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Proteomic study of activated *Taenia solium* oncospheres

S. Santivañez¹, A. Hernández-González², N. Chile³, A. Oleaga², Y. Arana³, S. Palma³, M. Verastegui³, A.E. Gonzalez⁴, R. Gilman⁵, H.H. Garcia¹, M. Siles-Lucas^{2,*}, and Cysticercosis Working Group in Peru

¹Cysticercosis Unit, Instituto Nacional de Ciencias Neurológicas, Lima, Peru

²IRNASA, CSIC. Cordel de Merinas, 40-52. 37008, Salamanca, Spain

³Departamento de Microbiología, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru

⁴School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru

⁵Department of International Health, Johns Hopkins University School of Public Health, Baltimore, Maryland, USA

Abstract

Taenia solium cysticerci are a major cause of human seizures and epilepsy in the world. In the gastrointestinal tract of infected individuals, taeniid eggs release the oncospheres, which are then activated by intestinal stimuli, getting ready to penetrate the gut wall and reach distant locations where they transform in cysticerci. Information about oncospherical molecules is scarce, and elucidation of the oncosphere proteome could help understanding the host-parasite relationship during the first steps of infection. In this study, using liquid chromatography and tandem mass spectrometry (LC-MS/MS) analysis, we could identify a set of oncospherical proteins involved in adhesion, protein folding, detoxification and proteolysis, among others. In addition, we have characterized one of the identified molecules, the parasite 14-3-3, by immunoblot and immunolocalization. The identification of these oncospherical proteins represents the first step to elucidate their specific roles in the biology of the host-parasite relationship.

Keywords

Taenia solium; Proteome; Oncospheres; Activated

Introduction

Taenia solium and *T. saginata* are among the most prevalent human tapeworms in the world. The intermediate host of *T. solium* are mainly pigs. Humans act as definitive hosts for the parasite. Gravid segments from humans infected with the adult tapeworm are passed in the faeces each containing around 40,000 eggs that resist destruction in the environment for a relatively long period of time. After ingestion by an intermediate host, oncospheres hatch and activate in the intestine, penetrate the gut and reach different organs via the blood stream,

*Corresponding author: M. Siles-Lucas. IRNASA, CSIC. Cordel de Merinas, 40-52. 37008, Salamanca, Spain. Tel.: 0034923219606; FAX: 0034923219609. mmars.siles@irnasa.csic.es (M. Siles-Lucas).

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developing into cysticerci mainly in striated muscle. Humans are infected when ingesting raw or inadequately cooked pork containing viable cysticerci. The humans may also act as intermediate hosts for the parasite due to the accidental ingestion of *T. solium* eggs via unwashed hands or contaminated food, giving rise to human cysticercosis. While infection of the intestinal tract by adult worms is not life-threatening, infection with larval tapeworms from *T. solium* in man can be very serious and can lead to death when these localize in brain tissue.

Oncospheral hatching and activation have been achieved *in vitro*, showing that activated oncospheres are surrounded by parasite-derived excretory vesicles [1]. Little is known about the molecular crosstalk between taeniid oncospheres and its host when parasites are activated and ready to traverse the intestinal tissue. In this regard, genomic and ESTs studies have been recently reported for adult worms and cysticerci [2,3], but not for oncospheres. Similarly, no proteomic studies on *T. solium* have been performed to date, and thus only individual proteins have been identified and characterized, and from those, the majority have been investigated in cysticerci and adult worms. The cellular events occurring within seconds to minutes after oncospherical activation are probably similar to those found in other eukaryotes upon “activation”, and most probably include changes in intracellular physiology, signalling through kinases and secondary messengers, remodelling of the cytoskeleton and an increase in the overall metabolic rate of the organism [e.g. 4]. In addition, molecules present in the penetration glands of oncospheres, and also those related with adhesion, proteolysis, and sorting-folding of proteins in secreted granules, would be expected to play key roles in the mechanism of oncospherical gut invasion. Oncospheres are the parasite stage determining the infection success in their intermediate host, and thus the identification of proteins from activated oncospheres is the key to understand both the parasite biology and the host-parasite relationship. We initiate here the study of the proteome from *in vitro* activated oncospheres. To achieve this goal, *in vitro* hatched and activated oncospheres of *T. solium* were treated with trypsin and the peptides released were analysed by LC-MS/MS for their identification. In addition, we further characterize one of the parasite proteins –the 14-3-3- found during our proteomic approach.

Materials and methods

Parasites

Taenia solium adult worms fragments (gravid proglottids) were recovered from Peruvian patients' stools as described previously [5]. The proglottids were collected by sieving, washed thoroughly with distilled water and stored in 25% glycerol supplemented with penicillin (1,000 IU/ml) and gentamicin (100 µg/mL) at 4°C until used. Species identification was made by histology and PCR-RFLP as described before [5,6]. The eggs were obtained from gravid proglottids by gentle homogenization (using a manual homogenizer) in water.

Hatching and activation of oncospheres

The eggs obtained from gravid proglottids were washed three times in distilled water prior to hatching. *In vitro* hatching of oncospheres was performed using 0.75% sodium hypochlorite for 10 minutes at 4°C as previously described [5,7]. Immediately after hatching, parasites were activated *in vitro* with an artificial intestinal fluid (1 g pancreatin -Sigma Chemical Co., St. Louis, MO-, 200 mg Na₂CO₃, and 1 ml pig bile, with the volume adjusted to 100 ml with RPMI 1640 medium -pH 8.04) by incubation at 37°C for 1 h [5]. After activation, oncospheres were washed three times in PBS and stored at -20 C in 70% methanol in PBS.

Trypsin digestion of oncospheres

100,000 *T. solium* activated oncospheres frozen in 70% methanol were processed as follows. Trypsin digestion was performed as described before by us [8]. Briefly, oncospheres were washed three times, 1 min per wash, with 50mM NH₄HCO₃ and incubated with 5 ng/µl of

sequencing grade trypsin (Promega) in NH_4HCO_3 at 37°C for 30 min. The supernatant containing the released peptides was then subjected to DTT and iodoacetamide treatment as follows: supernatants were reduced with 10 mM dithiothreitol (DTT) in 50 mM NH_4HCO_3 in a microwave oven for 3 min at 560 W, and alkylated with 55 mM iodoacetamide in 50 mM NH_4HCO_3 for 30 min at room temperature in the dark. The reaction was stopped with 10% trifluoroacetic acid and the supernatants were preserved at -20°C.

