Comparative Transcriptome Analyses of the Developmental Stages of Taenia multiceps

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Cerebral coenurosis, caused by the larvae of Taenia multiceps (Coenurus cerebralis), is a fatal central nervous system disease in sheep and other herbivores and occasionally humans. Comparative transcriptomic profiles of the developmental stages of the parasite remain unknown. In this study, RNA sequencing was used to determine the transcriptome profiles of different stages of the life cycle of T. multiceps, including Oncosphere, Coenurus cerebralis (Pro with Cyst), and Adult (Adu), as well as scolex-neck proglottids (Snp), immature–mature proglottids (Imp), and gravid proglottids (Grp) of the adult stage. A total of 42.6 Gb (average 6.1 Gb) Illumina pair-end reads with a 125-bp read length were generated for seven samples. The total number of differentially expressed genes (DEGs) in the various life stages ranged from 2,577 to 3,879; however, for the tissues of the adult worm, the range was from 1,229 to 1,939. Kyoto Encyclopedia of Genes and Genomes analysis showed that the DEGs mainly participated in cellular and metabolic processes, binding and catalytic activity, genetic information processing, and environmental information processing. In addition, a large number of genes related to development and parasite–host interaction were identified. Quantitative reverse transcription-polymerase chain reaction confirmed that the levels of 28 selected DEGs were consistent with those determined using RNA sequencing. The present study provides insights into the mechanisms of the development and parasitic life of T. multiceps.

Keywords: comparative transcriptome, developmental stages, Coenurus cerebralis, differentially expressed gene, Taenia multiceps

KEYPOINTS

- A total of 42.6 Gb (average 6.1 Gb) Illumina pair-end reads with a 125-bp read length were generated for seven samples from T. multiceps.
- The total number of DEGs in the various life stages of T. multiceps ranged from 2,577 to 3,879; however, for the tissues of the adult worm, the range was from 1,229 to 1,939.
- Some differentially expressed genes are involved in the development of T. multiceps and the parasite–host interaction.
INTRODUCTION

*Taenia multiceps* is a tapeworm that inhabits the small intestine of dogs and other canids. The larval stage (known as *Coenurus cerebralis*) with a fluid-filled cyst is usually found in the nervous system of the intermediate host and often causes encephalitis and neurological symptoms in infected hosts, and sometimes even death (1, 2). Definitive hosts excrete egg-containing feces into the environment; when intermediate herbivorous hosts ingest these eggs during grazing, oncospheres spread from the eggs. Furthermore, through blood circulation, they reach various locations, including the central nervous system, where they could develop into mature *Coenurus* cysts of the larval stage within 2–3 months (3). The life cycle of *T. multiceps* is completed after the scoleces contained within the coenurus are ingested by the definitive host. The disease may last several months and is fatal (4, 5). *T. multiceps* infection in sheep results in significant economic losses in many parts of the world, predominantly in the developing countries of Africa, Europe, and Southeast Asia (1, 6, 7). Cases of human infections due to accidental ingestion of *T. multiceps* eggs have also been reported frequently and cause serious pathological conditions (8–12). Cestode development is well-known to involve complex morphological and physiological changes associated with changes in the host species (13, 14). Strobilation development (generation of serially repeated reproductive organs) is an interesting but difficult study subject, as it is difficult to obtain the parasites and maintain them in experimental hosts or *in vitro* (15). Recently, the genome of *T. multiceps* was sequenced to understand its biology and evolution (16). Extensive transcriptome analysis of *T. multiceps* larvae (17) and adults (18) provides substantial and useful information. Additionally, comparative transcriptomic analysis can identify key genes associated with the development, structure, and storage of carbohydrates, thereby contributing to the understanding of the biology of organisms (19–21). However, the mechanisms underlying the development of *T. multiceps* from larva to adult remain unclear. In the present study, comparative transcriptome analyses of the developmental stages of *T. multiceps* were performed to obtain important insights into the molecular basis of biological processes involved in worm development and reproduction.

