafted the manuscript and drew the figures. ZBY checked and modified the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

Department of Parasitology, School of Basic Medical Sciences, Zunyi Medical University, Zunyi, China

Data Availability Statement

The proteomic data had uploaded on the website (https://datadryad.org/search), but the data will be private during peer review process.

https://doi.org/10.5061/dryad.mcvdnck14

https://datadryad.org/stash/share/s21iDQXI3fI0OLnAdPXrGvtGPJC2vM5uasr56H5oM0U

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Figures

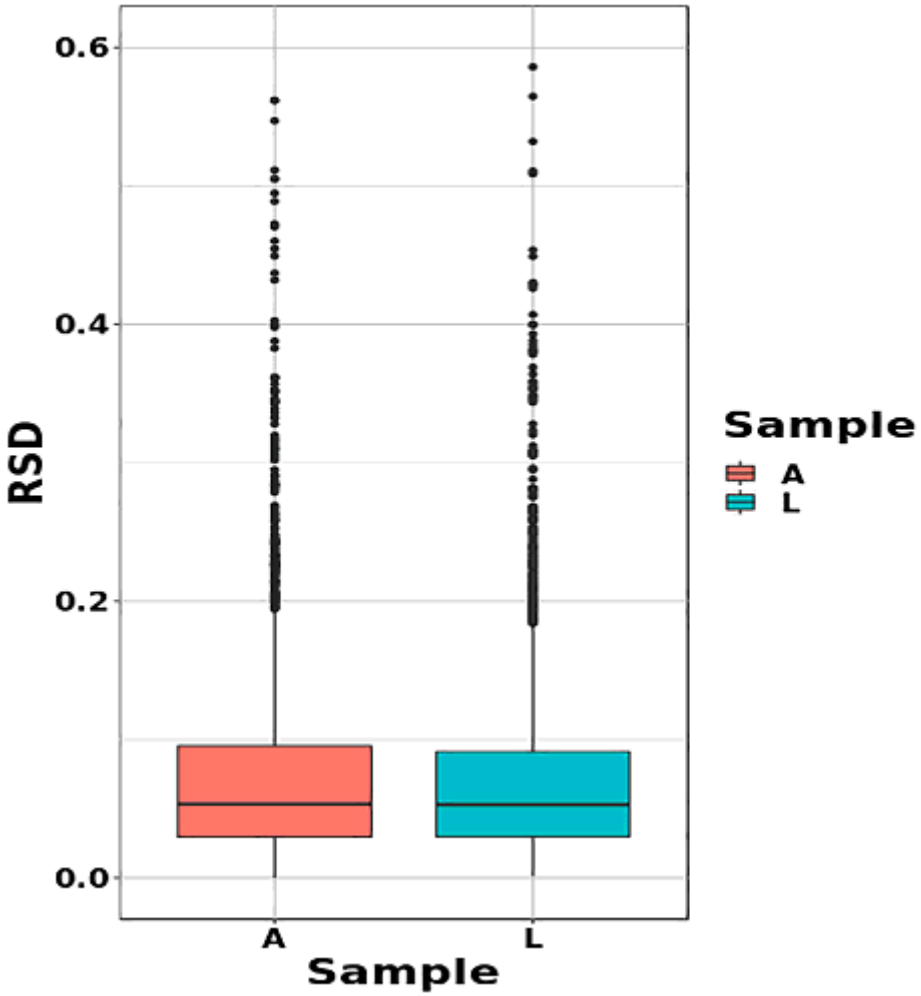

Figure 1

Quantitative repeatability of differentially expressed proteins

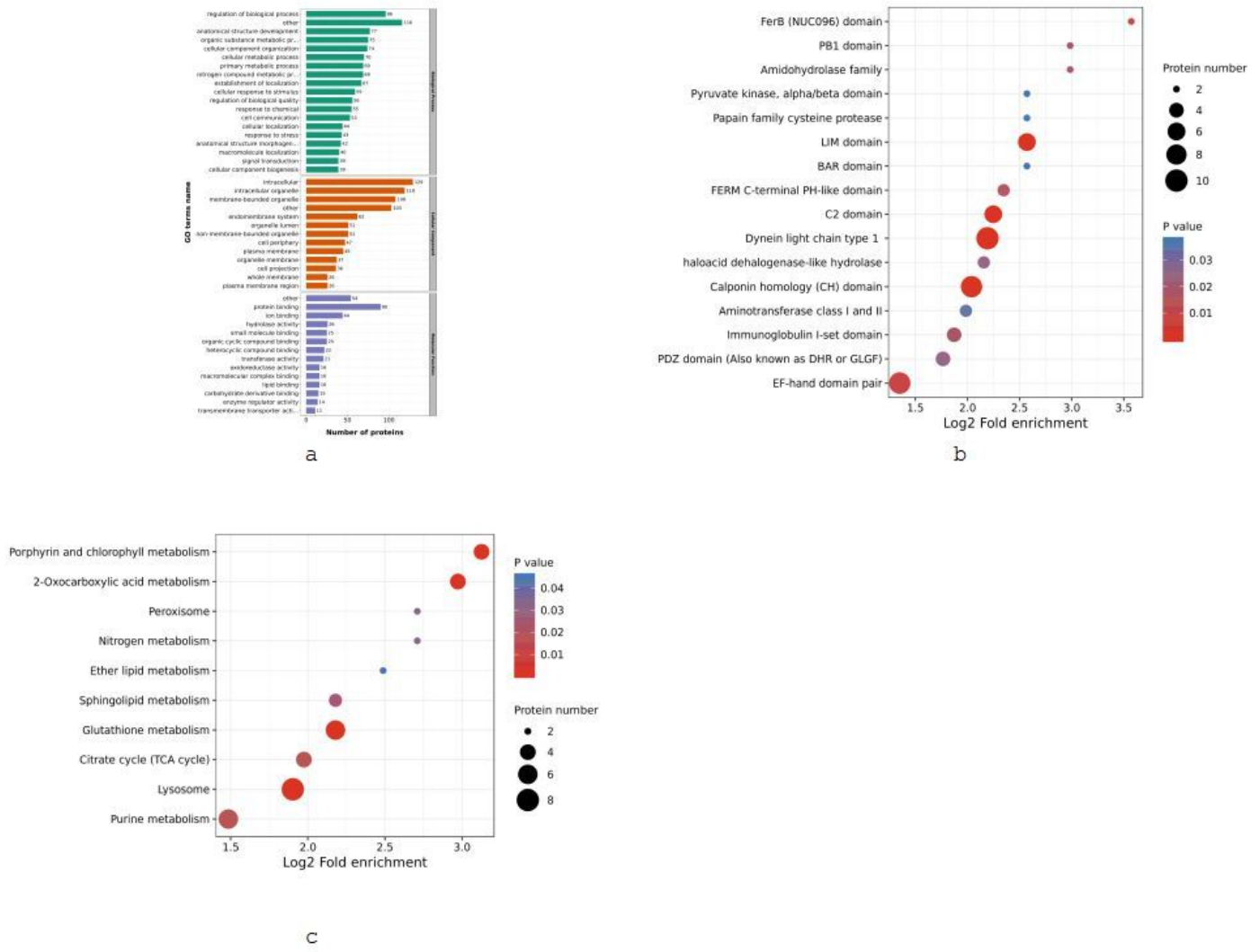

Figure 2

Cysticercus(down-regulated) proteins GO ontology.

KEGG, GO and protein domain enrichment analysis of Down-regulated proteins.A GO ontology; B Domain enrichment;C KEGG enrichment