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

The resulting peptides from the above-mentioned assay were treated and fractionated as described previously by us [8]. The resulting fractions were analysed in a 4700 Proteomics Analyzer (Applied Biosystems) in positive-reflector mode (2000 shots every position). For the MS/MS analyses, five of the most intense precursors according to the threshold criteria – minimum signal-to-noise 10; minimum cluster area, 500; maximum precursor gap, 200 ppm; maximum fraction gap, 4) – were selected for each position. The MS/MS information was sent to Mascot *via* the GPS Explorer software (Applied Biosystems). A database search was performed on the sequences available at NCBI nr, SwissProt-UniProt, GeneDB (*Schistosoma mansoni* genome), <http://www.nematodes.org> (nematode ESTs), <http://www.sanger.ac.uk/Projects/Echinococcus> (*Echinococcus* ESTs) and a selection of NCBI db on *Taenia*, including *T. solium* adult worm and cysticerci sequences generated in the genome project [2] and the ESTs recently published from cysticerci [3], using the Mascot search engine (Matrix-Science).

Searches were done with tryptic specificity, allowing one missed cleavage and a tolerance in the mass measurement of 100 ppm in MS mode and 0.8 Da for MS/MS ions. Protein identification with confidence scores of >95% were considered significant. When the score was close to the limit a manual inspection of the MS/MS spectra was done in order to accept the identification. The molecular function and biological process were assigned to the identified proteins according to AmiGO and the UniProtKB database (<http://www.uniprot.org>). Finally, a semi-quantitative estimation of the predicted proteins in each trypsin digestion was done using the Exponentially Modified Protein Abundance Index (emPAI) [9].

Immunolocalization and immunoblot

For the immunolocalization and immunoblot of the 14-3-3 protein in *T. solium* oncospheres, a polyclonal serum against the recombinant E14t 14-3-3 protein from *Echinococcus* was used [10,11]. Oncospheres stored at -20°C in 70% methanol in PBS were washed five times with PBS plus 0.1% sodium azide. Re-hydrated oncospheres were then incubated overnight at 4°C with 0.1% sodium azide plus 1% bovine serum albumin (BSA) in PBS (blocking solution). Parasites were further incubated with the anti-14-3-3 serum or serum from a naïve mouse diluted at 1:50 in blocking solution for 3 hours at room temperature. Parasites were then washed four times with 0.05% Tween 20 in PBS and once with PBS, and further incubated with an anti-mouse IgG marked with Alexa Fluor 594 (A11005; Invitrogen) at 1:300 in blocking solution overnight at 4°C. Then, oncospheres were washed as above-described and placed on a microscope slide. The luminescent reaction was studied in a fluorescence microscope with a filter at 594 nm under the 100× objective. Corresponding pictures were done with the program NIS Elements 3.0 (Nikon).

Immunoblot was done as follows: 2 µg of a total extract from activated oncospheres obtained as described before [11] was subjected to polyacrylamide electrophoresis in a 12% gel per duplicate. One extract lane was stained with silver nitrate. The second lane was transferred onto a nitrocellulose membrane as described [10]. The nitrocellulose transferred extract was incubated with 1% BSA in PBS (blocking solution) for 1h 30 min at 37°C. The membrane was then incubated with the anti-14-3-3 serum or a mouse normal serum at 1:100 dilution in

blocking solution for 1h at 37°C. The membrane was then washed five times with 0.05% Tween 20 in PBS and further incubated with an anti-mouse IgG marked with peroxidase (Sigma) diluted at 1:1000 in blocking solution for 1h at 37°C. After washing as above-mentioned, immunoblot was developed by standard procedures with the substrate orthophenylene-diamine.

Results

Appearance and viability of oncospheres after activation

Activated oncospheres (90% of hatched oncospheres) showed a round-shaped appearance, border-located hooks and the absence of the oncospherical membrane (physically disrupted and discarded, as expected), under a microscope at 100× (Figure 1a). In addition, activated parasites occasionally showed the presence of secretory vesicles around them (Fig. 1b). Around 10% of oncospheres showed the outer envelope and were considered as hatched but non-activated parasites (data not shown). Activated oncospheres showed 80% viability evaluated by active movements under a microscope. After trypsin treatment, some of the non-activated oncospheres were still intact, and many of the activated oncospheres were not intact.

Proteins identified in *Taenia solium* activated oncospheres

The analysis of peptides from activated oncospheres yielded 88 identified molecules. From these, 20 had no homology with known proteins, and 3 were contaminations added during oncospherical digestion (pig trypsin; gil136429) and from the manipulation of the material during the proteomic analysis (human keratins gil238054406 and gil239938886). The remaining 65 proteins are listed in Table 1. This set of proteins was classified into seven categories related with their biological function: proteolysis, cytoskeleton-motility-muscle, chromosome segregation-transcription-translation, metabolism-detoxification, signaling, stress-secretion, and biosynthesis-nutrient uptake. This classification was based on the theoretical function assigned to each protein in the AmiGO and UniProtKB databases. In each category, proteins are classified from the highest to the lowest score. Table 1 also includes the number of identified peptides for each match, the EmPAI value, and their classification as putative vaccine candidates and/or modulators based on the results already published by others for *Taenia* and related parasites. In addition, Table 1 indicates the presence or absence of each identified protein in secretions products from *Schistosoma mansoni* cercariae identified before in proteomic studies [12,13]. The first category includes components from the proteosome (VID24, RING finger proteins, ubiquitin and peptidase C19R, all from the ubiquitin pathway) and the lysosome (the cysteine-protease dipeptidyl-peptidase II, the serine-protease antigen 5, and the hydrolase acid phosphatase A). Additionally, a lipoprotein signal peptidase was identified in this group. The second group includes muscle-related (tropomyosin A and B, Tsol22a –troponin T- and troponin I, and paramyosin –antigen B-, and contraction-regulating proteins Ca-binding Sm20-like protein, calmodulin, integrin alpha-7 precursor and myophilin) and cytoskeleton/motor-related (actin 1 and 2, alpha and beta-tubulin, myosin 3, myosin light chain, myosin regulatory light chain, dynein light chain and GCP-2). Proteins related with cell division were grouped in the category number 3, containing histone H4, transcription modulators (HRX, MIER1) one reverse transcriptase (Polyprotein), two ribosomal components (Ts5 60S and 40S), one translation initiation factor (eIF5A), one ribonuclease (XRN2) and one ATPase related with chromosome segregation (ERC-1). Group number 4 comprises proteins related with glycolysis (beta enolase, GAPDH and fructose-bisphosphate aldolase), detoxification (glutathione-S transferase and superoxide dismutase) and ATP generation (cytochrome B, ATP synthase beta and one ATPase inhibitor). Signaling proteins related with cell growth and division were also found in our analysis, including annexins A5 and A6, 14-3-3zeta, macrophage colony stimulating factor 1 receptor, three kinases and the signalling mediator PDZ 8. Stress, mainly protein-folding related polypeptides in group 6 included Tsol-

