Comparative Proteomics of *Schistosoma Japonicum* Developed From Different Oncomelania Snails Permissive Areas

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Abstract

Background: Schistosomiasis is an important zoonotic parasitic disease that is widely prevalent in tropical and subtropical countries and regions in the worldwide.

Methods: We aimed to analyze the proteomic differences between adult *S. japonicum* worms in Weishan Lake of Shandong province and the Jiangsu Yangtse river. Isobaric tags for relative and absolute quantification (iTRAQ) assays were used to analyze the differential proteomic profiles between female and male adult worms.

Results: A total of 2364 adult *S. japonicum* proteins were identified, and 1901 proteins were quantified by isobaric tags for relative and absolute quantification (iTRAQ) technology. Our results revealed 68 differentially expressed proteins (DEPs) in female adult worms and 55 DEPs in male adult worms. LC-MS/MS and bioinformatics analysis indicated that these DEPs are enriched in cellular composition, molecular function, biological function and catabolism pathways. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Domain and Clusters of Orthologous Groups (COG) analyses indicated that several groups of DEPs were involved in regulating transport, metabolism, signal transduction, energy production and conversion, defense and biosynthesis in adult *S. japonicum* worms. Our findings indicated that adult *S. japonicum* worms derived from *O. hupensis* that were transferred from permissive to nonpermissive areas exhibited moderate changes at the proteomic level. Moreover, snails transferred to the Weishan Lake did not change their schistosomiasis transmission ability and remained pathogenic in mice. In addition, three upregulated proteins (peptidylprolyl isomerase, heat shock protein 90α and receptor expression-enhancing protein (Q5DBJ1)) and three downregulated proteins (histone H3, histone H4 and receptor expression-enhancing protein (C1L9D7)) were found in both female and male adult worms.

Conclusions: Proteomic analysis showed that differentially expressed proteins (DEPs) between adult *S. japonicum* worms in Weishan Lake of Shandong province and the Jiangsu Yangtse river. The results of this proteomics analysis of adult worms that hatched in two separate intermediate hosts help to improve our understanding of the growth and developmental mechanisms of *S. japonicum* in different environments. Under the South-to-North Water Diversion Project (SNWDP) framework, long-term surveillance is needed to prevent the diffusion of *O. hupensis* and to reduce the risk of schistosomiasis transmission.

Background

Schistosomiasis is an important zoonotic parasitic disease that is widely prevalent in tropical and subtropical countries and regions, seriously endangering human health and hindering social and economic development. World Health Organization data showed that 260 million people are infected with *S. japonicum*, and more than 700 million people are threatened worldwide [1]. With the implementation of effective prevention and control measures, schistosomiasis has been reduced or significantly blocked in

endemic countries [2]. However, most developing countries, especially in Sub-Saharan Africa, have experienced additional spreading of schistosomiasis because of extreme poverty, inadequate medical conditions and insufficient knowledge [2]. *Schistosoma mansoni* (*S. mansoni*) is prevalent in Sub-Saharan Africa, Central and South America, while *Schistosoma haematobium* (*S. haematobium*) is prevalent in Africa and the Arabian Peninsula. *S. japonicum* is prevalent in China, Japan, the Philippines and Indonesia, while *Schistosoma mekongi* (*S. mekongi*) and *Schistosomaintercalatum* (*S. intercalatum*) are endemic in specific local areas [3].

Schistosomiasis has been endemic in China for more than 2170 years. The discovery of *S. japonicum* eggs in a female corpse from the Hunan Changsha Mawangdui site and a male corpse from Hubei Jiangling confirmed that schistosomiasis emerged as early as the Han Dynasty of China [4]. Schistosomiasis is seriously endemic in the Yangtze River Basin and the twelve southern provinces and is a major parasitic disease that seriously endangers human health and economic development [4]. The transmission and epidemiology of schistosomiasis are affected by natural, social and biological factors. The prevention and control strategy for schistosomiasis is comprehensive and based on the control of infectious sources, including the elimination of infectious sources, the control of the only intermediate host, *Oncomelania* snails, improved management of human and livestock manure, and provisions for safe water and health education. As the main host and infectious source, cattle play an important role in the transmission of schistosomiasis.

The South-to-North Water Diversion Project (SNWDP) of the Eastern Route Project (ERP) is a major strategic project to transfer part of the abundant water resources in the Yangtze River Basin to Northern China [5]. The ERP origin is the city of Yangzhou in the Jiangsu province, which is heavily infected with *O. hupensis* [6]; it then crosses Baoying county in Jiangsu province (at 33°15' N), the current northern limit of *O. hupensis* in China [7-8], and then passes northward into Shandong and Hebei provinces [6], which are nonpermissive areas for *O. hupensis* survival.

Oncomelania snails are the only intermediate host for *S. japonicum*, however, *O. hupensis* are one of most popular snails in China [9-10]. The city of Gaoyou in Jiangsu province (35.15 degrees north latitude) is northernmost distribution area of *O. hupensis* in China [11]. The morphology of snails in Weishan Lake has evolved distinctly over the course of more than ten years of evolution. If the miracidia complete their development in their natural environment in snails and then develop into cercariae and invade mice, it will be of great significance to study the biology and pathogenicity of *S. japonicum* in nonpermissive areas.