MATERIALS AND METHODS

Sample Collection

(i) The larvae of *T. multiceps* (named *Coenurus cerebralis*) were obtained from the brains of naturally infected sheep at a local slaughterhouse in Gansu province, China. Freshly separated *Coenurus cerebralis* were washed repeatedly in sterile phosphate-buffered saline (PBS, pH 7.4) to remove the host components. Protoscolices (Pro) were then separated from the cyst wall (cyst) of *Coenurus cerebralis*. (ii) Adult *T. multiceps* (Adu) were collected from experimentally infected dogs at our laboratory animal center. The dog infection experiment was performed according to a previously reported protocol (18, 22). In brief, two 4-month-old parasite-free beagle dogs were infected orally with 40 *T. multiceps* protoscolices. When gravid proglottids (Grp) were found in the feces, the infected dogs were exsanguinated, and the worms were collected and washed. Thereafter, scolex-neck proglottids (Snp), immature–mature proglottids (Imp), and Grp were separated from each adult based on the proglottid characteristics. (iii) For the isolation of oncospheres, gravid tapeworm segments were obtained from the above collected adult worms, which were then homogenized, filtered, washed, and purified by Percoll density-gradient separation. Finally, oncospheres (Onc) were hatched according to the procedure described previously (23, 24). To prepare tissue samples from adult *T. multiceps*, intact adults were segmented into three parts: Snp, Imp, and Grp based on their proglottid characteristics. All prepared samples were washed with sterile PBS, preserved in five volumes of RNA-later (TaKaRa, Japan), and then immediately stored in liquid nitrogen at −80°C for RNA sequencing (RNA-seq) analysis.

Total RNA Isolation, Qualification, and Transcriptomic Library Construction

Total RNA was extracted from each sample using TRIzol Reagent (Invitrogen, USA), and contaminating genomic DNA was removed by treatment with RNase-free DNase I (Takara, Japan). RNA degradation and contamination were monitored on 1% agarose gels, and RNA integrity was assessed using an RNA Nano 6000 Assay Kit and Bioanalyzer 2100 system (Agilent Technologies, USA). RNA with an RNA integrity number (RIN) > 7.0 was considered to be of high enough quality for transcriptomic library construction and RNA sequencing, according to the manufacturer’s instructions. Subsequently, mRNA was isolated from total RNA, cut into fragments, and reverse-transcribed into first-strand cDNA using random hexamers. Briefly, double-stranded cDNA was synthesized and purified, and the ends were repaired. Finally, RNA sequencing libraries were constructed using the Illumina TruSeq RNA Sample Preparation Kit (Illumina, San Diego, CA, USA), according to the manufacturer’s instructions. Library quality was assessed using an Agilent Bioanalyzer 2100 system (Agilent Technologies).

Transcriptome Sequencing and Data Analysis

Seven transcriptomic libraries were pooled and sequenced using the Illumina HiSeq 2000 platform (Illumina, San Diego, CA, USA). Raw reads in fastq format were first processed through in-house Perl scripts to obtain clean reads by discarding reads containing adapters, reads containing poly-N, and low-quality reads. Finally, high-quality reads were generated after RNA-seq and filtering using the FastQC software (25). The filtered reads were then aligned to the draft of the *T. multiceps* genome sequence, the indexes of Burrows–Wheeler were constructed using Bowtie2 in default parameters (26), and the clean data from seven RNA-seq libraries were mapped to the assembled genome of *T. multiceps* using the BWT-MEM software.

To assess the differentially expressed genes (DEGs) in each life-cycle stage and tissue, transcript FPKM values from a replicate were plotted against transcript FPKM values from...
## TABLE 1 | qRT-PCR primers of differentially expressed genes.