sHSP35.6, Tsa p36, HSP-70, HSP-90, cyclophilin, alpha-crystallin and the universal stress protein, together with proteins IVY-1 and AAE, related with secretion. Finally, synthesis-related (phosphoenol pyruvate carboxykinase and serine incorporator) and nutrient uptake (aspartate/tyrosine/aromatic aminotransferase) components were classified into group 7. Regarding relative representation of each group in the totality of identified proteins, group 2 (muscle, cytoskeleton and motor-related proteins) were the most represented with 18 identified proteins. Relative representation of the remaining groups, from the most to the least, is: proteolysis (10); chromosome segregation, transcription and translation, and stress-secretion (9 each); signalling and metabolism-detoxification (8 each) and biosynthesis-nutrient uptake (3).

Relative quantification of the proteins identified in activated *Taenia solium* oncospheres

The emPAI index was applied to the identified proteins in each sample. Those with emPAI values <0.1 were disregarded. The obtained results are shown in Figure 2. *T. solium* activated oncospheres protein composition is dominated (53%) by cytoskeleton-motility-muscle-related polypeptides, and specifically by tropomyosins, actins and Tso22a (Troponin T). The next most abundant category is metabolism and detoxification (15%), led by fructose biphosphate aldolase. Less abundant were stress and secretion-related proteins (12%), dominated by cyclophilin and the small heat-shock protein Tso 35.6, chromosome segregation, transcription and translation-related molecules (10%) headed by histone H4, proteolysis (proteasome-related proteins; 5%) and signalling (3%). Remarkably, the less abundant was the biosynthesis-related group.

Immunolocalization and immunoblot of the 14-3-3 protein in *Taenia solium* activated oncospheres

Immunolocalization of the 14-3-3 protein in activated oncospheres showed a specific and intense red reaction around oncospheres (Figure 4a, D, F) No signal was detected when parasites were incubated with a naïve serum (Fig. 4a, B). Immunoblot on a total extract from activated oncospheres using the same anti-14-3-3 anti-serum resulted in the identification of a single band of around 25 kDa (Fig. 4b, 2), compatible with the apparent molecular weight described for 14-3-3 proteins [14]. No reaction was detected with a naïve serum on parasite's total extract in immunoblot (Fig. 4b, 3).

Discussion

In an attempt to give the first picture of the biology of invasion by *T. solium* oncospheres, we have performed a proteomic analysis from *in vitro* activated oncospheres. Our activation approach resulted in a parasite sample consisting of 90% activated oncospheres without oncospherical membrane, together with 10% activated but “unreleased” parasites presenting the oncospherical membrane. Although these were a minority, we cannot rule out that some of the identified proteins are present in the above-mentioned membrane. We have found a set of proteins not described before in *T. solium* oncospheres, with the exception of the oncospherical stage-specific antigen Tso22a [6]. Some others have been specifically characterized in *T. solium* adult worms and cysticerci, including actins [15], antigen 5 [16], paramyosin (AgB) [17], myosin [18], GST [19], SOD [20], cytochrome B [21], and Tsa p36 (crystallin) [22] and other heat-shock proteins [23]. Some of them have shown to play crucial roles in host-parasite relationships, e.g. the vaccine candidate paramyosin [24]. There are, however, several proteins that have been described before in *T. solium* oncospheres and not found here, e.g. TSOL16, 18 and 45 (rev. in [25]). It should be mentioned, nevertheless, that these proteins or their “precursors” in other *Taenia* species were firstly detected due to its immunogenicity, which does not imply high abundance. Thus, the absence of these and other proteins here could be attributed to their low abundance in our extract.

Only few examples of identification and description of individual specific oncospherical proteins are found to date for *T. solium*, thus direct biological comparison with our proteomic data with comparable studies is unfeasible. In spite of the data showing that consecutive parasite life-cycle stages are most likely to share a high percentage of proteins [26], the comparison of our proteome with the relatively comparable data obtained by transcriptomics in *T. solium* cysticerci is still hampered by the lack of further characterization (e.g., localization, biological function, etc.) of the described cysticerci ESTs. *Schistosoma mansoni* cercariae display mechanisms similar to those of oncospheres to infect its vertebrate host and data about their proteome repertoire is ample [12,13,26]. Cercariae penetrate host physiological barriers liberating acetabular glands content including immunomodulators, proteases and cytosolic proteins, molecules that are probably functionally similar to those present in the oncosphere penetration glands. After penetration, both parasites give rise to a permanent migration track, in which parasite surface, secreted and tegumental proteins have been found for cercariae [13]. Due to these similarities in the biology of oncospheres and cercariae, our proteomic data have also been compared here with those reported before for *S. mansoni* cercariae secretions and above-mentioned.