In a previous study, we transferred *O. hupensis* from Yangzhou in Jiangsu province (permissive area) to the city of Jining in the Shandong province (nonpermissive area north of 33°15' N), and kept the snails in the southern foothills of Dushan Island in the Weishan Lake in Shandong province where the ERP of SNWDP passes through. After 13 years of breeding and 12 generations of snails, the shell shape and population of the snails changed (Fig. 1) [12]. Unlike the results reported in other studies [13-16], a series of experiments showed that snails infected by miracidia and fed in the natural environment of the lakeshore released cercariae that were still pathogenic and could infect mice; furthermore, the offspring

of transferred snails were still able to be infected by *S. japonicum*. Until now, no study has compared on a proteomic level *S. japonicum* derived from *O. hupensis* transferred to nonpermissive areas with those derived from *O. hupensis* in their original habitat.

In this study, we aimed to analyze the proteomic differences between adult *S. japonicum* worms in Weishan Lake of Shandong province (nonpermissive area north of 33°15' N) and the Jiangsu Yangtse River (permissive area). Isobaric tags for relative and absolute quantification (iTRAQ) assays were used to analyze the differential proteomic profiles between female and male adult worms. Overall, our results provide an understanding of the regulation of differentially expressed proteins (DEPs) as well as invaluable information about the metabolic pathways and development of *S. japonicum.*

Methods

Parasite materials

O. hupensis were originally collected from the city of Yangzhou in the Jiangsu province (32°24' N, 119°26' E; hereafter referred to as "Jiangsu snails"), and then transferred to a pond at the southern foot of Dushan Island in the Weishan Lake in the city of Jining in the Shandong province (35°05' N, 116°44' E) . The transferred snails survived and spawned for 13 years (12 generations; hereafter referred to as "Shandong snails"). The Jiangsu and Shandong snails were infected with *S. japonicum* miracidia (Jiangsu isolate), then bred in the marshland of the Weishan Lake. Infectious snails were selected to release cercariae for infecting Institute for Cancer Research (ICR) mice. The mature adult worms were recovered by hepatic perfusion from the infected mice 50 days postinfection. Male and female worms were manually separated under a dissecting microscope and pooled together into four groups, namely, adult male or female worms derived from infectious Jiangsu snails or Shandong snails (hereafter referred to as "T-M" (male worms from Shandong snails), "CK-M" (male worms from Jiangsu snails), "T-FM" (female worms from Shandong snails) and "CK-FM" (female worms from Jiangsu snails)). All *Oncomelania* snails were removed from the feeding plate of a 25°C incubator. A 50 ml beaker was added to the room temperature laboratory environment. The ICR mice of the two groups were infected with 20 cercariaes, which were released from the *Oncomelania* snails. Fifty days after infection, adult male and female worms were randomly separated from 5 mice of each group.

Protein extraction and Digestion

Proteins from five female and five male adult worms from the two ICR mouse groups ("T-M", "CK-M", "T-FM" and "CK-FM") were precipitated with cold acetone for 3 h at -20 °C. After centrifugation at 4 °C, 12000 g for 10 min, the protein pellet was resuspended in lysis buffer (8 M urea, 100 mM triethylammonium bicarbonate (TEAB)). The protein concentration was determined using a modified Bradford Protein Assay Kit according to the manufacturer's instructions. A total of 100 μg of protein from each sample was reduced, alkylated, and sequentially diluted. For digestion, each sample was digested at a ratio of 100:1 with Sequencing Grade Modified Trypsin (1:50 dilution) for a continuous digestion at 37 °C overnight for 4 h.

Peptide Isobaric Labeling

After trypsin digestion, peptides were desalted using a Strata X SPE column and then vacuum-dried. iTRAQ labeling was performed using the iTRAQ Reagent 8-plex Multiplex Kit according to the manufacturer's protocol. Proteins from the worms in the four groups were labeled with reagents 113, 115, 117 and 119. Four independent replicates were performed for each group. The labeled peptides were separated into 10-20 components by HPLC on a C18 column. Forty peptide fractions were collected, dried in a vacuum and separated into 16 components by mass spectrometry.

HPLC Fractionation

The dried and labeled peptides were reconstituted and then fractionated by high pH reverse-phase HPLC using Waters Bridge Peptide BEH C18 column (130 Å, 3.5 μm, 4.6 × 250 mm). Briefly, peptides were, separated, combined into 8 fractions and dried by vacuum centrifugation. Next, the peptide fractions were desalted using a Ziptip C18 column according to the manufacturer's instructions, and then finally dried under a vacuum before storage at -20 ℃ until MS analyses were performed.

High-resolution LC-MS/MS analysis

Mass spectrometry was performed using a Thermo Nano LC 1000 high-performance liquid chromatography (HPLC) system with Proxeon EASY-nLC 1000 coupled to a Q Exactive. An Acclaim PepMap® 100 C18 (3 μm, 100 Å, 75 μm × 2 cm) trap column and an Acclaim PepMap® RSLC C18 (2 μm, 100 Å, 50 μm × 15 cm) analysis column were used in the mass spectrometric analysis. Trypsin digested fractions were reconstituted and eluted from the trap column and then loaded onto a reversed-phase analytical column. The eluent was sprayed via an NSI source at a 1.8 kV electrospray voltage and then analyzed by MS/MS in Q Exactive. The mass spectrometer was operated in data-dependent mode, automatically switching between MS and MS/MS.