| Gene ID      | Forward primer sequence (5′-3′)                           | Reverse primer sequence (5′-3′)                           | Gene annotation                                                                 |
|--------------|-----------------------------------------------------------|-----------------------------------------------------------|---------------------------------------------------------------------------------|
| Tmu003811    | TAGTAGGAAAGCAGCGCAAGAGC                                     | TGACACTGCTCCTCAACGTAACT                                    | Oncosphere protein Tso31b [Taenia solium]                                       |
| Tmu005434    | CACAGCTTATGCGCTTTGCG                                         | TCTTGCGTGCTGCTATTGC                                        | Oncosphere protein Tm18 [Taenia multiceps]                                      |
| Tmu012228    | CAGTCAGGCTGACAAATGG                                        | TGTGTTGCTTGGGAAGGATTG                                      | Oncosphere protein Tm18 [Taenia multiceps]                                      |
| Tmu007899    | TCTGAGTTCGGCAGAGTCCTCA                                     | GATTTACGCTGCTGCTTGCAT                                      | Oncosphere protein Tm16 [Taenia multiceps]                                      |
| Tmu005154    | GTGCCCTACAGTTGCTCTTT                                      | TTGCTTTCAACGATGGGTCT                                       | 8 kDa glycoprotein [Taenia multiceps]                                           |
| Tmu005158    | GTGCCCTACAGTTGCTCTTT                                      | CGGTTCTGCAAGTGGAGGATG                                      | 8 kDa glycoprotein [Taenia hydatigena]                                          |
| Tmu006823    | GGAAGCAGCTGACAAATGG                                        | CTTCGCTTGGCAAGCAGTGT                                      | 8 kDa glycoprotein [Taenia hydatigena]                                          |
| Tmu003810    | TGACTTCCAACGCAAGAC                                       | GCTGCTTACAGTGGCAAGG                                       | 45m oncosphere protein [Taenia multiceps]                                      |
| Tmu007808    | ATGACGACATCAACTTGAGG                                       | TGATACTCCTTTGATGCGAG                                      | Heat shock protein HSP 90-alpha [Echinococcus granulosus]                        |
| Tmu010897    | CATCAGGATCAGATCAAGG                                        | GCGCTCTACGCTTCTTTGC                                       | Fushi tarazu Antennapedia Ultrabithorax abdominal A [Echinococcus granulosus]   |
| Tmu010896    | CATCAGGATCAGATCAAGG                                        | GCGCTCTACGCTTCTTTGC                                       | Aquaporin-9 [Echinococcus granulosus]                                           |
| Tmu012595    | AGAAGGAAAGCAGGGTGGAGG                                      | GCGCTCTACGCTTCTTTGC                                       | Heat shock cognate protein[Echinococcus granulosus]                             |
| Tmu003182    | AGCCACACGGACGAGGGG                                        | GACACCATTCATTCCTTCC                                       | Heat shock cognate protein [Echinococcus granulosus]                            |
| Tmu005684    | AGCAGAAGGTACAGATCGG                                        | GCACTTCTTGCGCTTTAACA                                      | Heat shock cognate protein [Echinococcus granulosus]                            |
| Tmu010513    | TGTGTTGCTGCTTCTTCTTTG                                     | TGAGTGTTGAGGTTGCAGC                                       | Heat shock cognate protein [Echinococcus granulosus]                            |

**another replicate using cutflinks (27).** The false discovery rate (FDR) was used to determine the threshold P-values in multiple tests for comparing the differential expression. We used \[|\log_{2}(FPKM_{sampe1}/FPKM_{sampe2})| \geq 1 \text{ and FDR} \leq 0.001\] as the threshold to judge the significance of DEGs.

### Functional Classification of DEGs

Functional annotation of DEGs was performed using the UniProt database and BLASTP v2.3.0+ (28) and InterProScan (29) analyses. Gene ontology (GO) enrichment analysis of DEGs was performed using the GOseq R package (v1.10.0).
**RESULTS**

**Summary of RNA-Seq Data and Transcript Assembly**

The seven RNA samples from the developmental stages of *T. multiceps* had prominent 18S and 28S ribosomal peaks on agarose gels (data not shown). All RNA samples possessed high integrity and purity with RINs > 7.0, according to Bioanalyzer 2100 analysis, and were considered of sufficient quality for further experiments. Raw read sequences were filtered to discard adaptors and low-quality reads (error rate > 0.001) from all seven RNA-seq libraries. The clean data from seven RNA-seq libraries with a matching ratio > 97% were mapped to the genome of *T. multiceps* (Table 2). Overall, a total of 42.6 Gb (average 6.1 Gb) Illumina pair-end reads with a 125-bp read length were generated for seven samples, including oncospheres, protoscolices and cyst wall, Imp, Snp, and Grp. A total of 8,208 to 10,498 predicted genes in all samples with FPKM value > 1, 4,396–6,652 genes with FPKM value > 10, and 752 to 1, and 290 genes with FPKM value > 10 were detected (Table 3).