Identified proteins were classified here into several categories regarding their biological function assigned by specific servers. The most striking finding is the concentration of those proteins also found in *S. mansoni* cercarial secretions into two main categories: cytoskeleton-motility-muscle and metabolism-detoxification (20 out of 26). Furthermore, the four most abundant proteins found here in activated oncospheres (Troponin B, actin 2 and 1, and FBA) belong to the above-mentioned groups. These two categories also include the majority of proteins already described as vaccine candidates and modulators of the host-parasite relationship either in taeniids, schistosomes or other parasite helminths, which seems to point out these molecules as those preferentially “seen” by the host during infection. The identification of cytosolic proteins like those related with metabolism and detoxification in cercariae secretions could be attributed to its presence in the secretory glands of these two parasites and presumably holosecreted from these glands. On the other hand, the “secretion” of some of the structural proteins is difficult to explain, although many of these proteins have been repeatedly found in parasites’ secretions, e.g., cyclophilin whose presence in the host-parasite interface could be explained by a high demand for its protein folding activity during gland vesicles content synthesis [27].

Invasion and penetration of host tissue by oncospheres is likely to be mediated by adhesion, proteolytic and modulatory molecules. Here, we have identified three proteins with potential adhesion properties: antigen 5, integrin alpha-7 precursor and AAE protein. The antigen 5 possesses a cell-adhesive motif in its 22 kDa subunit, similar to that present in thrombospondin and in the circumsporozoite protein from *Plasmodium falciparum* [28]. Integrins are transmembrane proteins that mediate interactions between adhesion molecules on adjacent cells and/or the extracellular matrix binding to laminin [29]. A simple touch of some cells including parasitic protozoa to laminin can result in drastic alterations in cellular morphology (e.g., microvilli formation) which is almost always followed by proteolytic activation, e.g. [30]. Regarding this, it has been shown that oncospheres display morphological changes when adhering to cells *in vitro* [1]. The AAE protein has been shown to be involved in adhesion of some pathogenic bacteria to host tissues [31]. Whether these molecules are playing an adhesion role during oncospherical infection could be addressed in the *in vitro* adhesion model described by us before [1].

Proteolytic activity has been detected before in the excretory/secretory products from *T. solium* oncospheres, showing cysteine, serine, and aminopeptidase activities [32]. Nevertheless, none of the proteolytic molecules involved in these activities has been identified to date. Surprisingly, we have identified only two proteins potentially related either with host

tissue digestion or with hatching, namely dipeptidyl-peptidase II (DPPII) and acid phosphatase. The DPPII found here in oncospheres is the first record of a specific serine protease in *T. solium*. This could be used by the parasite either for the degradation of nutrients, immune molecules or structural proteins from the host. It has been described that DPPII degrades collagen [33], thus a possible role for DPPII in the degradation of host tissue to facilitate oncospherical gut penetration could be hypothesized. This is reinforced by the reports of DPPII as a secreted molecule (included in secretory granules) in some organisms, suggesting extracellular functions for this lysosomal protease –rev. in [33]–. Antigen 5, highly similar to other members of the trypsin family, has been also grouped within the proteolysis group here. Nevertheless, it should be pointed out that the homologous Ag5 from *Echinococcus* presents a substitution in the catalytic triad that makes it inactive as protease, which includes Ag5 in a unique group of inactive serine proteases homologues to snake venom proteins and known for displaying allergen immunomodulatory properties [34]. Intriguingly, venom-like proteins have been also found in cercariae secretions [13], thus its functions in *T. solium* oncospheres merit further investigations.

Modulation of the host responses is crucial for the highly labile oncospherical stage as well. We have already mentioned that several molecules found here have been related with immunomodulation in taeniids or in related parasites, e.g. antigen 5, paramyosin and tropomyosin. Paramyosin is itself very immunogenic and has been proposed to protect cercariae from immune attack by “decoy” binding proteins of the complement pathway, and believed to be released from acetabular (secretion) glands [13]. Similarly, immunomodulation by oncospheres could be mediated by this and additional molecules. Additionally, a group of “modulatory” molecules related with infection and pathogenesis in infectious agents have been also found during our proteomic study. These include mainly glycolytic enzymes that, once on the host-parasite interface, could interact with host components, such as fibronectin and plasminogen, or interact directly with the host cells, to trigger signal transduction and thereby enable the pathogen to colonize, persist and invade the host tissue [35].

An additional set of relatively abundant proteins found here are those related with the sorting and degradation of unneeded or damaged proteins. In response to cellular stress, heat shock proteins (HSPs) target misfolded proteins for proteasomal degradation. These HSPs have been implicated in increasing activity of the ubiquitin-proteasome system, e.g. [36], matching with our finding in AO of five components from this system plus five HSPs.

The least represented group was that including biosynthesis and nutrient uptake-related molecules. It has been suggested that like some trematode cercariae, the oncospheres are non-feeding during the hexacanth penetration phase [37]. This, together with our results, could indicate a quiescent stage regarding “feeding” for the oncospheres during the first steps of infection.

Signaling proteins identified here included the 14-3-3 ζ molecule. This has been found to be present in soluble oncospherical extracts from a related parasite (*Echinococcus multilocularis*) and playing a key role in the biology of this and other parasites [38,39]. This, together with the availability of specific tools e.g., antibodies, prompted us to perform a preliminary characterization of the homologous protein in *T. solium* oncospheres. As mentioned, the 14-3-3 protein identified in activated oncospheres by LC-MS/MS belongs to the zeta isoform. It has been found that the same 14-3-3 isoform in different organisms presents a higher conservation than different 14-3-3 isoforms from the same organism [40]. Accordingly, antisera raised against a specific 14-3-3 isoform cross-react with this isoform regardless the organism from which the 14-3-3 protein is investigated, e.g. [41]. Here, we have used a mouse hyperimmune serum obtained after immunization with the E14t (*Echinococcus* 14-3-3 zeta) recombinant protein [10,11] for the localization and immunoblot characterization of the corresponding

14-3-3 zeta protein in *T. solium* oncospheres. This resulted in the detection of a specific band in activated oncospherical extracts with an apparent molecular weight of around 25 kDa, similar to that found before in *Echinococcus* oncospherical extracts [38]. The same antiserum showed to be reactive at the surface of activated *T. solium* oncospheres. The 14-3-3 proteins are cytosolic molecules, thus our findings showing its surface location in oncospheres was not expected. Nevertheless, paradoxical location of several cytosolic and structural proteins has been described in other parasites. One classical example of this is the finding of the vaccine candidate paramyosin in the surface of several parasites, e.g., *Fasciola hepatica* [42]. Similarly, the 14-3-3 protein has been found in secretions from *E. granulosus* and *S. japonicum* adult worms [10,43] and from *S. mansoni* schistosomulae [44], as well as in the tegument of *F. gigantica* adult worms [45]. Whether and how the 14-3-3 detected here gets to the host-parasite interface during infection should be further investigated.

