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Comparative analysis of microRNA in schistosomula isolated from non-permissive host and susceptible host

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ABSTRACT

The reed vole *Microtus fortis* is the only known mammal in which the schistosome is naturally prevented from maturing and schistosome infection does not cause significant pathogenesis. However, the mechanism behind this phenomenon remains unknown. In the present study, Solexa deep sequencing technology was used to carry out high-throughput sequencing and comparative analysis of microRNA (miRNA) between small RNA libraries isolated from 10 days oldschistosomula of *M. fortis* and BALB/c mice.In total, 10d schistosomula from *M. fortis* and BALB/c mice yielded 13.37 and 10.84 million reads, respectively, and nearly 39% and 40% of reads could be mapped to selected miRNAs in miRbase. Based on a bioinformatic analysis, we found that most of the miRNAs identified in *Schistosoma japonicum* were detected in our study. Further analysis revealed that 24 miRNAs were differentially expressed between the schistosomula from the two rodents, of which 21 were down-regulated and three were up-regulated in schistosomula from *M. fortis*. Also, six novel miRNAs were predicted and identified in this study. Target genes were mapped and filtered by correlating them with differentially expressed genes obtained from *S. japonicum* oligonucleotide microarray analyses performed in previous studies. miRNAs such as miR-10-3p, miR-10-5p, and miR-2b-5p may affect the growth, differentiation, and metabolism of worms via regulation of the expression of target genes such as enolase, aquaporin, TGF-beta-inducible nuclear protein, and paramyosin. Gene Ontology analysis of the predicted target genes of these six differentially expressed miRNAs revealed that some important biological pathways, such as metabolic processes,glycolysis, and catalytic activity, were involved. The results of this study highlight the function of miRNAs in the development and survival of the schistosome, and provide valuable information to increase our understanding of the regulatory function of miRNAs in schistosome development and host-parasite interactions in a differentially susceptible host environment.

1. Introduction

Schistosomiasis is the most prevalent tropical diseases caused by blood flukes of the genus *Schistosoma*. It is estimated that more than 200 million individuals are infected in more than 70 countries and close to 800 million are at risk of infection [1,2]. There are more than 40 species of mammals, including cattle, water buffalo, sheep, goat, rabbit, and mouse, that are naturally infected by *Schistosoma japonicum* (*S. japonicum*) in China. The reed vole, *Microtus fortis* (*M. fortis*), is the only known mammal found in endemic areas of China in which the schistosome is naturally prevented from maturing and completing its life cycle [3]. Most of the worms in *M.*

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fortis are consumed in the lung, and the rest are eliminated in the liver. The maintenance of the infection state is limited to around 15 days after a challenge with cercariae [4–6]. Based on these observations, *M. fortis* has been used as a non-permissive animal model to investigate the molecular mechanisms associated with its anti-schistosome ability. Previous studies indicated that a stronger humoral and/or cellular immune response may play an important role in the early phase of the infection in *M. fortis* [7]. Other factors, such as apoptosis, nutrition, neuroendocrine hormones, signaling pathways, and other environmental parameters that might affect parasite survival and development, have been reported in previous studies [4,5].

MicroRNAs (miRNAs) are a class of small single-stranded non-coding RNA that are 18–25 nucleotides long, generated from endogenous transcripts that form hairpin loops, and function in the transcriptional and post-transcriptional regulation of gene expression [8,9]. They regulate gene expression by binding to 3' untranslated regions (UTRs) of target cellular mRNA, causing translation inhibition and mRNA degradation. miRNAs play fundamental roles in diverse biological and pathological processes, including growth, metabolism, development, cell differentiation, and cellular response to environmental and developmental signals [10–12]. Recently, some miRNAs that function in the schistosome have been identified, providing insight into the role of miRNA in schistosome development, growth, maturation, and host-parasite interaction [13–15].

In the current study, we aimed to identify differentially expressed miRNAs from the transcriptome of 10 days old schistosomula from *M. fortis* and BALB/c mice using a deep sequencing approach. Our results provide useful information to better define specific miRNAs involved in the development, growth, and maturation of the schistosome, and facilitate the screening of new drugs and vaccine targets for the control of schistosomiasis.

2. Materials and methods

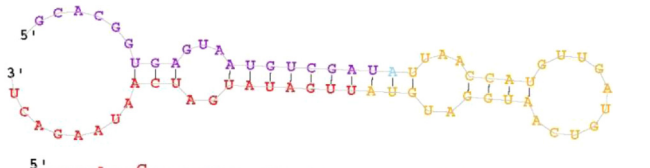
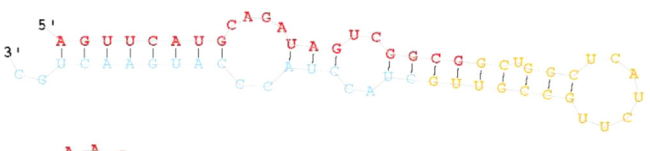
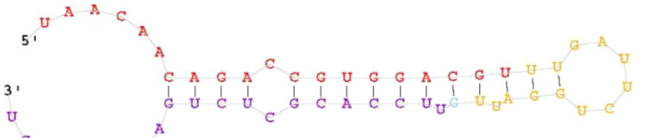
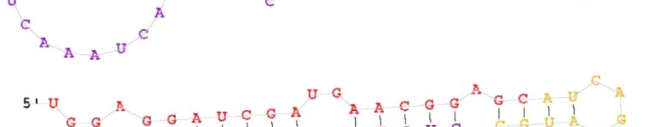
2.1. Host animals and parasites

All animal care and experimental procedures were conducted according to the guidelines for animal use in toxicology. The study protocol was approved by the Animal Care and Use Committee of the Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences (CAAS). Eight-week-old specific-pathogen-free (SPF) female *M. fortis* (60 g) and BALB/c mice (20 g) were purchased from Xipu'erbikai Experimental Animal Co., Ltd (Shanghai) and Shanghai Laboratory Animal Center, Chinese Academy of Sciences (Shanghai), respectively. The life cycle of *S. japonicum* (Chinese mainland strain, Anhui isolate) was maintained in New Zealand rabbits and *Oncomelaniahupensis* snails at the Shanghai Veterinary Research Institute, CAAS. *M. fortis* and BALB/c mice were infected with 3000 and 200 cercariae, respectively, and were perfused with 37 °C pre-warmed phosphate buffered saline (PBS) at 10 days post-infection when the schistosomula were collected. Special attention was paid to removing host tissue from the isolated parasites through multiple washings with 37 °C pre-warmed PBS.

2.2. Total RNA isolation, small RNA library construction, and sequencing

Small RNA was isolated from schistosomula using the mirVana isolation kit, according to the manufacturer's instructions (Ambion, USA). The RNA quality was measured using a Nanodrop-2000 spectrophotometer (Thermo Scientific, USA) and was stored at −80 °C.

Table 1
Primarily analysis of the identified differential expressed novel miRNAs.

novo microRNAs location at genome	example miRBase miRNA with the same seed	Secondary structure
SJC_S019938:1123.1191:+	smo-miR1085-3p	
SJC_S005013:7093.7180:–	dme-miR-7-5p	
SJC_S026307:2680.2740:+	hsa-miR-1206	
SJC_S000447:13966.14020:+	tae-miR1134	
SJC_S004051:17257.17309:+	ppt-miR902i-3p	
SJC_S001666:32997.33075:–	–