**Identification and Annotation of DEGs in the Developmental Stages and Different Tissues of *T. multiceps***

To comprehensively understand the dynamics of gene expression in *T. multiceps*, we compared the transcriptome data between different life cycle stages and different tissues of adult worms. The total number of DEGs in the various life stages ranged from 2,577 to 3,879; however, for the tissues of the adult worm, the range was from 1,229 to 1,939 (Supplementary Table 1; Figures 1A,B). A total of 2,437 DEGs were selected at different developmental stages (Figure 2A). Of these, 664 were oncosphere stage-specific and upregulated, including oncosphere protein Tm18 (Tmu005436), Tm16 (Tmu007899), 45m (Tmu003810), and fibronectin type III domain-containing protein (Tmu000145), and inhibitor of apoptosis protein (Tmu004095). Four hundred eighteen of the DEGs were protoscolices stage-specific and upregulated, including immunogenic proteins (Tmu003750), neuronal nitric oxide synthase protein inhibitor (Tmu000145), and inhibitor of apoptosis protein (Tmu004095). In addition, 237 of the DEGs were cyst wall-specific and upregulated, including 8 kDa glycoproteins (Tmu005741, Tmu005823), diagnostic antigen gp50 (Tmu00752), and Hsp90

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**TABLE 2 | RNA-seq reads for transcriptomes aligned to the assembled genome of *T. multiceps***

| Sample | Average GC% | Raw reads | Clean reads | Ratio of clean reads | Matching reads | Matching ratio |
|--------|-------------|-----------|-------------|---------------------|---------------|---------------|
| Onc    | 48.5        | 51,022,862| 44,815,122  | 87.83%              | 43,922,159    | 98.0%         |
| Pro    | 47.5        | 51,802,274| 45,942,166  | 88.69%              | 44,568,458    | 97.0%         |
| Cyst   | 47.0        | 47,781,894| 41,028,226  | 85.87%              | 40,039,080    | 97.6%         |
| Adu    | 48.5        | 54,360,816| 49,654,526  | 91.34%              | 49,152,028    | 99.0%         |
| Snp    | 50.0        | 56,183,654| 47,765,165  | 84.99%              | 46,938,444    | 99.0%         |
| Imp    | 49.0        | 49,695,832| 46,823,629  | 94.22%              | 46,338,444    | 99.0%         |
| Grp    | 49.0        | 54,779,876| 47,353,255  | 86.44%              | 46,721,666    | 98.7%         |

**TABLE 3 | Statistics of expression genes in seven RNA-seq samples with FPKM > 1, FPKM > 10, and FPKM > 100***

| Sample | No. of genes | FPKM > 1 | FPKM > 10 | FPKM > 100 |
|--------|--------------|----------|-----------|------------|
| Onc    | 8,208        | 5,545    | 1,197     |            |
| Pro    | 9,169        | 6,347    | 1,248     |            |
| Cyst   | 8,318        | 4,396    | 752       |            |
| Adu    | 10,405       | 6,572    | 1,290     |            |
| Snp    | 8,729        | 5,809    | 1,106     |            |
| Imp    | 10,498       | 6,652    | 1,255     |            |
| Grp    | 8,724        | 5,228    | 1,153     |            |
| Total  | 11,317       | 8,170    | 2,218     |            |

FPKM means fragments per kilobase of exon per million fragments mapped.
In addition, 1,118 of the DEGs were adult-specific and upregulated, including the tegumental antigen (Tmu000235), ornithine aminotransferase (Tmu000506), heat shock protein 70 (Tmu01113), and tetraspanin (TSP) (Tmu000409). These phase-specific upregulated genes were annotated using GO. Furthermore, there were 456 specific upregulated genes during the oncosphere stage that could be annotated to 31 GO terms. Two hundred thirty-four protoscolices stage-specific upregulated genes were annotated to 32 GO terms, 60 cyst wall-specific upregulated genes to 23 GO terms, and 446 adult-specific upregulated genes to 31 GO terms.