Protein Functional Annotation and Enrichment

All identified proteins were classified by Gene Ontology (GO) annotation based on three categories: biological process, cellular component and molecular function. Annotation and enrichment results were used to annotate the proteins' KEGG database descriptions using the online KEGG automatic annotation server (KAAS) and the KEGG pathway database service tools. A two-tailed Fisher's exact test was applied to the GO annotation information for the identified proteins. We calculated the number of DEPs for each GO term at a level of 2. The GO annotation proteome was derived from the UniProt-GOA database (www.http://ebi.ac.uk/GOA/). After obtaining a protein ID based on a protein search, the ID was converted into the ID of the UniProtKB database; GO annotation information for *S. japonicum* was located based on the UniProKB ID. We carried out the correction for multiple hypothesis testing with a corrected p-value < 0.05 considered significant.

Data processing

Proteome Discoverer 1.3 (Thermo Scientific) software was used to transform the original mapped file (raw.data) into an mgf file, which was compared against the reviewed Swiss-Prot database combined with the unreviewed TrEMBL database, and then the data were submitted to the MASCOT 2.3.0 server. The server performed database retrieval, and a library file (.dat article) was used to achieve an FDR < 0.01 standard to obtain highly credible qualitative results.

Results

Oncomelania snails

Compared with the Jiangsu snails, the body size of the Shandong snails in the Weishan Lake region decreased, and their shells became thinner (Sup Figure 1). We cannot exclude the possibility that environmental factors affect snail breeding, which leads to a natural tendency toward decay over time.

SDS-PAGE analysis of adult schistosome proteins

Protein content and quality of four different samples of adult worm proteins were initially assessed by 12% SDS-PAGE analysis ("T-M", "CK-M", "T-FM" and "CK-FM"). The samples showed a complex mixture of proteins ranging from 150 kDa to < 10 kDa. Each sample exhibited slightly distinct band patterns and were subsequently prepared for LC-MS/MS analysis (Sup Figure 2).

LC-MS/MS identification of proteins

A total of 2364 adult worm proteins were identified and 1901 proteins were quantified in this experiment. Our results showed that there were 68 DEPs in the T_FM-vs-CK_FM group, including 24 upregulated proteins and 44 downregulated proteins, and 55 DEPs in the T_M-vs-CK_M group, including 25 upregulated proteins and 30 downregulated proteins. The DEP distribution ratios obtained from our analysis are displayed and were defined based on threshold protein quantifications (1.2 & 0.05) (Figure 1).

Functional classification of DEPs

DEPs were classified by gene ontology (GO) term analysis based on cellular component, molecular function, biological progress and subcellular location. Our results indicated that 9 cellular components were enriched among the 68 DEPs from the T_FM-vs-CK_FM group, which included 32 upregulated proteins and 36 downregulated proteins (Sup Figure 3). In addition, 10 cellular components were enriched among the 73 DEPs from the T_M-vs-CK_M group, which included 25 upregulated proteins and 48 downregulated proteins (Sup Figure 3).

Our statistical analysis showed that the 38 DEPs from the T_FM-vs-CK_FM group (19 upregulated proteins and 19 downregulated proteins) were enriched for three molecular functions (binding, molecular transducer activity and catalytic activity). Of the 38 DEPs, the largest number of proteins were enriched for catalytic activity, which accounted for more than a half, followed by binding and molecular transducer

activity (Sup Figure 3). The T_M-vs-CK_M group was enriched for 5 molecular functions (binding, structural molecule activity, transporter activity, antioxidant activity and catalytic activity), with 16 upregulated proteins and 14 downregulated proteins. Of the 40 DEPs, the number of proteins enriched for catalytic activity and binding was almost the same, which were followed by structural molecule activity, antioxidant activity and transporter activity (Sup Figure 3).

The 52 DEPs from the T_FM-vs-CK_FM group (30 upregulated and 22 downregulated proteins) were enriched for biological processes including localization, biological regulation, metabolic processes, response to stimulus, cellular component organization or biogenesis, cellular processes and single-organism processes (Sup Figure 3). Compared with the T_FM-vs-CK_FM group, the 41 DEPs from the T_M-vs-CK_M group (13 upregulated and 28 downregulated proteins) were enriched for biological processes including biological regulation, metabolic processes, response to stimulus, cellular component organization or biogenesis, regulation of biological processes, cellular processes, signaling and single-organism processes (Sup Figure 3).

Our statistical analysis of the subcellular location category showed that the distribution of the subcellular distributions of the 68 DEPs were different between the T_FM-vs-CK_FM group and T_M-vs-CK_M group (Fig.2). A larger number of DEPs were enriched in the cytosol (38.18%), extracellular space (30.91%), cytoskeleton (5.45%) and plasma membrane (9.09%) in the T_M-vs-CK_M group than in the T_FM-vs-CK_FM group (cytosol, 30.88%; extracellular space, 30.91; cytoskeleton, 5.45%; and plasma membrane, 8.28%). Meanwhile, fewer DEPs were enriched in the nucleus (7.27%) and cytosol-nucleus (1.82%) in the T_M-vs-CK_M group compared to the T_FM-vs-CK_FM group (nucleus, 11.76% and cytosol-nucleus, 10.29%).

