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Comparative proteome profiling of hydatid fluid from Algerian patients reveals cyst location-related variation in *Echinococcus granulosus*

Razika Zeghir-Bouteldja[a,b], Andy Polomé[c], Sabrina Bousbata[c], Chafia Touil-Boukoffa[a,*]

[a] Laboratory of Cellular and Molecular Biology, Team 'Cytokines and NO Synthases' Faculty of Biological Science University of Sciences and Technology Houari Boumediene, USTHB, PB 32 El-Alia, Algiers 16111, Algeria
[b] Department of Biological Science, Akli Mohand Oulhadj University, Bouira, Algeria
[c] Proteomic Platform, Microbiology Laboratory, Department of Molecular Biology, Université Libre de Bruxelles, Belgium

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ABSTRACT

Human cystic echinococcosis, an endemic zoonosis in Algeria, is caused by larvae of the cestode *Echinococcus granulosus*. Parasitic modulation of the immune response allows *E. granulosus* to persist in intermediate hosts. Previous *in vitro* and *in vivo* immunological studies have shown differences in host immune responses according to the status and location of the hydatid cysts in the body. In this study, a proteomic analysis of human hydatid fluids was performed to identify the proteins in hydatid cyst fluids. Hydatid fluid was obtained after cystic surgical removal from three patients with these cysts. The study was conducted on fertile hydatid fluids from lungs, vertebra, and infertile paravertebral fluids. Comparisons of the protein compositions of these fluids revealed differences in their protein profiles. These differences are probably related to the cyst location and fertility status of the parasite. Notably, our analysis identified new proteins from the parasite and human host. The identification of host proteins in hydatid fluids indicates that the hydatid walls are permeable allowing a high protein exchange rate between the metacestode and the affected tissue. Interestingly, our study also revealed that parasite antigenic protein expression variations reflect the differences observed in host immunostimulation.

1. Introduction

Human echinococcosis is a parasitic disease caused by *Echinococcus granulosus* metacestodes. It is characterized by long-term growth of the larval stage in the intermediate host (Zhang et al., 2003). It constitutes a major health problem in North Africa, particularly in Algeria. The annual incidence is 1.11/100,000 Algerian population. *Echinococcus granulosus* is a small tapeworm that lives firmly attached to the mucosa of the small intestine in their definitive hosts (e.g. dogs and wild carnivores).It grows into adult stage. The shedding of gravid proglottids resulting into eggs production in the feces occurs within 4–6 weeks after infection of the definitive host. Ingestion of eggs by intermediate host animals such as sheep or human results in the release of an oncosphere into the gastrointestinal tract, which travels via blood or lymph to reach the most frequent organs the liver and lungs, and less frequent in spleen, soft tissues, bones, breast, heart and spinal extradural space where cystic development begins (Farmer et al., 1990; Zhang et al., 2003; Siracusano et al., 2012a,b). In most cases, the larvae remain viable within their host for many years despite the host's immune response. The hydatid cyst is unilocular, filled with hydatid fluid which might be fertile or sterile. Fertile cysts contain protoscoleces parasites produced by the germinal layer via asexual reproduction. The cyst is entirely covered by a laminated layer with an acellular carbohydrate-rich surface of variable thickness, which protects the parasite from the immunological and physiological reactions occurring in the host (Diaz et al., 2011). Hydatid fluid is a complex mixture of excretory and secretory products derived from protoscoleces and the germinal layer which confers antigenic properties to the parasite (Siracusano et al., 2012a,b). Parasite molecules play an important role in the chronic establishment of the infection and interfere with the functional activity of the host's immune cells (Hewitson et al., 2009). *E. granulosus* has evolved a range of strategies to impair the host immune

Abbreviations: *E. granulosus*, *Echinococcus granulosus*; *E. multilocularis*, *Echinococcus multilocularis*; PHF, pulmonary hydatid fluid; VHF, vertebral hydatid fluid; PVHF, paravertebral hydatid fluid; ESP, excretory/secretory products; *S. kowalevskii*, *Saccoglossus kowalevskii*; *D. dendriticum*, *Dicrocoelium dendriticum*

* Corresponding author at: University of Sciences and Technology Houari Boumediene, Faculty of Biological Science, Laboratory of Cellular and Molecular Biology, Team 'Cytokines and NO Synthases', PB 32 El-Alia, Algiers, 16111, Algeria.
E-mail addresses: bouteldja_raz@yahoo.fr (R. Zeghir-Bouteldja), andy.polome@ulb.ac.be (A. Polomé), sabrina.bousbata@ulb.ac.be (S. Bousbata), touilboukoffa@yahoo.fr (C. Touil-Boukoffa).

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response, such as antigenic variation, protease production, immunosuppression, and skewing of the Th1/Th2 cytokine profile (Touil-Boukoffa et al., 1997, 1998; Zhang et al., 2008; Mezioug and Touil-Boukoffa, 2009).

In 2013, Zheng et al., sequenced the genome of *E. granulosus* revealing new genes which products are essential for host interaction and immune system perversion opening the field for new therapeutic targets which may help for future antihelmintic drug development (Zheng et al., 2013). The post-genomic studies were conducted and based on proteomic characterization of the different development stages of *E. granulosus*. Chemale et al. (2003). This team was the first to identify protoscoleces and hydatid fluid proteins by coupling two-dimensional gel electrophoresis and mass spectrometry. This work was completed by Monteiro et al. (2010) who identified proteins from the components of *E. granulosus* metacestode (protoscolex, germinal layer and hydatid cyst fluid) from bovine as well as host proteins in association with the hydatid cyst revealing new insights into parasite-host interaction. Most recently, the excretory/secretory products and antigenic proteins of *E. granulosus* adult worms from infected dogs were also characterized using two-dimensional LC–MS (Cui et al., 2013; Wang et al., 2015) providing new insights into the mechanisms involved in the establishment of *E. granulosus* infection and the modulation of the immune response.

