Veterinary Parasitology 252 (2018) 35–46

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Comparative transcriptome analysis of *Eimeria necatrix* third-generation merozoites and gametocytes reveals genes involved in sexual differentiation and gametocyte development

Shijie Su[a,b,c], Zhaofeng Hou[a,b,c], Dandan Liu[a,b,c], Chuanli Jia[a,b,c], Lele Wang[a,b,c], Jinjun Xu[a,b,c], Jianping Tao[a,b,c,*]

[a] *College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, China*
[b] *Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou 225009, China*
[c] *Jiangsu Key Laboratory of Zoonosis, Yangzhou University, Yangzhou 225009, China*

A R T I C L E I N F O

Keywords:
Eimeria necatrix
Gametocytes
Merozoites
Comparative transriptomics
Genes
Sexual differentiation and development

A B S T R A C T

Eimeria necatrix is one of the most pathogenic parasites causing high mortality in chicken older than 8 weeks. *Eimeria* spp. possess a coccidian lifecycle including both sexual and asexual stages. Sexual differentiation and development occupies a central place in the life cycle of the *Eimeria* parasite. However, our knowledge of the sexual differentiation and gametocyte development of *Eimeria* is very limited. Here using RNA sequencing, we conducted a comparative transcriptome analysis between third-generation merozoites (MZ-3) and gametocytes (GAM) of *E. necatrix* to identify genes with functions related to sexual differentiation and gametocyte development. Approximately 4267 genes were differentially expressed between MZ-3 and GAM. Compared with MZ-3, 2789 genes were upregulated and 1478 genes were downregulated in GAM. Approximately 329 genes in MZ-3 and 1289 genes in GAM were further analyzed in the evaluation of stage-specific genes. Gene Ontology (GO) classification and KEGG analysis revealed that 953 upregulated gametocyte genes were annotated with 170 GO assignments, and 405 upregulated genes were associated with 231 signaling pathways. We also predicted a further 83 upregulated gametocyte genes, of which 53 were involved in the biosynthesis of the oocyst wall, and 30 were involved in microgametocyte development. This information offers insights into the mechanisms governing the sexual development of *E. necatrix* and may potentially allow the identification of targets for blocking parasite transmission.

1. Introduction

Avian coccidiosis is an intestinal disease caused by *Eimeria* and occurs worldwide. Infection with *Eimeria* impairs growth, suppresses the immune system, and causes significant mortality with an estimated global cost to the poultry industry of more than USD 3 billion annually (Blake and Tomley, 2014). In China, the annual loss due to in-feed medications or live vaccines used in the control of *Eimeria* was estimated at USD 30–60 million (Hao et al., 2007). Current approaches to limit avian coccidiosis include anticoccidial chemicals, vaccines, and natural products. Anticoccidial chemicals, coccidiocides, coccidiostats, and ionophores have long been used as a mainstream strategy to control avian coccidiosis in modern poultry production systems (Muthamilselvan et al., 2016). Although this strategy is cost-effective and successful, drug resistance and public demands for residue-free meat have encouraged the development of alternative control strategies (Chapman, 2014).

Eimeria spp. undergo the simplest life cycle with the completion of merogony and gametogony within a single host, and completion of sporogony *in vitro*. The exact number of merogony cycles varies between species but the average is three in poultry *Eimeria*. The final-generation merozoites enter the sexual stage of reproduction by forming male microgametes and female macrogametes in host cells. Following fertilization, the zygotes develop into oocysts and are excreted into poultry stool. Despite the lack of sexual dimorphism, final-generation merozoites may be sexually mature (Klimes et al., 1972). The commitment of final-generation merozoites to sexual differentiation is often considered genetically programmed and not reliant on environmental cues (Smith et al., 2002). Because both sexes of gametocytes (GAM) are produced from cloned lineages that originated from a single haploid parasite, sexual development in *Eimeria* depends on the differential expression of stage-specific genes rather than the presence

