Expression and Analysis of *Microtus fortis* against *Schistosoma japonicum* CD36 Gene

Yang Xiang¹, Xue-Qin Zheng¹, Zhen Xiang¹, Dong-Song Nie¹, Yu Liu¹, Xian-Lei Li¹, Dian-Dian Lv¹, Jun-Jian Hu² and Pei He²

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: December 19, 2012 / Accepted: July 22, 2013 / Published: August 30, 2013.

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* CD36 gene probe to hybridize analysis of CD36 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* CD36 gene and CD36 protein structural domains were analyzed by using bioinformatics. The results showed that the CD36 expression levels in the liver tissue of *Microtus fortis* after being infected were significantly higher than before being infected. The *Rattus norvegicus* CD36 cDNA sequence of a total length is 1625 bp and encoded 472 amino acid residues and *Rattus norvegicus* CD36 protein containing a CD36 superfamily domain.

Key words: *Microtus fortis*, *Schistosoma japonicum*, CD36, expression, analysis.

1. Introduction

Schistosomiasis is the world's second-largest parasitic disease after malaria, and is 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 main Chinese pop schistosomiasis 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 is 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 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 days, and all *Schistosoma japonicum* demise after infected *Microtus fortis* 20 to 28 days *in vivo* [2]. The major organ *Schistosoma japonicum* was perished in the liver [3]. Experiments showed that rat, *Rattus*, the bandicoot rat, *Rattus norvegicus*, Hainan, roof rats infect 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 model is 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

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

scientists did a lot of research work on *Microtus fortis* in humoral immunity and 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. CD36 is a transmembrane protein which is macrophage MIF (migration inhibitory factor) receptor molecules, and related to a variety of human diseases and parasitic infections [9], and detected in CD36 and MIF interactions in 57 tumor cells, and have different combination and interaction mechanism in the modulation of host cell effect with the host MIF [10, 11]. In this paper, the authors compared the differential expression of CD36 in the *Microtus fortis* liver tissues which were infected with *Schistosoma japonicum* before and after, and analyzed the CD36 by using bioinformatics.

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 for infecting *Microtus fortis*, and total RNA was extracted [12] 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*) CD36 gene probes were used hybridization [13] with *Microtus fortis* uninfected and infected after 10 d 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 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 was 2 µg (Fig. 1).

- (1) The *Microtus fortis* not infected with *Schistosoma japonicum*;
- (2) The *Microtus fortis* liver tissue after 10 days infected with *Schistosoma japonicum*;
- (3) The *Microtus fortis* liver tissue after 15 days infected with *Schistosoma japonicum*.

3.2 Differential Expression Analysis of CD36 in *Microtus fortis* Liver Tissue Infected with *Schistosoma japonicum* after 10 d and 15 d with Normal Control Group

The expression of CD36 in *Microtus fortis* liver tissue infected with *Schistosoma japonicum* after 10 d and 15 d significantly increased (Fig. 2) than the

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

Fig. 2 Northern blot analysis of CD36.

(A) 1: The *Microtus fortis* liver tissue not infected with *Schistosoma japonicum*; 2: The *Microtus fortis* liver tissue infected with *Schistosoma japonicum* after 10 days; 3: The *Microtus fortis* liver tissue infected with *Schistosoma japonicum* after 15 days. (B) GAPDH.

normal control group, after Northern blot analysis with Rat CD36 gene probe.

3.3 Bioinformatics Analysis of the Complete Open Reading Frame of CD36 Gene

The full length of cDNA sequence of rat CD36 contains 1625 bp, encoding 472 amino acid residues, as shown in Fig. 3.

3.4 Bioinformatics Analysis of CD36 Protein Domain

Rat CD36 proteins contain 472 amino acid residues, containing a CD36 superfamily domains, as shown in Fig. 4.