Functional enrichment of DEPs

Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was used to classify the DEPs by their GO enrichment analysis. First, we calculated the GO enrichment of cellular components associated with the DEPs, including terms such as protein-DNA complex, nucleosome, DNA packaging complex, chromosome, chromosomal part, chromatin, extracellular region and extracellular space (Sup Figure 4). The results revealed 25 DEPs in the T_FM-vs-CK_FM group and 27 DEPs in the T_M-vs-CK_M group among 934 total proteins (Sup Table1, 2); of the identified DEPs, 11 proteins from each group were upregulated and 14 and 16 proteins were downregulated, respectively (Sup Figure 4). The results from the GO enrichment analysis for cellular components showed that the T_FM-vs-CK_FM group was enriched for terms including protein-DNA complex and nucleosome and DNA packaging complex, while the T_M-vs-CK_M group was enriched for terms such as extracellular space and extracellular region (Sup Table1, 2).

Next, GO enrichment analysis was used for molecular functions associated with the DEPs, including oxidoreductase activity, ferroxidase activity, ferric iron binding, iron ion binding, transition metal ion binding, cysteine-type peptidase activity, glutathione transferase activity, peroxiredoxin activity, lipid binding, transferase activity, oxidoreductase activity, peroxidase activity, calcium-dependent phospholipid binding, peptidase activity, antioxidant activity and phospholipid binding (Figure 3). The results revealed

that there were 29 DEPs from the T_FM-vs-CK_FM group and 31 DEPs from the T_M-vs-CK_M group out of a total of 1216 proteins (Sup Table3, 4). This included 14 and 12 DEPs that were upregulated and 15 and 19 DEPs that were downregulated in the two groups, respectively (Figure 3). The results from the GO enrichment analysis for molecular functions showed that the T_FM-vs-CK_FM group was enriched for oxidoreductase activity, ferroxidase activity and ferric iron binding, while the T_M-vs-CK_M group was enriched for cysteine-type peptidase activity and glutathione transferase activity.

GO enrichment analysis was then used for biological processes associated with DEPs, including cellular homeostasis, homeostatic processes and transition metal ion homeostasis (Sup Figure 5). The results revealed 19 DEPs in the T_FM-vs-CK_FM group and 19 DEPs in the T_M-vs-CK_M group among the 881 total proteins, which included 11 and 7 DEPs that were upregulated and 8 and 12 DEPs that were downregulated in two groups, respectively (Sup Figure 5). The results from the GO enrichment analysis for biological processes showed that the T_FM-vs-CK_FM group was enriched for homeostatic processes and cellular homeostasis, while the T_M-vs-CK_M group was enriched for protein folding and actomyosin structure organization.

Finally, KEGG enrichment analysis revealed 19 DEPs in the T_FM-vs-CK_FM group and 17 DEPs in the T_M-vs-CK_M group among the 1009 total proteins (Sup Figure 6). The DEPs from the T_M-vs-CK_M group were involved in mineral absorption, systemic lupus erythematosus, antigen processing and presentation, alcoholism and biosynthesis of amino acids. In contrast, the DEPs from the T_M-vs-CK_M group were involved in signaling pathways of drug metabolism, metabolism, chemical carcinogenesis, the peroxisome, glutathione metabolism, systemic lupus erythematosus and the lysosome (Sup Figure 6). A total of 10 and 5 DEPs were upregulated in females and males, respectively, while 9 and 12 DEPs were downregulated in females and males, respectively (Sup Figure 6). GO enrichment analysis for KEGG pathway results showed that the T_FM-vs-CK_FM group was associated with mineral absorption, while the T_M-vs-CK_M group was associated with protein folding.

Discussion

Because of the special geographical location, the transferred snails (Shandong snails) lived in Weishan Lake in the Shandong province more than ten years (Sup Figure 2). In our study, snails were infected by miracidia and fed in their natural environment, and then cercariae were released from infectious snails to infect mice. Our results showed that the offspring of Shandong snails still maintained the ability to be infected by *S. japonicum.* Moreover, the cercariae released by the snails showed no significant difference in their ability to transmit schistosomiasis compared with *O. hupensis* collected in permissive areas. An additional 30 *Oncomelania* snails were randomly selected from the experimental group and the control group early in the experiment for assessment by histoanatomy. No other *Clonorchis*, *Clonorchis exodidymis*, *Clonorchis exophylla* or *Clonorchis circulans* parasites were found in the Shandong snails. Although a series of experiments have focused on the proteomics and transcriptomics of *S. japonicum* [17-19], no studies have been published comparing *S. japonicum* derived from *O. hupensis* transferred to nonpermissive areas on a proteomic level.