In conclusion, the experimental approach used in this work affords valuable information on the protein composition from *T. solium* oncospheres. A range of oncospherical proteins was identified, most of which had not been identified previously in this parasite stage. The new information obtained here provides clues of the mechanisms of gut penetration and immune evasion that could aid to define the crosstalk between activated oncospheres and its host during the first steps of infection.

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References

1. Verastegui M, Gilman RH, Arana Y, Barber D, Velásquez J, Farfán M, Chile N, Kosek JC, Kosek M, Garcia HH, Gonzalez A, Cysticercosis Working Group in Peru. *Taenia solium* oncosphere adhesion to intestinal epithelial and Chinese hamster ovary cells *in vitro*. *Infect Immun* 2007;75:5158–66. [PubMed: 17698575]
2. Aguilar-Díaz H, Bobes RJ, Carrero JC, Camacho-Carranza R, Cervantes C, Cevallos MA, Dávila G, Rodríguez-Dorantes M, Escobedo G, Fernández JL, Frago G, Gaytán P, Garciarubio A, González VM, González L, José MV, Jiménez L, Laclette JP, Landa A, Larralde C, Morales-Montor J, Morett E, Ostoa-Saloma P, Sciutto E, Santamaría RI, Soberón X, de la Torre P, Valdés V, Yáñez J. The genome project of *Taenia solium*. *Parasitol Int* 2006;55:S127–30. [PubMed: 16337432]
3. Almeida CR, Stoco PH, Wagner G, Sincero TC, Rotava G, Bayer-Santos E, Rodrigues JB, Sperandio MM, Maia AA, Ojopi EP, Zaha A, Ferreira HB, Tyler KM, Dávila AM, Grisard EC, Dias-Neto E. Transcriptome analysis of *Taenia solium* cysticerci using Open Reading Frame ESTs (ORESTES). *Parasit Vectors* 2009;31:35–46. [PubMed: 19646239]
4. Whitaker M. Calcium at fertilization and in early development. *Physiol Rev* 2006;86:25–88. [PubMed: 16371595]
5. Mayta H, Hancock K, Levine MZ, Gilman RH, Farfán MJ, Verastegui M, Lane WS, Garcia HH, Gonzalez AE, Tsang VC, Cysticercosis Working Group in Peru. Characterization of a novel *Taenia solium* oncosphere antigen. *Mol Biochem Parasitol* 2007;156:154–61. [PubMed: 17850901]
6. Verastegui M, Gilman RH, Garcia HH, Gonzalez AE, Arana Y, Jeri C, Tuero I, Gavidia CM, Levine M, Tsang VC, Cysticercosis Working Group in Peru. Prevalence of antibodies to unique *Taenia solium* oncosphere antigens in taeniasis and human and porcine cysticercosis. *Am J Trop Med Hyg* 2003;69:438–44. [PubMed: 14640505]
7. Stevenson P. Observations on the hatching and activation of fresh *Taenia saginata* eggs. *Ann Trop Med Parasitol* 1983;77:399–404. [PubMed: 6685462]

8. Pérez-Sánchez R, Valero ML, Ramajo-Hernández A, Siles-Lucas M, Ramajo-Martín V, Oleaga A. A proteomic approach to the identification of tegumental proteins of male and female *Schistosoma bovis* worms. *Mol Biochem Parasitol* 2008;161:112–23. [PubMed: 18638509]
9. Ishihama Y, Oda Y, Tabata T, Sato T, Nagasu T, Rappsilber J, Mann M. Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. *Mol Cell Proteomics* 2005;4:1265–72. [PubMed: 15958392]
10. Siles-Lucas M, Nunes CP, Zaha A, Breijo M. The 14-3-3 protein is secreted by the adult worm of *Echinococcus granulosus*. *Parasite Immunol* 2000;22:521–8. [PubMed: 11012978]
11. Siles-Lucas M, Merli M, Mackenstedt U, Gottstein B. The *Echinococcus multilocularis* 14-3-3 protein protects mice against primary but not secondary alveolar echinococcosis. *Vaccine* 2003;21:431–9. [PubMed: 12531641]
12. Knudsen GM, Medzihradsky KF, Lim KC, Hansell E, McKerrow JH. Proteomic analysis of *Schistosoma mansoni* cercarial secretions. *Mol Cell Proteomics* 2005;4:1862–75. [PubMed: 16112986]
13. Hansell E, Braschi S, Medzihradsky KF, Sajid M, Debnath M, Ingram J, Lim KC, McKerrow JH. Proteomic analysis of skin invasion by blood fluke larvae. *PLoS Negl Trop Dis* 2008;16:1–11.
14. Aitken A. 14-3-3 proteins: a historic overview. *Semin Cancer Biol* 2006;16:162–72. [PubMed: 16678438]
15. Campos A, Bernard P, Fauconnier A, Landa A, Gómez E, Hernández R, Willms K, Laclette JP. Cloning and sequencing of two actin genes from *Taenia solium* (Cestoda). *Mol Biochem Parasitol* 1990;40:87–93. [PubMed: 2348833]
16. Liu D, Rickard MD, Lightowlers MW. Analysis of taeniid antigens using monoclonal antibodies to *Echinococcus granulosus* antigen 5 and antigen B. *Parasitol Res* 1993;79:82–5. [PubMed: 7682330]
17. Laclette JP, Landa A, Arcos L, Willms K, Davis AE, Shoemaker CB. Paramyosin is the *Schistosoma mansoni* (Trematoda) homologue of antigen B from *Taenia solium* (Cestoda). *Mol Biochem Parasitol* 1991;44:287–95. [PubMed: 2052029]
18. Cruz-Rivera M, Reyes-Torres A, Reynoso-Ducoing O, Flisser A, Ambrosio JR. Comparison of biochemical and immunochemical properties of myosin II in taeniid parasites. *Cell Biol Int* 2006;30:598–602. [PubMed: 16733094]
19. Vibanco-Pérez N, Jiménez L, Merchant MT, Landa A. Characterization of glutathione S-transferase of *Taenia solium*. *J Parasitol* 1999;85:448–53. [PubMed: 10386436]
20. González R, Mendoza-Hernández G, Plancarte A. Purification of *Taenia solium* cysticerci superoxide dismutase and myoglobin copurification. *Parasitol Res* 2002;88:881–7. [PubMed: 12209327]
21. Nakao M, Sako Y, Ito A. The mitochondrial genome of the tapeworm *Taenia solium*: a finding of the abbreviated stop codon U. *J Parasitol* 2003;89:633–5. [PubMed: 12880275]
22. Benitez L, Harrison LJ, Parkhouse RM, Garate T. Sequence and preliminary characterisation of a *Taenia saginata* oncosphere gene homologue of the small heat-shock protein family. *Parasitol Res* 1998;84:423–5. [PubMed: 9610643]
23. Vargas-Parada L, Solís CF, Laclette JP. Heat shock and stress response of *Taenia solium* and *T. crassiceps* (Cestoda). *Parasitology* 2001;122:583–8. [PubMed: 11393832]
24. Vázquez-Talavera J, Solís CF, Terrazas LI, Laclette JP. Characterization and protective potential of the immune response to *Taenia solium* paramyosin in a murine model of cysticercosis. *Infect Immun* 2001;69:5412–6. [PubMed: 11500411]
25. Lightowlers MW. Vaccines for prevention of cysticercosis. *Acta Trop* 2003;87:129–35. [PubMed: 12781387]
26. Curwen RS, Ashton PD, Johnston DA, Wilson RA. The *Schistosoma mansoni* soluble proteome: a comparison across four life-cycle stages. *Mol Biochem Parasitol* 2004;138:57–66. [PubMed: 15500916]
27. Curwen RS, Ashton PD, Sundaralingam S, Wilson RA. Identification of novel proteases and immunomodulators in the secretions of schistosome cercariae that facilitate host entry. *Mol Cell Proteomics* 2006;5:835–44. [PubMed: 16469760]
28. Lorenzo C, Salinas G, Brugnini A, Wernstedt C, Hellman U, González-Sapienza G. *Echinococcus granulosus* antigen 5 is closely related to proteases of the trypsin family. *Biochem J* 2003;369:191–8. [PubMed: 12358601]