The aim of our current study was to characterize the proteome of the fluids from human hydatid cysts and to compare the protein composition of them according to their location and fertility status. In this work, the proteomic analysis was conducted on pulmonary, vertebral and paravertebral hydatid cystic fluids with the aim of clarifying the host-parasite interactions occurring in the different stages and how the parasites adapt to the different host tissue locations. Comparing the protein compositions of the hydatid fluids from different host locations could potentially lead to improvements in the diagnosis, prognosis and treatment of human cystic echinococcosis.

2. Materials and methods

2.1. Sample preparation

Human cysts were obtained from three Algerian patients with primary infection. The patients were admitted at the thoracic surgery department of Mustapha Bacha University Hospital, Algiers, Algeria. Patients were from the high plateau and north-central provinces. They came from rural and grazing areas with a common presence of dogs. The patient with pulmonary cysts suffered cough, fever and chest pain. The patient with paravertebral hydatidosis was admitted with symptoms of paraplegia and showed an osteolysis in hydatid cyst area. The pulmonary, vertebral and paravertebral human hydatid cysts containing fertile and infertile fluids (3–8 cm in diameter) were collected from patients after their surgical removal. None of the patients had received pharmacological treatment. All subjects were informed of the study objectives and signed official consent forms. The study was conducted according to the guidelines of the local Ethics Working Group of the Thematic Research Agency in Health Science. Hydatid fluids were aseptically aspirated from individual cysts and centrifuged at 10,000 × g for 15 min at 4 °C to recover the fluid from the pelleted protoscoleces. Proteins present in the hydatid fluids were precipitated overnight with ice-cold acetone (1 V/3 V), and then recovered by centrifugation at 10,000 × g for 15 min at 4 °C. The protein pellet was washed three times with ice-cold acetone, air-dried, and stored at −80 °C until use.

2.2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and protein identification by liquid chromatography-tandem mass spectrometry (LC–MS/MS)

Proteins were resuspended in SDS-PAGE loading buffer (60 mM Tris-HCl, pH 6,8; 8% SDS; 10% glycerol; 0,01% bromophenol blue; 5% β-mercaptoethanol) and boiled for 5 min. Proteins were separated by SDS-PAGE (12%) run at 30 V for 120 min, 90 V o/n and 120 V for 120 min. Proteins were revealed by home-made Coomassie blue-staining (0,1% R250, 50% methanol). Protein bands were manually excised from the gels, reduced with 10 mM dithiothreitol, alkylated with 55 mM iodacetamid, and trypsin digested with sequencing-grade modified trypsin (Promega, Leiden, Holland) as described by Shevchenko et al. (1996). The resulting tryptic peptides were fractionated on a nano-HPLC EASY-nLCII instrument (Thermo Fisher Scientific, Odense, Denmark) coupled to a QTOF Ultima Global instrument (Waters, Zellik, Belgium). Peptides were loaded on a 10 cm long column with a 75 µm inner diameter, packed with 3 µm C_{18} particles. Reverse-chromatography was performed with a binary buffer system consisting of 0.1% formic acid (FA) (Buffer A) and 95% acetonitrile in 0.1% FA (Buffer B) for a one hour gradient run with a flow rate of 300 nl/min. The QTOF instrument was operated in the data-dependent mode and the three most abundant peptides with +2 and +3 charges were selected. The raw data files were processed using the Mascot Daemon platform (Matrix Science Ltd, London, UK). The fragmentation spectra were searched against NCBInr-All (https://www.ncbi.nlm.nih.gov/guide/all/) with the parent ion mass tolerances set to 100 ppm. Database search parameters were the following: trypsin as the digestion enzyme with 1 tryptic miscleavage allowed; carbamidomethylation of cysteine was set as fixed modification and methionine oxidation and pyroglutamic acid as variable modifications. For database searching, Mascot individual search algorithms internal estimates using a 95% confidence cutoff were used to calculate Mascot scores. Hence, proteins with scores greater than 54 were significant. Protein identifications were then manually inspected and protein hits were retained when matched with at least two peptides.

2.3. Bioinformatic analysis

Host proteins, identified by searching the NCBInr database, were organized in groups by the PANTHER® classification system (Protein ANalysis THrough Evolutionary Relationships Version 9.0, release date Jan 20, 2014). The classification System Version 9.0 was designed to facilitate high-throughput analysis. Proteins in this database are classified according to their molecular functions and biological processes. The parasite proteins were identified by NCBInr database searches and then organized in groups by gene ontology (http://www.geneontology.org).Each protein was organized in groups related to biological processes and molecular functions.

3. Results

3.1. Protein profiles of the hydatid fluids

The SDS-PAGE and Coomassie-stained protein profiles of the three hydatid fluids (pulmonary hydatid fluid, PHF; vertebral hydatid fluid, VHF; and paravertebral hydatid fluid, PVHF) are shown in Fig. 1. The SDS-PAGE analysis of the human hydatid fluids showed, in each case, a complex mixture of proteins ranging from 170 kDa to less than 10 kDa with a major band of 40–70 kDa corresponding to albumin (Fig. 1). A protein band with a molecular weight larger than 170 kDa can be seen in the fertile fluid samples (corresponding to ferritin as shown in Fig. 1). Interestingly, the infertile PVHF preparation showed fewer bands compared with fertile PHF and VHF, although the albumin signal is conserved.

3.2. Proteomic analysis

The proteomic analysis of the different hydatid fluids from the larval stage of *E. granulosus* revealed the presence of both host and parasite proteins as already observed by previous studies. In such