A total of 1,228 DEGs were selected from different developmental tissues (Figure 2B). Ninety-seven of the DEGs were Snp tissue-specific and upregulated, including diagnostic antigens (Tmu006941, Tmu012211, and Tmu012074), TSP (Tmu004996), and profoldin (Tmu010836). Eight hundred forty-seven of the DEGs were Imp tissue-specific and upregulated, including Tmu002740 (similar to ubiquitin/40S ribosomal protein S27a fusion protein) and putative thioredoxin-2 (Tmu000756), whereas 284 of the DEGs were Grp tissue-specific and upregulated, such as the fatty acid-binding protein (FABP) 3 (Tmu003178), insulin-like protein (Tmu004894), epidermal growth factor receptor kinase (Tmu008864), and oncosphere...
protein Tm18. These tissue-specific upregulated genes were annotated using GO. The 45 Snp tissue-specific upregulated genes were annotated to 21 GO terms, 328 Imp tissue-specific upregulated genes were annotated to 32 GO terms, and 124 Grp tissue-specific upregulated genes were annotated to 27 GO units.

To gain more insight into the specifically upregulated genes in each stage and tissues of the adult *T. multiceps*, the GO database was used to annotate the functions of these specific and upregulated genes. In summary, GO enrichment of the upregulated DEGs in stage-specific and tissue-specific conditions was analyzed. A total of 456 (68.7%), 234 (56.0%), and 446 (39.9%) specifically upregulated genes were assigned to one or more GO categories for Onc, Pro, and Adu, respectively. A total of 45 (46.4%), 328 (38.7%), and 124 (43.7%) specifically upregulated genes were assigned to one or more GO categories for Snp, Imp, and Grp, respectively, as shown in Figures 2A, B. Notably, the number of DEGs among the different stages was higher than that among the different tissues and was consistent with the extent of the phenotypic differences.

GO classifications enriched in genes for biological process were involved in cellular processes, metabolic processes, pigmentation, and biological regulation; GO classifications enriched in genes for molecular function process were involved in binding, catalytic, and transcription regulation; and GO classifications enriched in genes for the cellular component were involved in the regulation of macromolecular complexes and organelle development (Figure 3). The above observations suggest that the process of development from oncospheres to protoscolices and then to adults involves numerous molecular functions and requires extensive biological processes.
In addition, genes coding for an 8-kDa glycoprotein and the oncosphere proteins Tm16 and Tm18 showed obvious stage-specific variation in expression at different developmental stages of *T. multiceps*. In total, 15 of the 16 genes coding for the 8-kDa glycoprotein were expressed with FPKM > 1 (Supplementary Tables 2, 3).

**DEGs Related to Protease Inhibitor**

A total of 29 protease inhibitor-associated genes were found in the transcriptomes of *T. multiceps* and consisted mostly of a trypsin inhibitor, followed by serine and cysteine protease inhibitors, with varying expression levels in the different developmental stages. A comparative analysis of the gene expression levels between Adu, Pro, and Onc showed that Tmu006090, Tmu006091, and Tmu008198 were upregulated in Onc; Tmu010089, Tmu010102, Tmu004311, Tmu010010, Tmu008761, Tmu005998, Tmu002313, Tmu010090, and Tmu007272 were upregulated in Pro; whereas Tmu007271, Tmu012421, Tmu010103, and Tmu004242 were upregulated in Adu. However, Tmu002208, Tmu007199, Tmu005489, Tmu010101, Tmu003186, Tmu002646, Tmu008502, and Tmu004708 were highly expressed in Adu and Pro, in contrast to Onc (Figure 4).