29. Takagi J. Structural basis for ligand recognition by integrins. *Curr Opin Cell Biol* 2007;19:557–64. [PubMed: 17942298]
30. Avila EE, Calderon J. *Entamoeba histolytica* trophozoites: a surface-associated cysteine protease. *Exp Parasitol* 1993;76:232–241. [PubMed: 8500583]
31. Rose JE, Meyer DH, Fives-Taylor PM. Aae, an autotransporter involved in adhesion of *Actinobacillus actinomycetemcomitans* to epithelial cells. *Infect Immun* 2003;71:2384–93. [PubMed: 12704108]
32. Zimic MJ, Infantes J, López C, Velásquez J, Farfán M, Pajuelo M, Sheen P, Verastegui M, Gonzalez A, Garcia HH, Gilman RH. Comparison of the peptidase activity in the oncosphere excretory/secretory products of *Taenia solium* and *Taenia saginata*. *J Parasitol* 2007;93:727–34. [PubMed: 17918349]
33. Maes MB, Scharpé S, De Meester I. Dipeptidyl peptidase II (DPPII), a review. *Clin Chim Acta* 2007;380:31–49. [PubMed: 17328877]
34. Fry BG, Roelants K, Norman JA. Tentacles of venom: toxic protein convergence in the Kingdom Animalia. *J Mol Evol* 2009;68:311–21. [PubMed: 19294452]
35. Pancholi V, Chhatwal GS. Housekeeping enzymes as virulence factors for pathogens. *Int J Med Microbiol* 2003;293:391–401. [PubMed: 14760970]
36. Garrido C, Brunet M, Didelot C, Zermati Y, Schmitt E, Kroemer G. Heat shock proteins 27 and 70: anti-apoptotic proteins with tumorigenic properties. *Cell Cycle* 2006;5:2592–601. [PubMed: 17106261]
37. Smyth, JD.; McManus, DP. *The Physiology and Biochemistry of Cestodes*. Upd Sub. Cambridge University Press; 1989.
38. Siles-Lucas M, Gottstein B. The 14-3-3 protein: a key molecule in parasites as in other organisms. *Trends Parasitol* 2003;19:575–81. [PubMed: 14642768]
39. Siles-Lucas M, Merli M, Gottstein B. 14-3-3 proteins in *Echinococcus*: their role and potential as protective antigens. *Exp Parasitol* 2008;119:516–23. [PubMed: 18316081]
40. Wang W, Shakes DC. Molecular evolution of the 14-3-3 protein family. *J Mol Evol* 1996;43:384–98. [PubMed: 8798343]
41. Siles-Lucas M, Felleisen RS, Hemphill A, Wilson W, Gottstein B. Stage-specific expression of the 14-3-3 gene in *Echinococcus multilocularis*. *Mol Biochem Parasitol* 1998;91:281–93. [PubMed: 9566521]
42. Cancela M, Carmona C, Rossi S, Frangione B, Goñi F, Berasain P. Purification, characterization, and immunolocalization of paramyosin from the adult stage of *Fasciola hepatica*. *Parasitol Res* 2004;92:441–8. [PubMed: 14963769]
43. Liu F, Cui SJ, Hu W, Feng Z, Wang ZQ, Han ZG. Excretory/secretory proteome of the adult developmental stage of human blood fluke, *Schistosoma japonicum*. *Mol Cell Proteomics* 2009;8:1236–51. [PubMed: 19299421]
44. El Ridi R, Tallima H. *Schistosoma mansoni* ex vivo lung-stage larvae excretory-secretory antigens as vaccine candidates against schistosomiasis. *Vaccine* 2009;27:666–73. [PubMed: 19056448]
45. Chaithirayanon K, Grams R, Vichasri-Grams S, Hofmann A, Jorge G, Viyanant V, Upatham ES, Sobón P. Molecular and immunological characterization of encoding gene and 14-3-3 protein 1 in *Fasciola gigantica*. *Parasitology* 2006;133:763–75. [PubMed: 16938151]

Figure 1.

