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Molecular cloning and functional characterization of a homolog of the transcriptional regulator CSL in *Litopenaeus vannamei*

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ABSTRACT

The Notch signaling pathway transcriptional regulator, CSL (also called as CBF1, Suppressor of Hairless or Lag-1 in different species, generally designated as CSL1), is not only associated with cell proliferation and differentiation but also involved in tumorigenesis, inflammation and immune regulation in vertebrates. We recently showed that Notch signaling was involved in the immune response of *Litopenaeus vannamei* shrimp. However, as an important transcriptional regulator of this pathway, whether or not shrimp CSL was also involved in immune response had not been explored. Here, we cloned and characterized the CSL gene in *L. vannamei* (LvCSL), which has a 2271 bp open reading frame (ORF) encoding a putative protein of 756 amino acids, and contains two conserved Lag1-DNA bind as well as beta trefoil domains (BTD). LvCSL clustered with invertebrates in the phylogenetic tree and closely related to the RBP Jk X1 of *Parasteatoda tepidariorum*. The transcript level of LvCSL analyzed by quantitative polymerase chain reaction (qPCR) showed that LvCSL was widely expressed in all tissues tested, with induced levels observed in the hepatopancreas and hemocytes following immune challenge with *Vibrio parahaemolyticus*, *Streptoccocus iniae*, lipopolysaccharide (LPS), and white spot syndrome virus (WSSV), therefore, suggesting LvCSL involvement in shrimp immune response to pathogens. Besides, LvCSL knockdown decreased the expression of proliferation-related genes (LvHey2 and LvAstakine), and attenuated the expression of immune-related genes *L. vannamei* hypoxia inducible factor alpha (LvHIF-α), LvLectin and *L. vannamei* small subunit hemocyanin (LvHMCS) in shrimp hemocytes, as well as significantly decreased total hemocyte count. Moreover, high cumulative mortality was observed in LvCSL depleted shrimp challenged with *V. parahaemoliticus*. In conclusion, our present data strongly suggest that LvCSL is an important factor in shrimp, vital for shrimp survival and contributing to immune resistance to pathogens.

1. Introduction

Litopenaeus vannamei also known as Pacific white shrimp, is an important global aquaculture shrimp species with huge annual output and great economic benefits. The global shrimp production has increased in recent decades due to increase demand for seafood. It is estimated that the world production of *L. vannamei* reached 3.6 million tons in 2014 (Food and Agriculture Organization, FAO, 2018). Unfortunately, the shrimp aquaculture industry suffers from various diseases and infections including Acute Hepatopancreatic Necrosis Syndrome (AHPNS) (Theethakaew et al., 2017), Taura syndrome virus (Tumburu et al., 2012), white spot syndrome virus (Amarakoon et al., 2016) and white feces syndrome (WFS) (Tang et al., 2016) infections, resulting in severe economic losses (Kalaimani et al., 2013). There is therefore the need to properly understand the shrimp immune system so as to be able to devise innovative and effective preventive measures for a sustainable shrimp aquaculture development (Thitamadee et al., 2016).

The Notch signaling pathway is a highly conserved pathway from invertebrates to vertebrates (Artavanis-Tsakonas and Fortini, 1995), and was originally discovered as a cell fate regulation pathway in Drosophila (Muskavitch, 1994). Although primordially considered a pathway that plays important roles in cell development (Bolos et al., 2007), as in blood cells (Terrientefelix et al., 2013), lymphocytes (Tanigaki and Honjo, 2007) and neurons (Louvi and Artavanistsakonas, 2006), an increasing number of reports have shown that the Notch signaling pathway is also involved in innate and adaptive immunity (Ito et al., 2012; Radtke et al., 2013). For instance, genes involved in this

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Abbreviations

ACD	precooled acid citrate dextrose
AHPNS	Acute Hepatopancreatic Necrosis Syndrome
ATFS-1	activating transcription factor associated with stress-1
BTD	beta trefoil domains
CSL, CBF1	Suppressor of Hairless or Lag-1
dsRNA	double-strand RNA
EGFP	enhanced green fluorescent protein
FAO	Food and Agriculture Organization
GBI	Beijing Genomics Institute
HIF-α	hypoxia inducible factor alpha
HMCS	small subunit of hemocyanin
hpi	hours post injection
HPT	proliferation of hematopoietic tissue
IL-6	Interleukin-6
LPS	lipopolysaccharides
LvATFS-1	*Litopenaeus vannamei activating transcription factor associated with stress-1*
LvCSL, CSL	gene in *Litopenaeus vannamei*
LvEF1α	elongation factor 1 alpha gene of *Litopenaeus vannamei*
LvHSF1	*Litopenaeus vannamei* heat shock transcription factor 1
MSCs	mesenchymal stem cells
NF-κB	nuclear factor kappa B
NJ	neighbor-joining
ORF	open reading frame
PBS	phosphate buffer saline
qPCR	quantitative polymerase chain reaction
RA	rheumatoid arthritis
RACE	rapid-amplification of cDNA ends
RNAi	RNA interference
SE	standard error
SMART	simple modular architecture research tool
THC	total hemocytes count
TRP120	tandem repeat protein 120
WFS	white feces syndrome
WSSV	white spot syndrome virus

