Gene 618 (2017) 42–48

Contents lists available at ScienceDirect

Gene

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

# Identification and functional characterization of a novel member of low-density lipoprotein receptor-related protein (LRP)-like family in amphioxus

Zhan Gao[a,b], Baozhen Qu[a], Zengyu Ma[a], Deyan Jiao[a], Guangdong Ji[a,b,*], Shicui Zhang[a,b,**]

[a] Laboratory for Evolution & Development, Institute of Evolution & Marine Biodiversity, Ocean University of China, Qingdao 266003, China
[b] Department of Marine Biology, Ocean University of China, Qingdao 266003, China

ARTICLE INFO

*Keywords:*
Amphioxus
*Branchiostoma japonicum*
Low-density lipoprotein receptor-related protein (LRP)
Pattern recognition receptor (PRR)
Innate immunity

ABSTRACT

Low-density lipoprotein receptor-related protein (LRP) is a group of important endocytic receptors contributing to binding ligands and maintaining internal environment. In this study, we identified a soluble LRP-like molecule in the amphioxus *B. japonicum*, BjLRP, with an uncharacterized domain structure combination of LY-EGF-CRD-EGF-CRD. It was mainly expressed in the gill, muscle, notochord and testis, and was significantly up-regulated following the challenge with bacteria. Recombinant BjLRP was capable of interacting with both Gram-negative and positive bacteria as well as PAMPs including lipopolysaccharide (LPS), lipoteichoic acid (LTA) and peptidoglycan (PGN). Interestingly, recombinant LY peptide was also able to bind to the Gram-negative and positive bacteria as well as the PAMPs LPS, LTA and PGN. By contrast, none of recombinant EGF1, EGF2, CRD1 and CRD2 had affinity to the bacteria and the PAMPs. In addition, BjLRPΔLY had no affinity to the PAMPs, although BjLRPΔLY showed slight affinity to the bacteria. These suggest that the interaction of BjLRP with the bacteria and PAMPs was primarily attributable to the LY domain. It is clear that BjLRP is a novel pattern recognition protein capable of identifying and interacting with invading bacteria in amphioxus.

## 1. Introduction

Low-density lipoprotein receptor (LDLR)-related protein (LRP) belongs to LDLR family, which includes very low-density lipoprotein receptor (VLDLR), ApoE receptor, megalin and multiple EGF-repeat-containing protein as well (Gliemann, 1998; Howell and Herz, 2001). LRP plays a major role in the bulk clearance of lipoproteins, plasma proteases and protease-inhibitor complexes (May et al., 2007; Lillis et al., 2008; Nykjaer and Willnow, 2002). It is also an immune-relevant molecule functioning in innate immunity. For example, in macrophages, LRP1 is able to regulate NF-κB activation and microRNA-155 expression to mediate the inflammatory response (Mantuano et al., 2016).

Members of LRP family usually have five conserved characteristic structural and functional domains, including LDLR class A repeats, EGF precursor-like repeats, LDLR class B repeats containing YWTD motif (LY domains), membrane-spanning region, and intracellular domain containing at least one internalization signal sequence (NPXY motif) (Grimsley et al., 1999; Lillis et al., 2005; Yochem and Greenwald, 1993). However, some LRP-like proteins are found to lack transmembrane and intracellular region, existing in soluble form (Grimsley et al., 1999; Grimsley et al., 1998; Quinn et al., 1999; Deane et al., 2008). Soluble LRP-like proteins have been shown to be present in human plasma (Quinn et al., 1997), which are linked with antiviral immunity or diseases such as Alzheimer's disease and atherosclerosis (de Gonzalo-Calvo et al., 2015, 2016; Fischer et al., 1993; Liang et al., 2013).

Twelve different classes of LRPs, including LRP1, LRP1B, LRP2, LRP3, LRP4, LRP5, LRP6, LRP8, LRP10, LRP11, LRP12 and LRP13, have been identified in vertebrate species such as humans, chicken and fishes (Nykjaer and Willnow, 2002; Reading et al., 2014; Stifani et al., 1991). LRP-like proteins have also been identified in invertebrates such as nematode *Caenorhabditis elegans* (Yochem and Greenwald, 1993), fruit