Proteomics research has been widely applied in the virology, bacteriology and parasitology fields. Proteomics can not only provide evidence for cell biology but also elucidates the molecular mechanisms of diseases. Proteomics investigates the differentially expressed proteins (DEPs) induced by physiological and pathological mechanisms, metabolic regulation, and protein expression profiles, revealing the response of an organism to internal and external environmental changes. Proteomics is widely applied to study eukaryotic parasites, such as *S. japonicum*. Previous proteomics research on *S. japonicum* used two-dimensional (2D) gel electrophoresis. In ultraviolet-attenuated and normal cercariae, 20 DEPs were identified by mass spectrometry [5]. DEP analysis in *S. japonicum* schistosomula and adult worms showed 22 spots from schistosomula and 11 spots from adult worms [20]. Compared with 2D gel electrophoresis, iTRAQ is more suiTableand sensitive for the comparison and identification of DEPs [21]. iTRAQ-based analysis of adult *S. japonicum* from water buffalo and yellow cattle showed that 131 DEPs were primarily involved in protein synthesis, transcriptional regulation, protein proteolysis, cytoskeletal structure and oxidative stress response processes [22]. Our results showed that a total of 123 *S. japonicum* DEPs were identified, including 68 DEPs from the T_FM-VS-CK_FM group and 55 DEPs from the T_M-VS-CK_M group. The bioinformatics analysis revealed that the DEPs were mainly involved in transport and metabolism, signal transduction, energy production and conversion, and chromatin structure and dynamics. Our findings may help to improve our understanding of the growth and developmental mechanisms of *S. japonicum.* Compared with the Jiangsu snails, the *S. japonicum* developed in Shandong snails displayed differences in their proteomes, including in subcellular location, cellular component, molecular function, and biological process categories and KEGG pathways. Of note, we observed significant changes proteins associated with homeostatic processes, cellular homeostasis and mineral absorption in female worms, and protein folding and actomyosin structure organization in male worms.

Interestingly, we identified several novel proteins, uncharacterized proteins and unknown proteins that have not been previously reported in *S. japonicum* (Sup Table9,10). Our findings showed that peptidylprolyl isomerase [23], HSP90α [24-25] and receptor expression-enhancing protein (Q5DBJ1) [22] were upregulated and histone H3, histone H4 and receptor expression-enhancing protein (C1L9D7) were downregulated in both the T_FM-VS-CK_FM and T_M-VS-CK_M groups, which indicated that these proteins are relatively conserved in *S. japonicum*. We also identified some interesting DEPs only in the T_FM-VS-CK_FM group, such as protein disulfide-isomerase, carbonyl reductase 1 and innexin, while tryparedoxin peroxidase and a cell death-regulatory protein (GRIM19) were identified only in the T_M-VS-CK_M group.

HSP90α is one of four members of the HSP90 protein family that includes HSP90α, HSP90β, glucose regulatory protein 96 (GRP96) and tumor necrosis factor receptor associated protein-1 (TRAP-1) [24-25]. HSP90α is an important molecular chaperone that is involved in the activation of disparate client proteins, and it is a homodimeric protein that has complex biological functions [26]. HSP90 is involved in essential cellular processes and regulatory pathways, such as apoptosis, cell cycle control, cell viability, protein folding and degradation, and signal transduction [27]. In addition, HSP90 can activate antigen-presenting cells and dendritic cells to trigger adaptive immunity, which plays an important role in protein

homeostasis, cell differentiation and development [28]. HSP90 also plays a role in cancer and some neurological diseases [29-30]. The overall molecular structure of HSP90 homologues consists of three main conserved domains: an N-terminal domain (NTD), which is responsible for binding to ATP; a C-terminal domain (CTD), which is responsible for protein dimerization and contains a special motif, MEEVD (C-terminal pentapeptide domain) or KDEL, depending on the fine structure of HSP90 in cytoplasm or endoplasmic reticulum (ER); and the middle domain (MD), which regulates HSP90 function by binding to ATP phosphates designated for NTD, thus regulating its ATPase activity [31]. The MD of HSP90, with a relative molecular mass of approximately 35000 Da, has been studied as a binding site for client proteins and nuclear localization signals, and it is involved in the recognition of cooperative proteins and the regulation molecular chaperone activation [32]. Furthermore, there have been several research reports on HSP90 in *S. japonicum* infection [33-36]. The first report on HSP90 was in the excretory/secretory proteome of the adult developmental stage of human blood flukes of *S. japonicum* [33]. TiO_2-based phosphoproteomic analysis revealed that HSP90 is one of the phosphoproteins that is codetected in the different stages and sexes of schistosomes, and it may play an important role in the regulation of schistosome development [34]. Research by Gong *et al*. showed that a novel HSP90α molecule was identified in *Microtus fortis* resistance to *S. japonicum* infection in vitro and in vivo [35]. Work from Wenkert *et al*. indicated that *S. japonicum* HSP90 may be the drug target of synthetic geldanamycin (GA) derivatives in vitro [36]. Our findings indicate that HSP90 may participate in antigen processing and presentation in the major histocompatibility class (MHC) I pathway (Sup Figure 7).

Tetraspanins are important membrane proteins that are found in all multicellular eukaryotes [37]. They are also referred to as transmembrane 4 superfamily proteins and they have four transmembrane alpha-helices and two extracellular domains that are often thought to act as scaffolding proteins, anchoring multiple proteins to one area of the cell membrane [37]. Tetraspanins were also found in the male worms in our T_M-vs-CK_M group (Sup table10). Tetraspanins have been widely reported in *S. japonicum,* and they are thought to be potential diagnostic antigens or vaccine candidates against *S. japonicum* [38-41].

Mineral absorption plays an important role in female adult *S. japonicum* specimens*,* and ferritin is an important DEP that regulates the transport and storage of the iron pool. Ferritin is a universal intracellular protein that stores iron and releases it in a controlled fashion, and it plays an important role in inorganic ion transport and metabolism [42]. Studies have shown that ferritin deficiency in myeloid compartments dysregulates host energy metabolism and increases susceptibility to *Mycobacterium tuberculosis* infection [43]. A recent study demonstrated that mitochondrial ferritin plays an important role in preventing neuronal damage by regulating iron metabolism and attenuating oxidative stress [44]. Increased expression of mitochondrial ferritin provides new insights into the antioxidant role of ferritin in the brains of Alzheimer's disease (AD) patients [45]. A *Listeria monocytogenes* ferritin-like protein plays an essential role in acid stress tolerance [46-47]. Our study showed that ferritin plays an important role in the iron metabolic pathway of *S. japonicum* (Sup Figure 8).

