International Research Journal of Biotechnology Vol. 13(5) pp. 1-4, October, 2022
Available online http://www.interesjournals.org/IRJOB

Review Article

Causative Agent Resistant to Wear Cyst Fluid Protein Purification Investigation by Shotgun LC-MS/MS Taenia Solium Cysts from Pigs' Skeletal Muscle and Central Nervous System were Subjected to Quantitative Multiplexed Proteomics

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Received: 01-Oct-2022, Manuscript No. IRJOB-22-77502; **Editor assigned:** 03-Oct-2022, Pre-QC No. IRJOB-22-77502(PQ); **Reviewed:** 17-Oct-2022, QC No. IRJOB-22-77502; **Revised:** 22-Oct-2022, Manuscript No. IRJOB-22-77502(R); **Published:** 29-Oct-2022, DOI: 10.14303/2141-5153.2022.25

Abstract

The larval stage (cysts) of the tapeworm Taenia solium, which causes human and porcine cysticercosis, can infest a variety of tissues, including the skeletal muscles and the central nervous system (CNS) (SM). The proteome alterations brought on by the cyst's tissue localisation in the host tissues have received less attention. The ability to assess global proteome alterations in response to various situations is strength of quantitative multiplexed proteomics. Here, we used a TMT-multiplexed method to identify and quantify more than 4,200 proteins, 891 of which were host proteins, in cysts isolated from the SM and CNS of pigs. This is the most comprehensive host and parasite protein mixing associated with tapeworm infections that have been documented to our knowledge. Skeletal muscle cysts were enriched in a number of cysticercosis antigens, including GP50, paramyosin, and a calcium-binding protein. Our findings pointed to the existence of tissue-enriched antigens that could help advance cysticercosis immunodiagnosis (Stryiński R et al., 2020). We chose 42 highly antigenic proteins enriched for each tissue localization of the cysts using a variety of epitope identification techniques. We chose 10 proteins and created synthetic peptides from the top 10 epitopes, taking into consideration fold alterations and antigen/epitope contents. Serum antibodies from cysticercotic pigs identified nine peptides, indicating that these peptides constitute antigens. Combinations of peptides from SM and CNS cysts produced better outcomes than combinations of peptides from a single tissue site, although it is yet unknown which tissue-enriched antigens are the ideal. We found that at least five distinct antigenic determinants were necessary for a valid immunodiagnostic test for porcine cysticercosis using machine learning techniques (Victor B 2014).

INTRODUCTION

Taenia solium cysts from pig skeletal muscle were taken out and subjected to a shotgun proteome analysis to examine host-parasite interactions and discover known proteins in the cyst fluid. Aseptically obtained cyst fluid was subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Using the Blast2GO programme, gene alignment and annotation were carried out, and then a gene ontology study of the proteins that had been annotated. The pathways were further examined using the KEGG database, and STRING software was used to create a network map of protein-protein interactions (PPIs). 158 known proteins in total were found, the majority of which were low-molecular-mass proteins. The majority of these proteins' molecular activities were mostly connected to catalytic activity and binding functions, which were engaged in cellular and metabolic processes. The pathway enrichment analysis showed that the PI3K-Akt and glycolysis/gluconeogenesis signalling pathways were were most enriched in the known proteins. Enzymes involved in sugar metabolism constituted the majority of the PPI network's nodes. The proteins found in the cyst fluid that were analysed in this study could be crucial in the interaction between the cysticerci and the

host. The cyst fluid proteome of cysticerci will be identified and analysed using the shotgun LC-MS/MS, gene ontology, KEGG, and PPI network map data, which will serve as a foundation for more research into the invasion and activities of T. solium (Embers ME et al., 2004).

A significant parasitic zoonosis brought on by Taenia solium larvae is cysticercosis. Most of Asia, including China, India, and the majority of Southeast Asia, as well as sub-Saharan Africa, has high rates of cysticercosis. By swallowing the fertilised eggs found in the excrement of infected animals, humans or pigs can contract the disease and turn into intermediate hosts. The embryos are actively released from the eggs in the gut after ingestion and go through the mucosa into the circulation, where they travel to peripheral tissues like the central nervous system and grow into cysticerci. The parasite creates long-lasting asymptomatic infection by colonising host tissue, controlling the host immune response. Headache, intracranial hypertension, and epilepsy are examples of infection symptoms when the central nervous system is impacted. In addition, host proteins have been confirmed to be present in the cyst fluid, the excreted secretion products, and the tissue of metacestodes, which is necessary for parasite survival in host tissues during cysticercosis in processes such as osmotic regulation of worms, self-metabolic processes, and protection of innate immune responses. IFN- and IL-2, IL-12, IL-18, and TNF- are only a few of the many inflammatory type-1 cytokines that manifest surrounding the cyst during parasitism. Furthermore, interferon-, IL-18, IL-4, IL-10, IL-13, and transforming growth factor levels all rise; in the late stage of parasitism, inflammation and edoema gradually disappear, and the vesicles collapse and eventually develop into calcified nodules. Fibrosis also surrounds the cyst and induces mixed Th1 and Th2 immune responses. Th2 is the predominant response at this stage, and IL-4, IL-5, and IL-13 expression levels rise. The majority of the worm's weight is made up of cyst fluid, which also contributes to several metabolic and immunological processes and is crucial to the parasitic cycle (Kanan JH et al., 2006). Therefore, it is essential to comprehend the protein structure of cyst fluid in order to examine how T. solium interacts with the host.