*Abbreviations:* BSA, bovine serum albumin; CRD, carbohydrate-recognition domain; DNA, deoxyribonucleic acid; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; h, hour; HRP, horseradish peroxidase; IPTG, isopropyl β-D-thiogalactoside; KDa, kilodalton(s); LB, Luria-Bertani (medium); LY, LDLR class B repeats containing YWTD motif; LPS, lipopolysaccharide; LRP, low-density lipoprotein receptor-related protein; LTA, lipoteichoic acid; M, mole per liter; min, minute; MW, molecular weight; ORF, open reading frame; PAGE, PA-gel electrophoresis; PAMPs, pathogen-associated molecular patterns; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PGN, peptidoglycan; pH, potential of hydrogen; PRR, pattern recognition receptor; qRT-PCR, quantitative real-time PCR; RACE, rapid-amplification of cDNA ends; RNA, ribonucleic acid; s, second; SDS, sodium dodecyl sulfate; TBS, tris-buffered saline; Tris, tris (hydroxymethyl) aminomethane

* Correspondence to: G. Ji, Room 302, Darwin Building, 5 Yushan Road, Ocean University of China, Qingdao 266003, China.
** Correspondence to: S. Zhang, Room 205, Ke Xue Guan, 5 Yushan Road, Ocean University of China, Qingdao 266003, China.
*E-mail addresses:* jamesdong@ouc.edu.cn (G. Ji), sczhang@ouc.edu.cn (S. Zhang).

http://dx.doi.org/10.1016/j.gene.2017.04.011
Received 27 December 2016; Received in revised form 27 March 2017; Accepted 7 April 2017
Available online 08 April 2017
0378-1119/ 

fly *Drosophila* (Sappington and Raikhel, 1998), giant clam (Mikhailenko et al., 1997) and scallop (Liu et al., 2014). Interestingly, most LRP-like proteins in invertebrates are found to possess structures similar to the soluble form of LRPs in vertebrates, and play immune roles. It has been shown that in nematode, LRP-like protein serves as a receptor related to the phagocytosis of apoptotic cells (Su et al., 2002), and in scallop, LRP-like protein acts as a receptor possibly involved in the recognition and elimination of invading pathogens (Liu et al., 2014). However, information regarding the functions of LRP-like proteins in invertebrates remains rather limited.

Amphioxus or lancelet, a cephalochordate, represents the most basal extant chordate lineage (Putnam et al., 2008). About 20 LRP and LRP-like genes have been annotated in the genome of the amphioxus *Branchiostoma floridae* (http://genome.jgi-psf.org//Brafl1/Brafl1.home.html), but none of them have been functionally characterized to date. In this study, we reported the identification and functional characterization of a novel member of LRP-like protein in the amphioxus *B. japonicum*.

## 2. Materials and methods

### 2.1. Cloning and sequencing of amphioxus LRP-like gene (Bjlrp)

Adult *B. japonicum* collected from the vicinity of Qingdao, China, were ground in RNAiso plus (TaKaRa, China), and total RNAs were extracted with TRIzol (TaKaRa, China) from *B. japonicum* according to the manufacturer's instructions. After digestion with the recombinant RNase-free DNase (TaKaRa) to eliminate the genomic contamination, the cDNAs were synthesized with reverse transcription kit (TaKaRa) with oligo d(T) primer. A partial fragment of *Bjlrp* cDNA was amplified by PCR with the primer pair P1 and P2 (Table 1), that were designed on the basis of a LRP-like gene sequence found in *B. belcheri* genome database (http://mosas.sysu.edu.cn/genome/). To obtain the complete cDNA sequence, 3′ and 5′ RACEs were performed using the BD SMART™ RACE cDNA amplification kit (Clontech, China) according to the instructions, with the gene-specific primer pairs, P3 and P4, and P5 and P6 (Table 1), respectively. The clones obtained were sequenced and the overlapping sequences assembled. According to the cDNA sequence assembled, the full-length ORF of *Bjlrp* was obtained by PCR with the primer pair P7 and P8 (Table 1) and verified by sequencing. The deduced protein was analyzed using the SMART program (http://smart.embl-heidelberg.de/).