tggactgttactctctctctcgttggatggctagctgattactctgtgtgtagtagcttacaatgattgcgaag
 tgttgaacctttctgtagctctcaatgaacctattctctacacaaactcagatagctgctgttcgatgcgaat
 tagttgaacccaggcccaatagaaagcattgtaatgtactgtgagttggcgaagaagcaagtgctctctc
 ctgtgattctgtctgcacgagaggaggagaaatggcgctgcgatacggaaactgtggcctcatctactgagccggtt
 M G C D R N C G L I T G A V
 attgtgtgtctcctgtgctgtttggaggacattctcactcggcttggagacactactcattggaagaca
 I G A V L A V F G G I L M P V G D L L I E K T
 atcaaaagggaagttgtccttgaagaaggaaaccattgcttcaaaaactgggtgaaaacggcgaccact
 I K R E V V L E E G T I A F K N W V K T G T
 gtgtacagacagtttggatctttgactgcaaaaacccagaggaaaggcgaagaatagcagcaagatc
 V Y R Q F W I F D V Q N P E E V A K N S S K I
 aaggttaaacacagagaggtctcttaccacatcacagattcgttattttagccaaggaanaataactcaggga
 K V K Q R G P Y T Y R V R Y L A K E N I T Q D
 cccaaggaacagcactgctctctttgtataaccaactaggagccattcttgagcctcactgtctattgga
 P K D S T V S F V Q P N G A I F E P S L S V G
 acagagaatgacaacttcacagttctcaaatctggctgtggcagctgcaccacatattctacacaaactca
 T E N D N F T V L N L A V A A A P H I Y T N S
 tttgttcaaggtgtgctcgaacgcttatacaaaagtcgaagttctctatgttccaacacagcaagtttg
 F V Q G V L N S L I K K S K S M F P T R S L
 aaggaactctgtgggtgtacaaagatccattcttgagttgttccatatctcatataagtaccacagtt
 K E L L W G Y K D P F L S L V P Y P I S T V
 ggtgtgttttctccttacaataaacactgtagatggagtttataaagtttcaatggaaggatatacata
 G V F Y P Y N N T V D G V Y K V F N G K D N I
 agcaaggttccataatgtatacctataadgggaagaaggaattgtctctattgggaagttatttgcac
 S K V A A I I D T Y K G K R N L S Y W E S Y C D
 atgatttaattggcaagatgcagctctcttccacttttggtagaagttctggaacactgaggttcttt
 M I N G T D A A S F P P F V E K S R T L R F F
 tctctgcacatttcgaggtccatctatctgctgtttggatctgaagtgaaccttaaaaggaatcccogtg
 S S D I C R S I Y A V F G S E V N L K G I P V
 tacagatttgttcttccagcaaacgctttgctctcccactccagaaccagacaacactgtttctgc
 Y R F V L P A N A F A S P L Q N P D N H C F C
 actgaaaaagtaactccaataaactgtactgctatggtgtctggacatggcaagtgcaagaagga
 T E K V I S N N C T S Y G V L D I G K C K E G
 aagcctgtgtacatttcttccacatttccacatcgaagtcctgtatgtcgaapcctatcgaagc
 K P V Y I S L P H F L H A S P D V S E P I E G
 ttgaatcctaacgaagatgacataggacatacttgatgtggaaacctaaacttgattcactctacag
 L N P N E D E H R T Y L D V E P I T G F T L Q
 ttgcaaaacgactgcaggtcaacatactggtcgaagccagctagaanaataagaagcactgaaagatctg
 F A K R L Q V N I L V K P A R K I E A L K N L
 aagaagccttaccatttctacttactgttgccttaaatgagactgggacctcggcgatgagaagaacagaa
 K R P Y I V P I L W L N E T G T I G D E K A E
 atgttcagaanaaccagtgacccgggaaaaataaagctctggcgctgtttgagatggtcttacttgggttt
 M F R N Q V T G K I K L L G L V E M V L L G V
 ggagtgatgattgttgttcttattatattctactgtgcttgcagatctaagaatggaaaatgaagta
 G V V M F V A F M I S Y C A C R S K N G K
 gtggtgagcctacattatgcactagctacatttttggtaaaaaccaatctccaaaacgaagaccttaaga
 catgctgttttttataaaacacactatctgagttgaagaanaacgggtgtgtagcgctctctctctct
 ttgcagatataattattttatataatgcaaacgacagcatattcgaaagcattaatatgattgcaact
 aagcctatatttttataaaaactctgtatttttgtaagtccatcatctgcaactgagtggaactcaat
 ttctgcagcaactaatctctttttgggtctgatttactgatttttttctcgtttggcgaatttcaaga
 atgtatatattctagaagaacgctttgtctcactcgaaatgaactgttcatgctctgggtggccctt
 tcatttatagcaaatgttcttctgtgactgtcagcacatgatattgcatagggtattatctattttaag
 atttaaggatgaaaaatgaaacattccacatgaacactgttgatattatgttttaattccctccctct
 ctggtgtctcgtgcacaacgaagccaggtatcacagatacttttttttttttactttctacacagac
 ctattatgttctgttctcgcacctgaaatgaactatttttagcacattttagctcttttttttaagt
 attgttcgaagttccatgctgctagctcttttgaacactgagtagattttctctctctgctgcgcgca
 actaatgttaactcagagagctgttattgtgtttaaagatgtaattttataataaatggtatgtatata
 gaactcttaaaaaagctagaattggctttaaatatgtatttgttggaatattattctgcttttataatcac
 ccagaanaatgctggttctaacatttaagatgttcttaaatataaaaaaanaaaaaaanaaaaaa
 aaaa

Fig. 3 The amino acid sequence encoded by the rat CD36 gene cDNA sequence.

Fig. 4 Analysis of the rat CD36 protein domain.

4. Discussion

Schistosomiasis, which is the world's second largest parasitic disease after malaria, causes the harm to human health seriously. China is one of the hardest hit of schistosomiasis. The patients infected by the Japanese schistosomiasis are 130 million, and 11.612 million people are threatened by schistosomiasis in China. *Microtus fortis* which has natural anti-*Schistosoma japonicum* capacity is *Schistosoma japonicum* non-suitability host. Rat CD36 gene probe used Northern blot analysis with the *Microtus fortis* liver tissue mRNA. The results showed the CD36 gene expression significantly up-regulated in the *Microtus fortis* liver tissue infected with *Schistosoma japonicum* after 10 d and 15 d compare with control. The CD36 is macrophage uptake of OX-LDL (oxidized low-density lipoprotein) receptor molecules, involved in immune regulation, which show that the immune system to get a positive response after *Microtus fortis* infected with *Schistosoma japonicum*. CD36 has a role against *Schistosoma japonicum*.

5. Conclusion

In summary, the results demonstrated that Rat CD36 gene probe used Northern blot analysis with the *Microtus fortis* liver tissue mRNA and the CD36 gene expression significantly up-regulated in the *Microtus fortis* liver tissue infected with *Schistosoma japonicum* after 10 d and 15 d compare with control.

Acknowledgment

This work was supported by grants from the National Nature Science Foundation of China (No. 31071091; No. 21172067) and Department of Education key project of Hunan Province, China (No:09A035).

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