* Corresponding author at: College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, China.
E-mail address: yzjptao@126.com (J. Tao).

https://doi.org/10.1016/j.vetpar.2018.01.019
Received 18 October 2017; Received in revised form 20 January 2018; Accepted 22 January 2018
0304-4017/ © 2018 The Author(s). Published by Elsevier B.V.

or absence of sex chromosomes (Walker et al., 2013). Although genome sequences are available for all seven species of *Eimeria* which infect domestic chicken (Reid et al., 2014) and transcriptome analysis data are available for most developmental stages, including unsporulated oocysts, sporozoites, and second-generation merozoites (Novaes et al., 2012; Matsubayashi et al., 2016), only a few stage-specific genes have been identified in *E. tenella*, *E. maxima*, and *E. acervulina* based on the profiling of quantitative changes in gene transcription (Walker et al., 2010; Katrib et al., 2012; Walker et al., 2015). In contrast to *Plasmodium* species, for which it is known that parasite gametocytogenesis and gametogenesis occur in humans and insect vectors, respectively, and male and female gametocytes can be cultured and isolated (Walker et al., 2013), the genes involved in sexual development of *Eimeria* species have been less studied in part as a result of the difficulties in obtaining sufficient quantitiesof parasite material for transcriptional sequencing and analysis.

Eimeria necatrix is one of the most important species of this genus, as it is highly pathogenic and can cause high mortality in susceptible birds, particularly in chicken older than 8 weeks raised on a litter floor (McDougald and Fitz-Coy, 2013). The lifecycle of *E. necatrix* is somewhat different from that of other species of avian coccidia, in which the first- and second-generation meronts are primarily located in the mid-intestinal area of the host chicken, and third-generation meronts and later gametogony only in the caecum (McDougald and Fitz-Coy, 2013). The second-generation merozoites (MZ-2) pass to the caecumof infected chicken, where they penetrate the epithelial cells, and turn into third-generation meronts, which then release third-generation merozoites (MZ-3). The MZ-3 then turn into GAM. The gametogony of *E. necatrix* is more synchronous in chicken infected by injecting MZ-2 directly into the caecum than in chicken infected by giving oocysts orally (Mcdonald and Rose, 1987). Therefore, using the method described above, it is relatively easy to obtain a good yield of high-purity *E. necatrix* GAM for transcriptional analysis. Moreover, because of the different sites of parasite development, the MZ-3 of *E. necatrix* cannot be contaminated with MZ-2.

In our previous study (Su et al., 2017), we conducted RNA sequencing (RNA-seq) to create a differentially expressed gene profile for MZ-2 and MZ-3 in *E. necatrix*, and observed that the molecular functions of the genes upregulated in MZ-3 were enriched predominantly for transcriptional activity, cell proliferation and cell differentiation. In the present study, the transcriptome of GAM from *E. necatrix* was sequenced, and then compared with that of MZ-3 to identify global changes in gene expression as *E. necatrix* undergoes sexual differentiation and gametocyte development from MZ-3 to GAM. This information will offer insights into the mechanisms governing the sexual development of *E. necatrix* and has the potential to identify targets for blocking parasite transmission.

2. Materials and methods

2.1. Animals and treatments

One-day-old yellow-feathered broilers were obtained from the Poultry Institute of China Agricultural Academy (Yangzhou, Jiangsu, China). Birds were housed in *Eimeria*-free isolation cages and were provided with complete feed and clean water without anticoccidial drugs until use. Chicken feces were collected and analyzed by salt flotation and light microscopy to confirm the absence of oocysts in chicken one day before the experimental inoculations (Shirley, 1995). Chicken between 4–5 weeks of age were used to prepare GAM. The Yangzhou strain of *E. necatrix*, originally isolated from a chicken that died from *E. necatrix* infection by the Key Laboratory for Avian Preventive Medicine at Yangzhou University, was used in this study. All study protocols were approved by the Animal Care and Use Committee of the College of Veterinary Medicine, Yangzhou University.