KEGG pathway analysis indicated that most of the upregulated DEGs were significantly involved in metabolism, genetic information processing, cellular processes, and environmental information processing, such as glutathione metabolism, nitrogen metabolism, endoplasmic reticulum protein processing, endocytosis, and ubiquitin-mediated proteolysis. Furthermore, upregulated DEGs related to the Wnt signaling pathway, the mitogen-activated protein kinase (MAPK) signaling pathway, and the TGF-β signaling pathway were significantly abundant in the adult stage. Those related to inositol phosphate metabolism, citric acid cycle, and adhesion-related genes were significantly abundant in oncospheres, while progesterone-mediated oocyte maturation and oocyte meiosis-related DEGs were significantly abundant in Imp.
DEG Confirmation Using Real-Time PCR

Twenty-eight genes from the DEGs designated Tmu003811 (oncosphere protein Tso31b), Tmu005434 and Tmu012228 (oncosphere protein Tm18), Tmu007899 (oncosphere protein Tm16), Tmu005154 (8 kDa glycoprotein), Tmu003810 (45 m oncosphere protein), Tmu003173 (cytosolic FABP), Tmu000409 (TSP family), Tmu011357 and Tmu012976 (T-Box domain signature), Tmu004894 (insulin-like growth factor), Tmu007808 (heat shock protein) were selected for qRT-PCR analysis to quantify their transcription levels (Figure 5). Of these genes, Tmu003811, Tmu005434, Tmu012228, Tmu007899, Tmu003810, Tmu012421, and Tmu006091 were downregulated in protoscoleces, cyst wall, and the adult stage compared with those in the oncosphere stage, whereas Tmu005042, Tmu003173, and Tmu003193 were upregulated. Meanwhile, Tmu005434, Tmu012228, Tmu003810, and Tmu004894 were upregulated in Grp compared with those in Snp and Imp. The qRT-PCR results of the 28 genes corroborated their RNA-seq results.

DISCUSSION

The life cycle of *T. multiceps* requires two hosts (dog and sheep). When the final host ingests the mature *Coenurus* cysts, *T. multiceps* reaches maturity in its intestine around 40–42 days. Thereafter, the dog starts to disseminate 3–4 proglottids daily, which could contain almost 37,000 eggs each (30). *T. multiceps* eggs are usually discharged/released to the environment through feces from the proglottids (31). When taken up by the intermediate host with contaminated food or water upon reaching the intestine, eggs with hexacanth larvae release oncospheres that penetrate the intestinal wall and migrate toward target organs (usually the brain) through the bloodstream (32). Why oncospheres have a predilection for certain sites, such as muscle, brain, and subcutaneous tissue, is not clear. The larval stage, known as *Coenurus cerebralis*, causes central nervous system disease in sheep, commonly known as coenurosis. The cyst in the brain of sheep is a large, white, translucent structure containing clear fluid with numerous protoscoleces attached to the wall (32). The biology of cestodes
FIGURE 5 | Relative expression levels of the 28 selected DEGs determined using qRT-PCR. (A,B) Confirmation of the DEGs in the developmental stages of *T. multiceps*. (C) Confirmation of the DEGs in different tissues in the adult stage of *T. multiceps*.