pathway are shown to be up-regulated in mesenchymal stem cells (MSCs) from rheumatoid arthritis (RA) mice (Zhang et al., 2014). The canonical Notch signaling pathway could be activated by type 1 secreted tandem repeat protein 120 (TRP120) effectors to promote the intracellular survival of *Ehrlichia chaffeensis* (Lina et al., 2016), while Notch inhibition in mature T cells impaired its anti-fungal functions (Neal et al., 2017). The role of Notch in immunity is not only limited to vertebrates, as our recent studies revealed that Notch played important roles in shrimp immune system (Ning et al., 2017).

The transcriptional regulator CSL (also called as CBF1, Suppressor of Hairless or Lag-1 in different species, generally designated as CSL) is a sequence-specific DNA binding protein that functions as a transcriptional repressor in the Notch signaling pathway (Mumm and Kopan, 2000). CSL also plays an important role in the development of many cells such as lymphocyes (Maskus, 2007), intra-embryonic hematopoietic cells (Robertmoreno et al., 2005), and neural stem cells (Ehm et al., 2010). Recent studies have reported that CSL also plays essential roles in the immune system. For example, it has been shown that CBF 1 and p65 could coregulate IL-6 expression via competitive binding to the same target site in the IL-6 gene (Kannabiran et al., 1997; Mann et al., 2002). It has also been reported that RBP Jk heterozygous disrupted Notch signaling, leading to aortic valve disease, while this phenotype was absent in Notch1 heterozygous mice (Nus et al., 2011). Given that we recently showed that *L. vannamei* Notch played important roles in shrimp immune response (Ning et al., 2017), we wondered whether or not CSL as a Notch signaling pathway transcriptional regulator was also involved in shrimp immunity.

In this study, full-length CSL from shrimp *L. vannamei* (LvCSL) was cloned and its role in immunity was characterized. It was observed that LvCSL was widely distributed in all the tissues tested and its expression

Table 1
Primers and dsRNA sequences used in this study.

Primer	Sequence (5′-3′)
RACE	
LvCSL-5RACE1	GGAGTCAAGGAGCCCGCAAGCAT
LvCSL-5RACE2	TCGTGGGTAGGTTCCGTCCAGTCC
LvCSL-3RACE1	CGCTGCCCAGACTCATCATCCGG
LvCSL-3RACE2	TTCACATACACACCCGAGCCTGGC
Real-time RT-PCR	
LvCSL-F	GAGAACACGCAAATTAACCCAG
LvCSL-R	GCCATCATTACGGACTAAAGACA
LvHey2-F	TGGTGGAGTCGGAGGGCTTCTTT
LvHey2-R	CTATTACCGTTGCCGTCGCTGGA
LvAstakine-F	GCCTGCCTTGTGGTGTCA
LvAstakine-R	ACGCAAGATTCAGCTCCC
LvHIF-α-F	TACAGACCCCACCCATTACCAG
LvHIF-α-R	CCCTCCGTCAAAGAACTTGCT
LvLectin-F	TCAGAACTGCCTTGCGATCAC
LvLectin-R	CACGCCATTTGCTCATCCA
LvHMCS-F	CCTGGCCTCATAAAGACAACA
LvHMCS-R	TTTTCCACCCTTCAAAGATACC
LvEF-1α-F	TATGCTCCTTTTGGACGTTTTGC
LvEF-1α-R	CCTTTTCTGCGGCCTTGGTAG
dsRNA	
dsLvCSL-F	TGGGTTCGTCAGCCTACACA
dsLvCSL-R	TTCGGGAGCATTTCACAGC
dsLvCSL-T7F	GGATCCTAATACGACTCACTATAGGTGGGTTCGTCAGCCTACACA
dsLvCSL-T7R	GGATCCTAATACGACTCACTATAGGTTCGGGAGCATTTCACAGC
dsEGFP-F	TACGGCGTGCAGTGCTTCAG
dsEGFP-R	CTTCACCTCGGCGCGGGTCTTG
dsEGFP-T7F	GGATCCTAATACGACTCACTATAGGCTTCACCTCGGCGCGGGTCTT
dsEGFP-T7R	GGATCCTAATACGACTCACTATAGGTACGGCGTGCAGTGCTTCAG