2.2. Preparation of GAM

MZ-2 were obtained from the small intestine of chicken at 136 HPI after oral inoculation with 2.0×10^4 oocysts of *E. necatrix*, and approximately $1.0–1.5 \times 10^8$ second-generation merozoites (in a volume of 1.5–2.0 mL) were injected into the caeca of chicken as described by Mcdonald and Rose (1987). At 32 ± 0.5 HPI, fifteen chicken were sacrificed and the caeca were removed and washed with cold SAC (1 mM phenylmethanesulfonyl fluoride, 1 mg/mL bovine serum albumin, 170 mM NaCl, 10 mM Tris-HCl pH 7, 10 mM glucose, and 5 mM $CaCl_2$). Then, the caeca were slit open, the mucosal tissues were scraped and incubated at 37 °C in a beaker for 2 h with 0.5 mg/mL of hyaluronidase in SAC. The digested mucosal tissues were filtered through a 100, 20, and 17-μm polymon mesh. The debris on the mesh was discarded and the filtrate was further filtered through a 10-μm polymon mesh. The gametocytes on the mesh were washed with cold SAC and centrifuged at 1400*g* for 5 min, the supernatant was discarded, and the gametocytes were then resuspended in 3 − 5 vols of erythrocyte lysis buffer (Solarbio, Beijing, China) at 4 °C for 15 min and washed with cold phosphate-buffered saline (PBS) three times by centrifugation. The gametocytes were purified by density-gradient centrifugation using the method described by Mo et al. (2014). Approximately $l0^8$ gametocytes were recovered from fifteen chicken as one sample. The purified gametocytes were frozen immediately in liquid nitrogen for future use (Supplementary Fig. 1). A total of three batches of gametocytes were collected for RNA isolation.

2.3. RNA isolation for RNA-seq, mapping and functional annotation

2.3.1. GAM

Total RNA was extracted from three biological replicates of GAM using RNAiso Plus Total RNA extraction reagent (Takara, Dalian, China). The quality of the RNA was measured using a NanoDrop ND-2000, and the integrity was evaluated with an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Illumina sequencing libraries were constructed using a TruSeq™ RNA Sample Pre Kit (Illumina, San Diego, CA, USA). The quantification of the cDNA libraries was performed using a Qubit™ dsDNA HS Assay kit on a Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). The libraries were sequenced on the Illumina HiSeq 2500 platform according to the manufacturer's instructions by Shanghai Biotechnology Corporation. The library was run on two lanes in a flow cell to maximize the total number of RNA-seq reads. Raw readswere trimmed by removing adapter sequences and low-quality reads with Seqtk. Genome mapping was performed based on the recently published *E. necatrix* Houghton strain reference genome sequence (https://www.ncbi.nlm.nih.gov/assembly/GCF_000499385.1/) and using Tophat (version 2.0.9) with a spliced-mapping algorithm. After genome mapping, reads with less than two-base mismatches and multi-hits ≤2 were retained. The relative expression levels of all the matched unigenes were normalized by transforming the clean data to fragments kilobase of exon per million fragments mapped (FPKM) using Cufflinks.

2.3.2. MZ-3

The MZ-3 transcriptome data was collected in our previous study (Su et al., 2017). However, the raw reads were processed with Seqtk in this study.

2.4. Identification and analysis of differentially expressed genes (DEGs)

DE-seq was used to detect DEGs. To control for multiple testing, a *q*-value [a false discovery rate (FDR)- adjusted *p*-value] was used to adjust the significance level for individual gene. The DEGs between the two libraries were selected with the following filter criteria: *q*-value ≤ 0.05 and the absolute value of $\log_2$ fold change ≥1 or fold change ≥2, indicating that the expression of each DEG in different libraries should be

Download English Version:

https://daneshyari.com/en/article/8506098

Download Persian Version:

https://daneshyari.com/article/8506098

Daneshyari.com