is unique in many aspects, especially regarding their unique development, which is difficult to compare to that of other organisms. In the present study, a comprehensive comparative transcriptome analysis of the different developmental stages of *T. multiceps* was carried out for the first time, and 2,437 DEGs were obtained, including oncosphere-specific proteins, immunogenic proteins, diagnostic antigens, heat shock proteins, and tegumental antigens. Podesta and Siddiqui (33) suggested that protein synthesis in flatworm parasites is controlled post-transcriptionally and that these intracellular regulatory mechanisms are activated or suppressed by effectors of the host’s immune response. It is evident that this phase-specific or upregulated gene is important for adapting to parasitic hosts and certain sites. This is similar to our previous study, in which the lifecycle process of the oncosphere, larvae, and adults to adapt to the new host environment is a complex
inhibitor-associated genes were upregulated in the oncosphere, purines, pyrimidines, nucleotides, and lipids. It might also serve ion transport, especially amino acids, hexose sugars, vitamins, cestode tegument contains specific systems for molecular and surface in the same way as waste materials. The complex lack a gut, all nutritive materials must pass through the body as a major site for catalytic activities and contains enzymes of and adult stages; some reports have suggested that the Hsp90 gene controls stage differentiation in *Leishmania donovani* (35). Moreover, the Hsp90 gene has been found to correlate with strobilization in *Echinococcus granulosus* (36). Therefore, we hypothesized that the three Hsp90 genes in *T. multiceps* correlate with strobilization in adult worm proglottids. Our study also revealed that FABP was upregulated in protoscoleces and the cyst wall. Similar to what we observed, Basika et al. (20) showed that FABP transcripts were abundant in *Mesocestoides corti*. FABP is known to participate in fatty acid transport to obtain essential lipids from the host and to modify them according to the parasite’s needs, as cestodes lack the ability to synthesize fatty acids and cholesterol de novo (37). Nie et al. (38) confirmed that FABPs were mainly distributed in the muscle layer in the protoscoleces and cells between the body wall and the parenchyma layer of the cestode in various life stages of *T. multiceps*, suggesting that the FABP is closely related to the formation or development of protoscoleces and their adaptation to a parasitic environment. Of note, the TSP (Tmu000409) gene was significantly upregulated in adults. TSPs have been used as vaccine candidates against schistosomiasis and echinococcosis as diagnostic antigens for cystercrosis (39–42). Tran et al. (43) demonstrated that TSPs in the tegument of schistosomula and adult worms can act as receptors for host ligands, including MHC molecules, allowing parasites to mask their non-self-status and escape host immune responses. Furthermore, Huang et al. (44) identified 11 amino acid sequences in the TSP superfamily of *E. multilocularis*, and the Em-TSP1 homologs were found to be highly expressed in early-stage metacestodes compared with those in non-activated and activated oncospheres; however, the expression level of Em-TSP3 varied within non-activated oncosphere samples. In summary, the stage-specific expression of the TSP gene in *T. multiceps* requires further verification.

In the present study, three, nine, and four protease inhibitor-associated genes were upregulated in the oncosphere, protoscoleces, and adult stage, respectively. Because tapeworms lack a gut, all nutritive materials must pass through the body surface in the same way as waste materials. The complex cestode tegument contains specific systems for molecular and ion transport, especially amino acids, hexose sugars, vitamins, purines, pyrimidines, nucleotides, and lipids. It might also serve as a major site for catalytic activities and contains enzymes of parasitic and possibly of host origin (45, 46). It is well-known that in these developmental stages, the tapeworm must resist the digestive action of gastric acid in the internal environment of the respective hosts to survive; therefore, the protease inhibitor-associated genes mentioned above may play a critical role in protecting them from the host digestion system.

We surprisingly found a highly expressed 8 kDa glycoprotein in Pro; some information is available concerning 8 kDa as a serodiagnosis antigen in *T. solium* cystercerosis (47). In addition, this 8 kDa glycoprotein from *Echinococcus multilocularis* has been identified as a potential diagnostic marker in patients with alveolar echinococcosis (48). We predict that the 8 kDa glycoprotein may be outside for the serodiagnosis of *T. multiceps* coenurosis in the future.

Although KEGG pathways involved in metabolism, genetic information processing, cellular processes, and environmental information processing were identified in the present study, unique pathways were observed in the different stages of *T. multiceps*. For instance, genes associated with the Wnt, MAPK, and TGF-β signaling pathways were significantly abundant in the adult stage, which suggested that the growth and development of the adult tapeworm rely on the above signaling pathways as they play key roles in the regulation of cell growth, differentiation, and tissue and organ formation (49, 50).

**CONCLUSIONS**

In summary, we employed an Illumina RNA-Seq platform to identify DEGs in different developmental stages and tissues of *T. multiceps*. We found that the differential expression of some important genes may contribute to the development of *T. multiceps* and parasite–host interaction.

**DATA AVAILABILITY STATEMENT**

The RNAseq data from this study have been deposited in NCBI’s Sequence Read Archive and are accessible through accession NumberSRRX2357663, SRRX2357664, SRRX2357665, SRRX2357666, SRRX2357667, SRRX2357668, SRRX2357669. https://trace.ncbi.nlm.nih.gov/Traces/sra/?run=SRR5032913.

**ETHICS STATEMENT**

The animal study was reviewed and approved by the Animal Administration and Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Science. Written informed consent was obtained from the owners for the participation of their animals in this study.

**AUTHOR CONTRIBUTIONS**

BF and WHL conceived and designed the study. WHL wrote the manuscript. YY performed the experiments. NZZ analyzed
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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