Lysosomes are organelles that contain a variety of enzymes that decompose proteins, nucleic acids, polysaccharides and biological macromolecules [48-49]. Cathepsin B is encoded by the CTSB gene and is

found in humans, mice and rats; this protein belongs to the lysosomal cysteine protease family and plays an important role in intracellular proteolysis [50]. Cathepsin B is involved in enhancing the activity of urokinase, matrix metalloproteinase and cathepsin D [51-53], and it is also associated with autophagy, catabolism and specific immune resistance in cancer [54]. Our results indicated that cathepsin B (Figure 4), as a lysosomal hydrolase, plays an important role in the regulation of autophagy and the transport of synthesized lysosomal enzymes.

Conclusions

In the present study, we used iTRAQ to identify DEPs in adult female and male worms after development in two *S. japonicum* intermediate hosts, *O. hupensis* and *O. hupensis* transferred to nonpermissive areas. The bioinformatics analysis showed that the DEPs differed significantly between females and males after development in these two intermediate hosts, and some DEPs may play important roles in signaling pathways and nutrient metabolism. Comparative proteomics provided new insights into the developmental mechanisms of worms and the relationships between worms and their hosts.

Abbreviations

Isobaric tags for relative and absolute quantification (iTRAQ); Differentially expressed proteins (DEPs); the South-to-North Water Diversion Project (SNWDP); Eastern Route Project (ERP)

Declarations

Funding

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Author Contributions

Xin Liu, Feng Miao and Yongbin Wang performed the experiments and participated in the design of the study. Gongzhen Liu designed and drafted the manuscript. Kun Yang, Wei Li, Chunrong Xiong, Jianfeng Zhang, and Xinyao Wang participated in the animal experiments. Haoyun Yan and Changyin Wei participated in collection of oncomelania snails. Ge Yan and Changlei Zhao provided suggestions for designing the experiments. All authors read and approved the final manuscript. All persons who have made substantial contributions to the work are reported in the manuscript.

Ethical approval and informed consent

All animal procedures complied with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). The animal (ICR mice) use protocol was approved by the Institutional Animal Care and Use Committee of Shandong Institute of Parasitic Diseases.

Consent for publication

Not applicable.

Availability of data and materials

All datasets are presented in the main paper.

Competing interests

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

We performed analysis on routine administrative data and no ethical approval was required.

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Figures

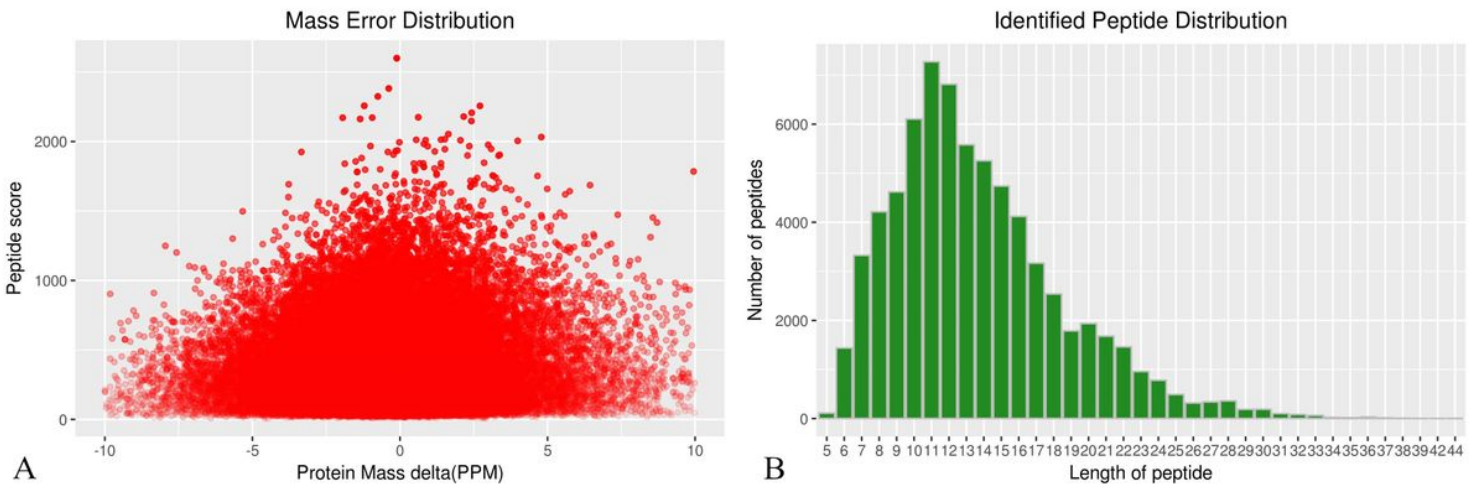

Figure 1

Total identified proteins distribution of adult worms.(A) The x-axis shows Protein Mass Delta (PPM); the y-axis shows peptide score of Mass Error Distribution. (B) The x-axis shows length of peptide; the y-axis shows number of peptide.