Representative microscopical images (100×) of activated *Taenia solium* oncospheres. The absence of envelopes (1a, b) and the presence of secretory vesicles (1b) can be seen. H, hooks; SV, secretory vesicles. Bars = 22 μ m.

Figure 2.

Comparative percentage of the relative abundance of *Taenia solium* activated oncospheres proteins calculated with the Exponentially Modified Protein Abundance Index (emPAI) and classified into seven functionally-related groups. The figure includes the total percentage of abundance for each functional group, as well as the abundance emPAI number and name of each protein, from the most to the least abundant, in the different groups.

Figure 3.

Immunolocalization and immunoblot of the 14-3-3 zeta protein in *Taenia solium* activated oncospheres. 3a. The mouse anti-14-3-3z serum reacted with the surface of activated oncospheres (D, F). No reactivity was found on oncospheres incubated with a negative mouse serum (B). Images A, C and E show the corresponding images from B, D and F, respectively, under visible light. ES, egg shell; H, hooks. Bars = 10 μ m. 3b. The same anti-14-3-3 serum reacted with a band of around 25 kDa by immunoblot in a total extract from activated oncospheres (2). The negative serum showed no reactivity at that molecular weight (3). The banding pattern from the parasite total soluble extract in a 12% polyacrylamide gel stained with silver nitrate is showed in (1). Molecular weight markers are indicated in the left of the figure, in kDa.

Table 1
Proteins from activated *Taenia solium* oncospheres identified by LC-MS/MS and classified regarding their biological function.

Family (name)	Process	Database ID nb.	Species	Score	NP	EmPAI	CS	VC, M
1. PROTEOLYSIS								
RING finger E3	Ubiquitin-protein ligase	gil129291298	<i>Taenia solium</i> (CY)	125	2	0.38		
Ubiquitin	Ubiquitination of proteins	gil129300688	<i>Taenia solium</i> (AD)	63	2	0.32	X	
VID24	Vaculolar import and degradation	sm_m21-38a02.q1ka	<i>Schistosoma mansoni</i>	53	3	0.14		
RING finger 121	Ubiquitin-protein ligase	sm_m21-17d01.q1ka	<i>Schistosoma mansoni</i>	47	2	--		
Peptidase C19R	Ubiquitin recycling	sm_r28-1b02.p1k	<i>Schistosoma mansoni</i>	44	2	--		
Dipeptidyl-peptidase II	Serine protease	SmlA20g02.p1k	<i>Schistosoma mansoni</i>	44	2	--		
LP signal peptidase	Aspartic protease	gil129285694	<i>Taenia solium</i> (CY)	42	2	--		
Antigen 5 precursor	Trypsin-like protease	EGPSPsl-16e05.p1k	<i>Echinococcus granulosus</i>	39	2	--		M
Acid phosphatase A	Hystidine acid phosphatase	sm_m28-12c11.q1ka	<i>Schistosoma mansoni</i>	39	2	--		
RING finger 11 (E3)	Ubiquitin-protein ligase	sm_m28-32g09.q1k	<i>Schistosoma mansoni</i>	38	2	--		
2. CYTOSKELETON, MOTILITY, MUSCLE								
Tropomyosin B	Muscle contraction	gil129298423	<i>Taenia solium</i> (AD)	474	16	2.03	X	VC, M
Actin 2	Cell motility	gil129295292	<i>Taenia solium</i> (AD)	432	8	2.00	X	
Actin 1 cytoplasmic	Cell motility	gil129299103	<i>Taenia solium</i> (AD)	371	7	1.66		
Tropomyosin A	Muscle contraction	gil129304455	<i>Taenia solium</i> (AD)	266	9	0.53	X	VC, M
Tsol22a (Troponin T)	Muscle contraction (thin filaments)	gil129283950	<i>Taenia solium</i> (CY)	264	6	0.86	X	VC
Troponin I	Muscle contraction	gil129284722	<i>Taenia solium</i> (CY)	113	6	0.26		
Dynein light chain	Motor protein	gil129283471	<i>Taenia solium</i> (CY)	83	2	0.35	X	VC
Myophilin	Actin-binding, regulating cell shape	gil129302386	<i>Taenia solium</i> (AD)	82	3	0.30		
Ca-binding Sm20-like	Muscle contraction	gil129298785	<i>Taenia solium</i> (AD)	64	2	--	X	VC
Paramyosin (Ag B)	Muscle component (thick filaments)	gil129293906	<i>Taenia solium</i> (AD)	58	3	0.27	X	VC, M
Calmodulin	Calcium trafficking at the sarcoplasm	gil129287777	<i>Taenia solium</i> (CY)	54	3	--	X	
Beta-tubulin	Major component of microtubules	sm_m21-32d12.q1ka	<i>Schistosoma mansoni</i>	54	2	--	X	
Myosin 3	Actin-dependent molecular motor	SmlA8e03.p1k	<i>Schistosoma mansoni</i>	51	2	--	X	
Myosin light chain	Actin-dependent molecular motor	gil129284301	<i>Taenia solium</i> (CY)	49	2	--	X	

Family (name)	Process	Database ID nb.	Species	Score	NP	EmPAI	CS	VC, M
Myosin regulatory LC	Actin-dependent molecular motor	gil129283561	<i>Taenia solium</i> (CY)	48	1	0.37	X	
GCP-2 (SPC97/98)	Tubulin-associated protein	sm_m21-51a08.q1ka	<i>Schistosoma mansoni</i>	46	2	--		
Integrin α -7 precursor	Maintain myofibers architecture	ITA7_MOUSE	<i>Mus musculus</i>	42	2	--		
Alpha-tubulin	Major component of microtubules	gil129293466	<i>Taenia solium</i> (AD)	40	2	--	X	