In a recent cysticercosis proteomics investigation, 2-dimensional electrophoresis was used to compare cysticercosis obtained from the central nervous system and skeletal muscle with serum from a cysticercosis patient. Quantitative research revealed that 11–13% of the cystic protein content was made up of host proteins. Using PDQuestTM (Bio-Rad, Hercules, California) and multivariate analysis, 2-dimensional electrophoresis was used to simultaneously analyse the tissue localization of cysts in various locations. It was discovered that the protein patterns of cysts obtained from the central nervous system and skeletal muscle were significantly different. Compared 7 distinct antigens from cyst fluid with their direct source homologs. Only annexin B1 and cAMP-dependent protein kinase can be developed as potential diagnostic antigens for cysticercosis due to their great specificity. Due to its excellent resolution, 2-dimensional electrophoresis (2-DE) in combination with mass spectrometry (MS) has emerged as a prominent technique for researching proteomics and is frequently used to generate differential protein expression profiles. The technique divides proteins into groups based on their molecular weight in the first and second dimensions and their isoelectric point. However, 2-DE has drawbacks, including a poor loading capacity, the inability to separate insoluble proteins, very acidic and alkaline proteins, as well as low abundance proteins; another major issue with this approach is the difference in protein gels (Harris JK et al., 2007).

Recent studies have demonstrated that, in contrast to the 2-DE/MS proteomics approach, the shotgun proteomics method only needs a single sample to identify hundreds of proteins. Trypsin hydrolyzes the protein, and MS is then utilised to effectively analyse each peptide. In the realm of parasites, the shotgun proteomic technique has proven particularly effective in building differential expression profiles and screening for particular antigens like schistosomiasis, Eimeria maxima, and Fasciola hepatica. The majority of recent researches have focused on single antigen screens; nevertheless, interactions between the host and the parasite include several proteins, and these proteins are coupled in a network. With the advancement of parasite proteomics research, new techniques should be applied to thoroughly study cysticercosis (Burgess and Burchmore, 2012). Shotgun proteomics was employed in this work to qualitatively examine the proteins from cysticerci cyst fluid, and then bioinformatics techniques were applied to thoroughly investigate the chosen proteins. Researchers will be able to learn a great deal from the findings of this study about the parasite's many developmental phases, invasion during colonisation, and immune evasion (Navarrete-Perea J et al., 2017).

By ingesting the eggs of this parasite, Taenia solium, one can get the disease that causes human and porcine cysticercosis. The oncospheres that penetrate the intestinal wall after being activated by a variety of gastrointestinal drugs eventually establish in many tissues and organs, including the brain and skeletal muscles (SM). Neurocysticercosis (NC), a dangerous and pleomorphic condition that can become very disabling in humans, is brought on by the development of cysts in the central nervous system (CNS). The amount and location of cysts in the CNS, as well as a variety of other characteristics, such as an intricate immunological reaction to various cyst antigens, have all been linked to human NC heterogeneity, at least in part. It is still unclear which molecular mechanisms contribute to the tissue localisation of T. solium cysts. Other pathogenic bacteria, including S. pneumoniae, Campylobacter jejuni, Escherichia coli, Trypanosoma brucei, and others, exhibit tissue preference that is connected to a variety of distinct pathogen proteins (Stryiński R et al., 2020).