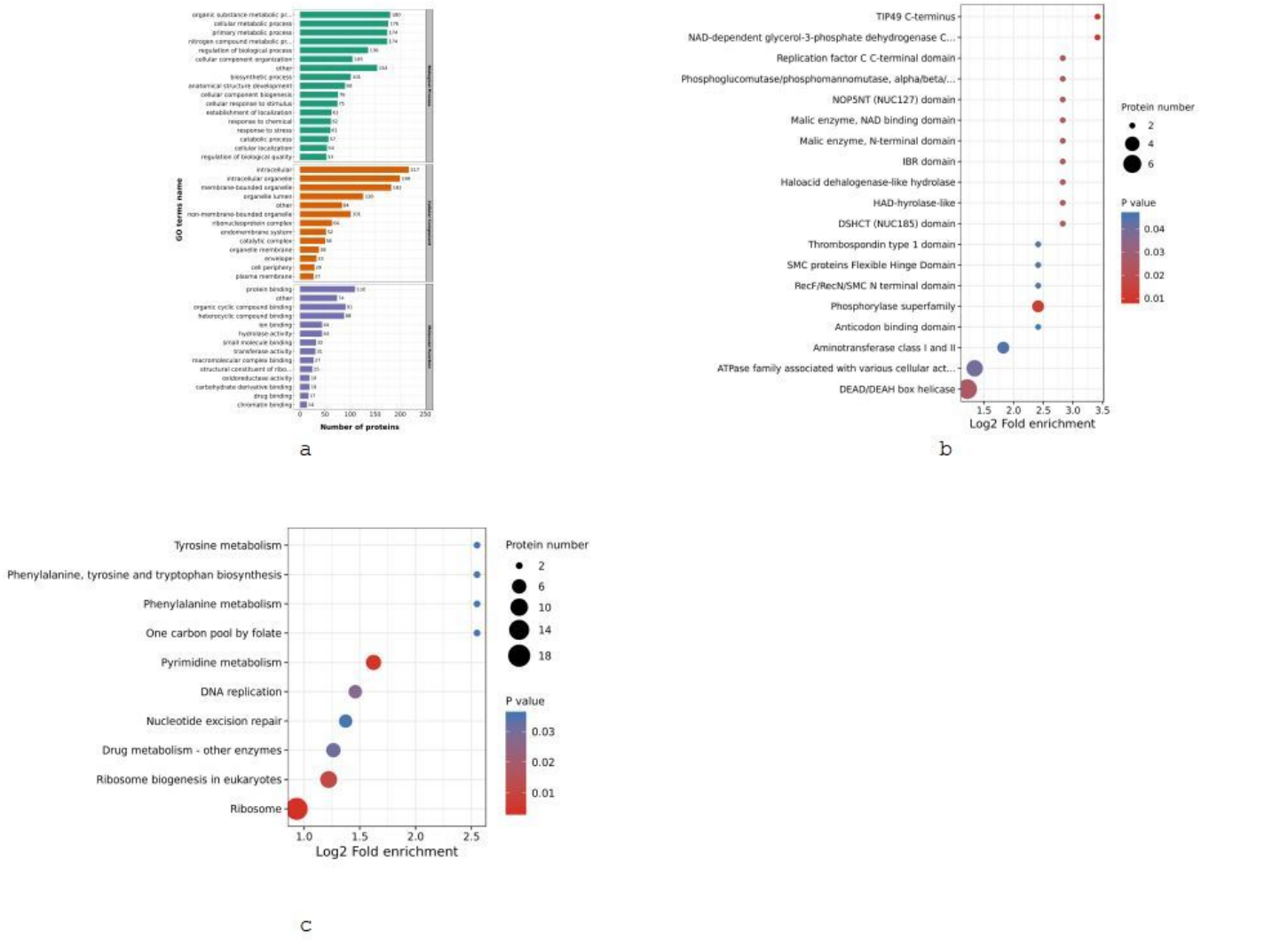

Figure 3

Adult(up-regulated) proteins GO ontology

KEGG, GO and protein domain enrichment analysis of Up-regulated proteins. A GO ontology; B Domain enrichment; C KEGG enrichment

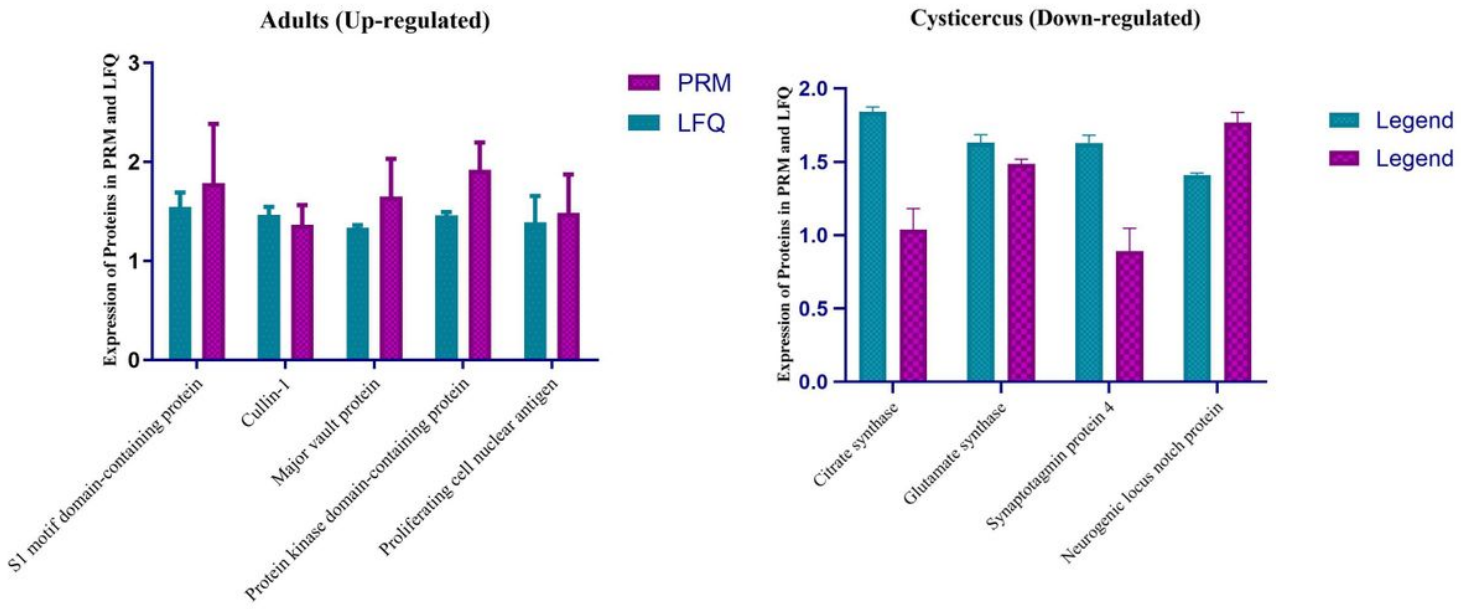

Figure 4

PRM analysis

Differentially expressed protein quantification by mass spectrometry-based targeted proteomics (PRM)

Supplementary Files

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- Downregulatedimmuneprotein.xlsx
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