### 2.2. Quantitative real-time PCR (qRT-PCR)

Total RNAs were extracted from the various tissues of *B. japonicum*, including the hepatic caecum, hind gut, gill, muscle, notochord, testis, and ovaries, and digested with RNase-free DNase. The cDNAs were synthesized as described above and used as template for qRT-PCR, which was performed on ABI 7500 real-time PCR system (Applied Biosystems) as described by Wang et al. (2009b). The gene-specific primers P9 and P10 (Table 1) were used to amplify a product of 129 bp. The *EF1α* gene (which has the gene-specific primers P11 and P12) was chosen as the reference for internal standardization (Wang and Zhang, 2012). The reaction of each sample was performed in triplicate. At the end of each PCR reaction, dissociation analysis was performed to confirm the amplification specificity. Data were analyzed using ABI 7500 SDS software (Applied Biosystems), and quantified with the comparative Ct method ($2^{-\Delta\Delta Ct}$) based on Ct values for *Bjlrp* and *EF1α* in order to calculate the relative mRNA expression level (Livak and Schmittgen, 2001).

qRT-PCR was also performed to assay the expression profiles of *Bjlrp* in response to the challenge with the Gram-positive bacterium *Micrococcus luteus* and the Gram-negative bacterium *Vibrio anguillarum* according to the method of Wang et al. (2009a). The animals *B. japonicum* were exposed to sterilized seawater with *M. luteus* ($10^8$ cells/ml) or *V. anguillarum* ($10^8$ cells/ml), respectively, and sampled at 0, 2, 4, 8, 12, 24 and 48 h after the exposure. The animals cultured in sterilized seawater were used as control. Total RNAs were prepared with Trizol from the whole animals, and the extraction of total RNAs, cDNA synthesis, and qRT-PCR were carried out as above.

**Table 1**
Sequences of the primers used in this study.

| Primer | Sequence (5′ to 3′) | Sequence information |
|---|---|---|
| P1 (S) | ATACACGACACGGGACTACAAGAG | cDNA fragment primer |
| P2 (AS) | TTGTTTGACAGATGTATCTTATACG | cDNA fragment primer |
| P3 (S) | AACTGCCGCTGGTCGATCCGACA | 3′-RACE PCR primer |
| P4 (S) | CCACCCATAACTACTTGAAGAGCA | 3′-RACE PCR primer |
| P5 (AS) | GTTTGCACGCACCACCCTG | 5′-RACE PCR primer |
| P6 (AS) | GGTCCGAGGACTCGATCTTGTTC | 5′-RACE PCR primer |
| P7 (S) | ATGGCCGTGCTTCAGCTCCTG | ORF primer |
| P8 (AS) | CTACACGGGGTTTGTTTGACAG | ORF primer |
| P9 (S) | GGCGGCACCTGTTACATCAC | Realtime PCR primer |
| P10 (AS) | GATATGGTAGCACTTCCCGTTG | Realtime PCR primer |
| P11 (S) | TGCTGATTGTGGCTGCTGGTACTG | Realtime PCR primer |
| P12 (AS) | GGTGTAGGCCAGCAGGGCGTG | Realtime PCR primer |
| P13 (S) | GGAATTCAGAGTTCTGGTTAAACGACAG | Recombinant primer (BjLRP) |
| P14 (AS) | CCGCTCGAGTTCATGTTTGACAGATGTACCTCA | Recombinant primer (BjLRP) |
| P15 (S) | GGAATTCCACACCATCGTCTACGAC | Recombinant primer (LY) |
| P16 (AS) | CCGCTCGAGTTCACCTGTTGCTGCCGTCCATG | Recombinant primer (LY) |
| P17 (S) | GGAATTCGGGTGCGACCCCAACCCGTG | Recombinant primer (EGF1) |
| P18 (AS) | CCGCTCGAGTTCATTCGCACATCCGTCCGGTGAA | Recombinant primer (EGF1) |
| P19 (S) | GGAATTCTGTGAGGATGGCTGGCATC | Recombinant primer (CRD1) |
| P20 (AS) | CCGCTCGAGTTCATTCGCAGATGAAAAAGTTC | Recombinant primer (CRD1) |
| P21 (S) | GGAATTCCCCTGCGACTCGGCGCCCTGT | Recombinant primer (EGF2) |
| P22 (AS) | CCGCTCGAGTTCATTGGCAGTTGTCTCCCGTGT | Recombinant primer (EGF2) |
| P23 (S) | GGAATTCTGCCCATCTGGCTACCAG | Recombinant primer (CRD2) |
| P24 (AS) | CCGCTCGAGTTCATGTTTGACAGATGTACCTCA | Recombinant primer (CRD2) |
| P25 (S) | GGAATTCGAGTCATTCCTGACCATCCAAGG | Recombinant primer (BjLRPΔLY) |
| P26 (AS) | CCGCTCGAGTTCATGTTTGACAGATGTACCTCA | Recombinant primer (BjLRPΔLY) |

The abbreviations, S and AS, represent Sense and Antisense, respectively.