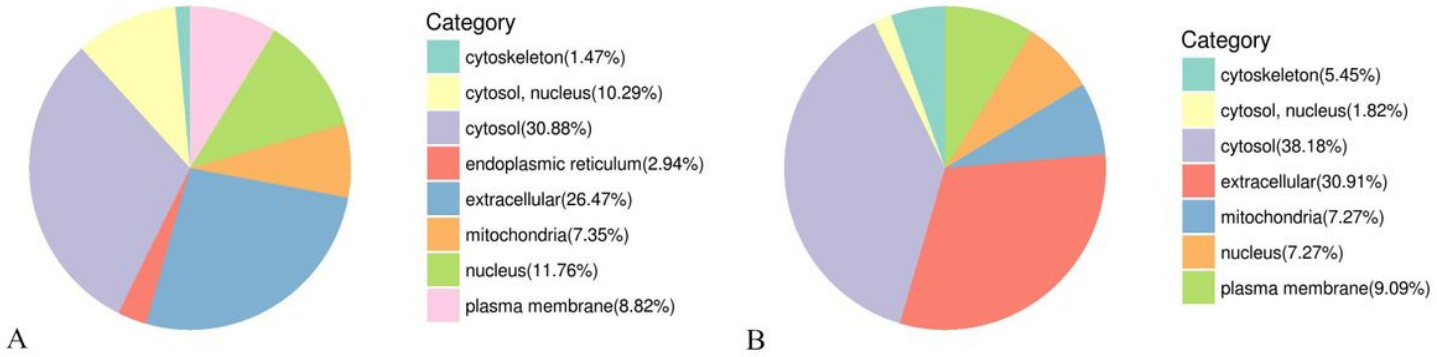

Figure 2

Subcellular location GO category distributions of DEPs from female (A) and male (B) adult worms.