3. CHROMOSOME SEGREGATION, TRANSCRIPTION, TRANSLATION

Histone H4	Core component of nucleosome	gil129306056	<i>Taenia solium</i> (AD)	63	3	0.94	X	
Ts5 60S	Ribosomal subunit component 60s	gil129295561	<i>Taenia solium</i> (AD)	59	2	0.44		
40S	Ribosomal subunit component 40s	gil129293421	<i>Taenia solium</i> (AD)	54	3	--		
HRX	Transcription modulation	HRX_MOUSE	<i>Mus musculus</i>	48	2	--		
MIER1	Transcriptional repressor	sm_m21-48d06.q1ka	<i>Schistosoma mansoni</i>	46	3	--		
Polyprotein	RNA-directed DNA polymerase	SmlA21d11.p1k	<i>Schistosoma mansoni</i>	43	3	--		
XRN2	5'-3' exoribonuclease	XRN2_MOUSE	<i>Mus musculus</i>	40	3	--		
ERC-1	ATPase, chromosome segregation	sm_m21-23g03.q1ka	<i>Schistosoma mansoni</i>	40	2	--		
eIF5A	Translation initiation, eIF5A	Emme68g03.p1k	<i>Echinococcus multilocularis</i>	39	2	--		

4. METABOLISM, DETOXIFICATION

FBA	Glycolysis	gil129285069	<i>Taenia solium</i> (CY)	205	5	1.00	X	VC, M
Beta enolase	Glycolysis	gil129293204	<i>Taenia solium</i> (AD)	106	4	0.39	X	M
GAPDH	Glycolysis	gil129291487	<i>Taenia solium</i> (CY)	63	2	0.37	X	M
ATPase inhibitor	ATP content regulation	gil129285665	<i>Taenia solium</i> (CY)	56	1	0.44		
GST26	Detoxification	EGCW-gr-6h05.q1k	<i>Echinococcus granulosus</i>	52	2	--	X	VC, M
SOD	Detoxification	Emme_24b11.p1k	<i>Echinococcus multilocularis</i>	43	3	0.31	X	VC, M
Cytochrome B	ATP generation	sm_m21-21d08.q1k	<i>Schistosoma mansoni</i>	42	2	--	X	
ATP synthase B	ATP synthesis	AT5F1_MOUSE	<i>Mus musculus</i>	40	4	--	X	

5. SIGNALING

Annexin A5	Signaling	gil129292673	<i>Taenia solium</i> (CY)	153	5	0.26		
14-3-3z	Intracellular signaling	Emme_39a08.p1k	<i>Echinococcus multilocularis</i>	50	4	--	X	VC

Family (name)	Process	Database ID nb.	Species	Score	NP	EmPAI	CS	VC, M
KIN2	Kinase	gil129284541	<i>Taenia solium</i> (CY)	50	3	--		
Annexin A6	Signaling	gil129298595	<i>Taenia solium</i> (AD)	49	2	--		
MCSF1 receptor	Growth, differentiation	CSFIR_DANRE	<i>Danio rerio</i>	44	2	--		
Kinase	Kinase	gil129302182	<i>Taenia solium</i> (AD)	44	2	--		
PDZ 8	Signaling mediator	gil129288918	<i>Taenia solium</i> (CY)	49	2	0.30	X	
PKB/Akt	Division, apoptosis, migration	sm_r28-25e07.q1k	<i>Schistosoma mansoni</i>	41	2	--		

6. STRESS, SECRETION

Tsol-sHSP35.6	ATP-independent protein folding	gil129295401	<i>Taenia solium</i> (AD)	117	2	0.51		
Cyclophilin (PPIase)	Protein folding	gil129301824	<i>Taenia solium</i> (AD)	103	3	0.76	X	
Tsa p36 (crystallin)	ATP-independent protein folding	TSP36_TAESA	<i>Taenia saginata</i>	68	2	0.17		
HSP-70	ATP-dependent protein folding	gil129290786	<i>Taenia solium</i> (CY)	59	2	0.33	X	VC, M
IVY-1	Vacuolar fusion	gil129302298	<i>Taenia solium</i> (AD)	55	2	0.26		
Universal stress protein	Stress endurance USP	sm_r28-20a02.q1k	<i>Schistosoma mansoni</i>	52	2	--		
HSP-90	ATP-dependent protein folding	gil187233130	<i>Taenia solium</i> (CY)	50	2	--	X	
AAE	Protein transport and secretion	sm_r28-27a01.q1k	<i>Schistosoma mansoni</i>	39	2	--		
Alpha crystallin	ATP-independent protein folding	sm_m28-18h03.q1ka	<i>Schistosoma mansoni</i>	38	2	--		

7. BIOSYNTHESIS AND NUTRIENT UPTAKE

PEPCK	Gluconeogenesis	gil129291584	<i>Taenia solium</i> (CY)	68	3	0.28	X	
Serine	Synthesis of sphingolipids	SmlA15e03.p1k	<i>Schistosoma mansoni</i>	39	2	--		
TyrB	Fatty acids uptake	SmlA50h04.p1k	<i>Schistosoma mansoni</i>	38	2	--		

LP, lipoprotein; LC, light chain; GCP2, gamma-tubulin complex component 2; HRX, zinc finger protein; MIER1, mesoderm induction early response 1 related protein; XRN2, 5'-3' exonuclease 2; ERC-1, RAB6-interacting protein 2; eIF5A, eukaryotic translation initiation factor 5A; FBA, fructose biphosphate aldolase; GAPDH, glyceraldehyde phosphate dehydrogenase; GST26, glutathione-S-transferase 26 kDa; SOD, superoxide dismutase; MCSF1, macrophage colony stimulating factor; PDZ, phorbol-ester/DAG-type zinc finger motif containing protein; PKB, protein kinase B; HSP, heat shock protein; PPIase, peptidyl-prolyl isomerase; IVY, interaction with VPS33 and YPT7 protein; AAE, autotransporter adhesion to epithelial cells protein; PEPCK, phosphoenol pyruvate carboxykinase; Serine, serine incorporator; TyrB, aspartate/tyrosine/aromatic aminotransferase.

NP, Number of matching peptides. CS, proteins described in *Schistosoma mansoni* cercarial secretions. VC, Vaccine candidate. M, modulatory parasite molecule.

AD, sequences identified in the genome project of *T. solium* adult worms. CY, sequences identified in the genome project or in the transcriptome of *T. solium* cysticerci.