There is little information on the proteome alterations caused by flatworm parasite infestations. However, we are aware that hormones, cytokines, and other chemicals from the host are reacted to by parasites. The accessibility of many tapeworm genomes has made it possible to describe the intricate host-parasite cross-communication involving, among other things, insulin, EGF/FGF, and TGF-b/BMP. Schistosoma mansoni, Taenia crassiceps, and Echinococcus multilocularis all exhibit insulin response (Chang SL 1978). It is also well known how different steroid hormones behave when a person has a parasite infection. Some parasites can also react to host cytokines; for instance, S. mansoni contains receptors for TNF- and TGF-, and it has been shown to undergo proteomic and genomic alterations in response to those cytokines. High throughput proteomic approaches have substantially expanded our ability to address these long-standing issues in molecular helminthology (Schwab SJ et al., 1987). Body fluids of the host may influence how an infectious agent's proteome is expressed in this setting; for instance, E. coli growing in medium supplemented with urine has a distinct proteome signature. Additionally, Streptococcus pyogenes has been observed to undergo a number of proteome alterations in response to serum supplementation. Less research has been done on the molecular aspects of helminth parasite tissue localisation; in the instance of Trichinella spiralis, numerous alterations have also been noted between parasites isolated from various host tissues (Schuster FL et al., 2004). However, significant developments in helminth proteomics have been documented, including the cystic/vesicular larval forms of metacestode. It is plausible that the molecular environment of the host tissue controls how pathogens, including parasites, express their proteins. As a result, particular tissue localizations of parasites may be linked to particular proteomic profiles of those parasites. Understanding the proteome alterations of parasites in various host tissues can offer insights into the molecular networking taking place in complex host parasite interactions, as well as help with the development of more potent vaccinations, medicines, and diagnostic tools (Berlana D et al., 2005).

We used isobaric quantitative proteomics in this study to clarify the proteome alterations of T. solium cysts isolated from SM and pig CNS. The identification of 42 tissue-enriched antigens and the creation of 14 synthetic antigenic peptides were made possible by the discovery of a protein profile linked to each tissue localization. These peptides were then tested for antibody recognition using sera from infected and uninfected pigs. Our findings suggested that at least five distinct epitopes from several tissue-enriched antigens are necessary for an ideal immunological diagnosis of porcine cysticercosis. The obvious and prodigious presence of host proteins in the protein extracts of the cysts—891 host proteins were identified and quantified—was a striking discovery. We provide preliminary data indicating that the physiology of tapeworms may be significantly influenced by a number of intact host proteins (Pier GB 2000).

CONCLUSION

After being aseptically removed, the cyst fluid from T. solium cysticerci was qualitatively examined by shotgun LC-MS/MS and then bioinformatically analysed of the 158 identified proteins. The PI3K-Akt signalling pathway and the glycolysis/ gluconeogenesis signalling pathway were the two primary pathways enriched. The majority of the interacting nodes in the PPI network were enzymes involved in glycolysis, and gene ontology analysis revealed that the majority of proteins had catalytic and binding functions. These actions could be crucial for preserving cysticerci's parasitism and metabolism. The relationship between the parasite and the host is dynamic and intricate. The huge quantities of proteins produced or released by the worm at each stage provide the parasite a distinct life cycle that is particular to the host and pathogenicity (Hansen LS et al., 1974). In order to characterise parasite processes, the systematic study of cyst fluid proteins has proven reference value. Over 4,200 proteins from the nine samples of T. solium cysts could be identified and quantified by high-throughput proteomics utilising a TMT-multiplexed approach. The proteome of the T. solium cyst is made up of a combination of host and parasite proteins (one of each 5 proteins were of host origin). Numerous proteins and antigens that are enriched for SM or CNS localizations may be found in T. solium cysts isolated from naturally infected or experimentally infected pigs. The host proteins that were discovered were extremely varied and had a wide range of metabolic and signalling functions (Savka MA et al., 1990). We discovered that different host proteins' immuno-localization investigations revealed where they are located in various cyst tissues (tegumentary or subtegumentary tissues in the bladder wall or in the scolex). The vesicular fluid included additional host proteins that were found. Here, we also demonstrated the intact absorption of a number of host proteins; IgG, for instance, kept its ability to bind antigens. Additional research should be done on the functional activity of host proteins in the cyst's tissue (Agudelo Higuita NI et al., 2016).

Combining the peptides/proteins obtained from SM and CNS enriched antigens would enable the invention of extremely efficient immunodiagnostic procedures using the parasite's antigens that were shown to be enriched for a certain tissue. We were able to distinguish between cysticercotic and non-cysticercotic pigs using a variety of peptide mixtures and machine-learning models with an efficiency that is comparable to the current diagnostic methods using crude extracts from complex cysts; however, the proper subset of tissue-enriched antigens has yet to be identified. The utilisation of SM and CNS enriched antigens is necessary for the development of an ideal immunodiagnostic test for human and porcine cysticercosis; however, variation of those antigens in the cysts recovered from various endemic locations still has to be examined. The effectiveness of the present diagnostic procedures may greatly benefit from this evaluation. In addition to designing more effective

immunological tests for human and porcine cysticercosis, characterising the antigenic proteins associated with each tissue localization of the cysts is important because they may be involved in intricate tissue-specific immunomodulatory processes.

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