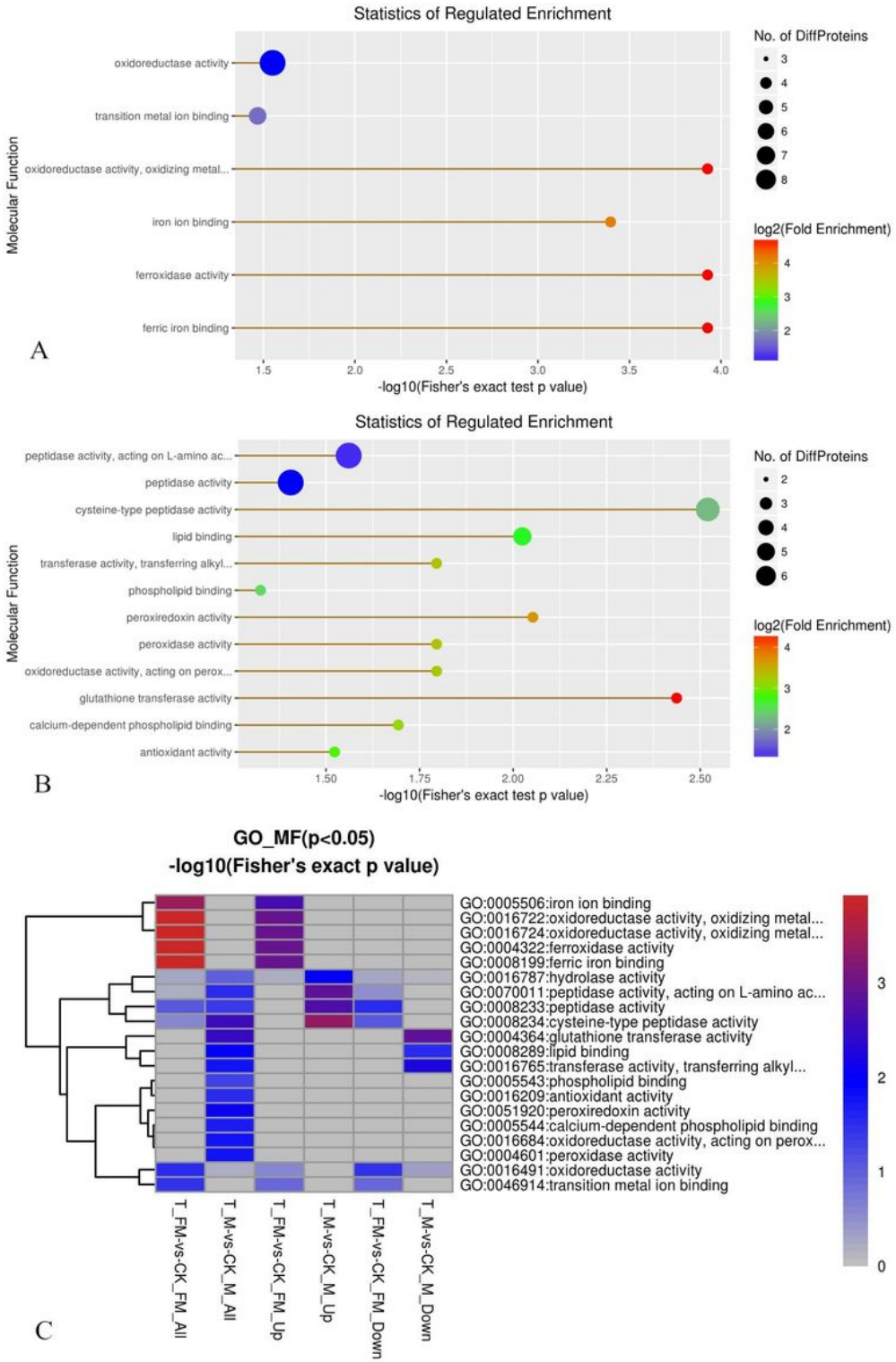

Figure 3

GO enrichment analysis for molecular function in the T_FM-vs-CK_FM (A) and T_M-vs-CK_M (B) groups, and the ratios (C) of regulated proteins. (A) and (B): The x-axis shows the –log10(Fisher's exact test p value) and the y-axis shows the molecular function score. The color indicates the score of the log2(Fold Enrichment); the circle size represents the number of DEPs. (C) The x-axis shows the -log10(Fisher's exact

test p value0.05) ratio of the T_FM-vs-CK_FM and T_M-vs-CK_M groups and the y-axis shows the GO molecular function score.

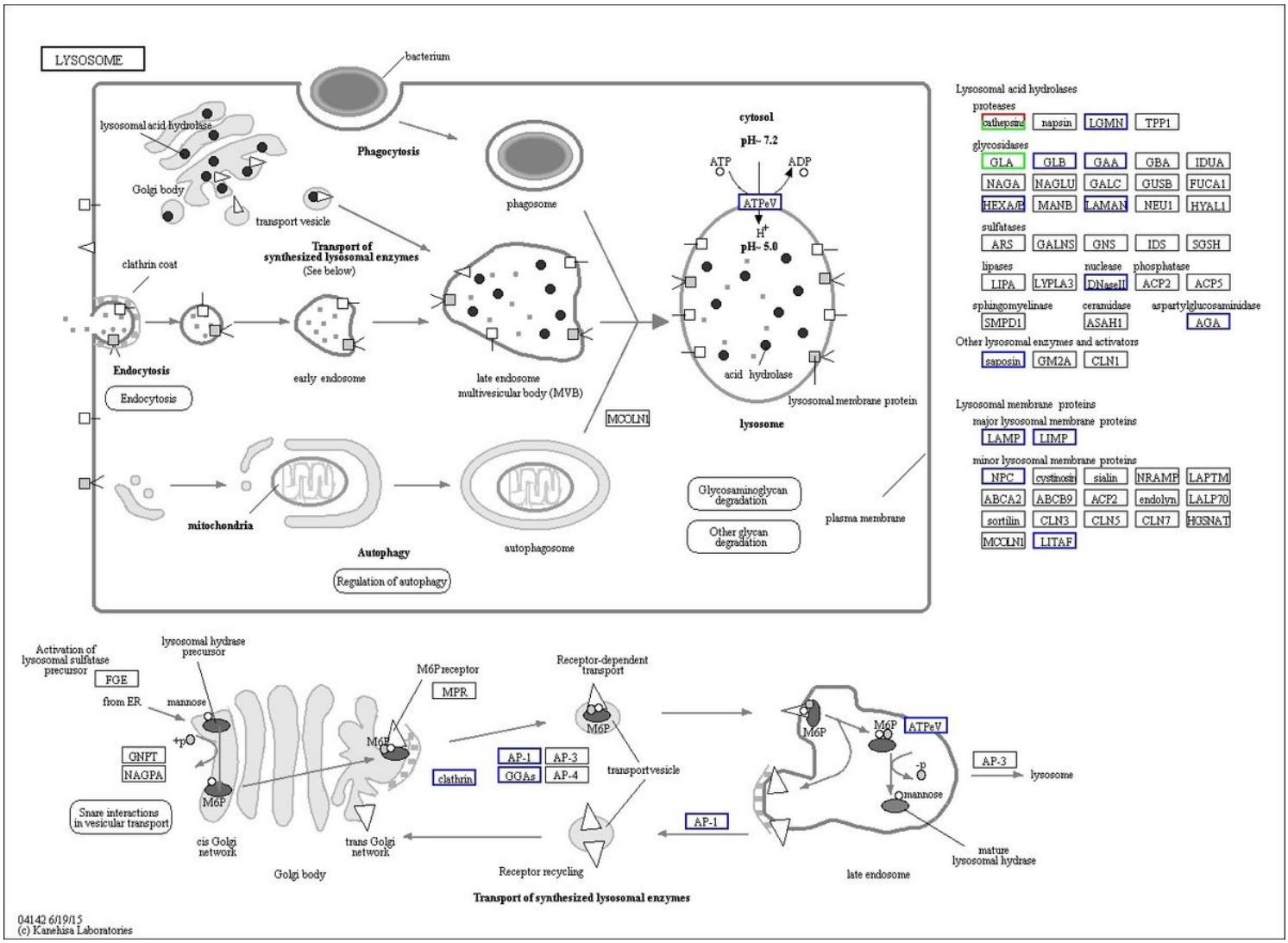

Figure 4

Representative KEGG pathway of downregulated lysosomal compartment (T_M-vs-CK_M, map04142). The rectangular nodes in the Figure represent gene products. The blue borders indicate background proteins, and white boxes indicate proteins not identified in this experiment. The red/green borders in the Figure indicate the DEPs detected in this study, with red representing upregulated proteins and green representing downregulated proteins. Half red and half green borders indicate gene products with both upregulated and downregulated proteins.

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