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Identification and characterization of transforming growth factor β-activated kinase 1 from *Litopenaeus vannamei* involved in anti-bacterial host defense

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ABSTRACT

LvTAK1, a member of transforming growth factor β-activated kinase 1 (TAK1) families, has been identified from *Litopenaeus vannamei* in this study. The full length of LvTAK1 is 2670 bp, including a 2277 bp open reading frame (ORF) that encoded a putative protein of 758 amino acids with a calculated molecular weight of ~83.4 kDa LvTAK1 expression was most abundant in muscles and was up-regulated in gills after LPS, Vibrio parahaemolyticus, Staphhylococcu saureus, Poly (I:C) and WSSV challenge. Both *in vivo* and *in vitro* experiments indicated that LvTAK1 could activate the expression of several antimicrobial peptide genes (AMPs). In addition, the dsRNA-mediated knockdown of LvTAK1 enhanced the susceptibility of shrimps to Vibrio parahaemolyticus, a kind of Gram-negative bacteria. These results suggested LvTAK1 played important roles in anti-bacterial infection. CoIP and subcellular localization assay demonstrated that LvTAK1 could interact with its binding protein LvTAB2, a key component of IMD pathway. Moreover, over-expression of LvTAK1 in *Drosophila* S2 cell could strongly induce the promoter activity of Diptericin (Dpt), a typical AMP which is used to read out of the activation of IMD pathway. These findings suggested that LvTAK1 could function as a component of IMD pathway. