Expression and Analysis of *Microtus fortis* Against *Schistosoma japonicum* CD72

Yang Xiang¹ and Junjian Hu²

1. College of Chemistry and Chemical Engineering, Hunan Institute of Science and Technology, Yueyang 414006, China

2. Hunan Provincial Institute of Schistosomiasis Control, Yueyang 414006, China

Received: September 30, 2014 / Accepted: September 30, 2014 / Published: September 30, 2014.

Abstract: The total RNA was extracted from *Microtus fortis* liver tissue which before being infected and after being infected 10 d and 15 d by the *Schistosoma japonicum* cercariae. Using *rattus norvegicus* CD72 gene probes were used to hybridize analysis of CD72 difference expression in the *Microtus fortis* liver tissues which were infected with *Schistosoma japonicum* before and after being infected. At the same time, the cDNA sequence and encoded amino acid sequence of the *rattus norvegicus* CD72 gene and CD72 protein structural domains were analyzed by using bioinformatics. The results showed that the CD72 expression levels in the liver tissue of *Microtus fortis* after being infected was significantly higher than before being infected. The *rattus norvegicus* CD72 cDNA sequence of a total length is 1479 bp and encode 364 amino acid residues and *rattus norvegicus* CD72 protein containing a CD72 superfamily domain.

Key words: *Microtus fortis*, *Schistosoma japonicum*, CD72, expression, analysis.

1. Introduction

Schistosomiasis is the world's second-largest parasitic disease after malaria, serious harm to human health. At least 76 countries and regions to be endangered, some 600 million people are threatened, 200 million people are infected, and China is one of the hardest hit of schistosomiasis. The Chinese pop schistosomiasis mainly is schistosomiasis japonica, a population of 11.612 million people infected schistosomiasis in China, a population of 130 million people threatened by schistosomiasis in the world. Schistosomiasis in China are mainly distributed in Jiangsu, Hunan, Hubei, Sichuan and Yunnan Province, Dongting Lake area as a national one of heavy disaster area, infected population occupies countrywide 1/4. *Microtus fortis* is a no suitable host for *Schistosoma japonicum*, with natural completely against schistosomiasis characteristics [1]. Oriental vole

distribution in China is mainly concentrated in the Yangtze River Basin, especially in endemic area of schistosomiasis japonica in Dongting Lake Island, is a dominant rat in the area. *Schistosoma japonicum* growth stagnation after infected *Microtus fortis* 12 d, and all *Schistosoma japonicum* demise after infected *Microtus fortis* 20 to 28 d *in vivo* [2]. The major organ *Schistosoma japonicum* was perished is in the liver [3]. Experiments showed that rat, *rattus*, the bandicoot rat, *Rattus norvegicus*, Hainan, roof rats to infection with *Schistosoma japonicum* susceptibility from high to low [4]. This reveals the different rat species of *Schistosoma japonicum* have different resistance, rat as unsuitable host of *Schistosoma japonicum*. So far, almost all of the schistosome infection animal models are positive model, but *Microtus fortis* to infection with *Schistosoma japonicum* model is a negative model. Therefore, use of *Microtus fortis* in natural resistance to *Schistosoma japonicum* infection in animal models, Chinese scientists did a lot of research work on *Microtus fortis* in humoral immunity and

Corresponding author: Yang Xiang, Ph.D., professor, research field: molecular and cell biology. E-mail: xiangyangbio@126.com.

cellular immunity [5], natural antibody associated antigen gene [6], screening resistance gene [7], gene expression profiling using microarrays of *Microtus fortis* to infection with *Schistosoma japonicum* and differences in gene [8] and function research. CD72 is a transmembrane protein is macrophage migration inhibitory factor (MIF) receptor molecules, and related to a variety of human diseases and parasitic infections [9]. This paper compares the Fortis to infection with *Schistosoma japonicum*, expression difference and bioinformatics analysis of CD72, for further research of CD72 in *Microtus fortis* against schistosomiasis mechanism lays a foundation.

2. Materials and Methods

2.1 Animal

The *Microtus fortis*, as indoor propagation of the Dongting Lake in Hunan, was provided by the Hunan Provincial Institute of Schistosomiasis Prevention and Control Research. *Schistosoma japonicum* cercariae, provided by the Hunan Provincial Institute of Schistosomiasis Prevention and Control Research, were used artificial quantitative infection experiment.

2.2 Sample Collection and RNA Extraction

1100 *Schistosoma japonicum* were used infected fortis, and total RNA was extracted from *Microtus fortis* liver tissue and positive control after infection 10 d and 15 d.

2.3 Northern Blot Experiments

The experimental chip hybridization experiment operated as follows: Rat (*Rattus norvegicus*) CD72 gene probes were used hybridization with *Microtus fortis* uninfected and infected after 10d and 15 d the liver tissue samples mRNA, respectively.

2.4 Bioinformatics Analysis

U.S. National Center for Biotechnology Information NCBI EST database (<http://www.ncbi.nlm.nih.gov/>) and Unigene database

(<http://www.ncbi.nlm.nih.gov/HomoloGene/nih.gov/Unigene>) were used for bioinformatics analysis. Identification of the Reading frame: Using the ExPASy server Translate and NCBI ORF finder. The protein domain analysis: protein blast in NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

3. Results

3.1 The RNA Extraction from the *Microtus fortis* Liver Tissue after Infected and not Infected *Schistosoma Japonicum*

The total RNA were extracted according to the description extraction in Molecular Cloning from *Microtus fortis* liver tissue infected with *Schistosoma japonicum* ago and after infected 10 d and 15 d. Sample mRNA in each lane approximately 2 µg (Fig. 1).

(1) The *Microtus fortis* not infected with *Schistosoma japonicum*;

(2) The *Microtus fortis* liver tissue after 10 d infected with *Schistosoma japonicum*;

(3) The *Microtus fortis* liver tissue after 15 d infected with *Schistosoma japonicum*.

3.2 The Differential Expression Analysis of CD72 in *Microtus fortis* Liver Tissue Infected with *Schistosoma japonicum* after 10 d and 15 d with Normal Control Group

The expression of CD72 in *Microtus fortis* liver tissue infected with *Schistosoma japonicum* after 10 d and 15 d significantly increased (Fig. 2) than the normal control group, after Northern blot analysis with rat CD72 gene probe.

Fig. 1 Total RNA extraction results from *Microtus fortis* liver tissue after Infected and not Infected with *Schistosoma japonicum*.

Department of Education key project of Hunan Province, China (No: 09A035).

References

- [1] He, Y., Luo, X., Zhang, X., Yu, X., Lin, J., and Li, Y. 1999. "Immunological Characteristics of Natural Resistance in *Microtus fortis* to Infection with *Schistosoma japonicum*." *Chin Med J.* 112 (7): 649-654.
- [2] Yu, Y. J., Hu, W. X., Wang, Y., Su, Z. J., Yi, X. Y., and Peng, X. H. 2003. "The Primary Studies on Breeding *Microtus fortis* as Laboratory Animal and Protecting Their Germ Plasm." *Laboratory Animal Science and Management* 20: 13-17.
- [3] Li, H., He, Y. Y., Lin, B. F., Lin, J. J., Chen, Y. J., and Wang, Y. J. 2001. "The Preliminary Research of *Microtus fortis* Superinfection *Schistosoma japonicum*." *Chinese Journal of Veterinary Parasitology* 9 (3): 11-17.
- [4] Liu, J. S., Yong, S. M., Chen, P. C., Liu, Z. H., and Mo, G. Y. 1992. "The Several Rodents Artificial Experimental Observation of *Schistosoma japonicum* Infection." *Journal of Schistosomiasis Control in China* 4 (6): 350-351.
- [5] Jiang, S. H., Wei, M. X., Lin, J. J., Pan, C. E., Qiu, Q. W., and He, Y. Y. 2008. "Research of IgG3 Antibody Anti-schistosomiasis." *Chinese Journal of Parasitology and Parasitic Diseases* 26 (10): 34-36.
- [6] Sun, Y., Sun, H., Jia, R. C., Liu, J. M., Yuan, C. X., and Shi, Y. J. 2008. "Relative Target Genes Screening of *Microtus fortis* Against *Schistosoma japonicum*." *Chinese Journal of Schistosomiasis Control* 20 (1): 26-31.
- [7] Qin, Z. Q., Hu, W. X., Wu, G. J., Xu, B., Shen, Q. X., and Gong, Q. 2004. "Oriental Vole Bone Gene Pool and *Schistosoma japonicum* Resistance Related Gene Screening." *Life Science Research* 8 (4): 333-338.
- [8] Sun, J., Lin, J. J., Cheng, G. F., Li, H., Shi, Y. J., and Lu, K. 2004. "Gene Difference Expression Research Profiling using Microarrays to Study Oriental Voles and Mice Infected before and after with *Schistosoma japonicum*." *Journal of Peking University (natural science edition)* 40 (4): 532-537.
- [9] Kleemann, R., Hausser, A., and Geiger, G., et al. 2000. "Intracellular Action of the Cytokine MIF to Modulate AP1 Activity and the Cell Cycle through Jab1." *Nature* 408 (6809): 211-216.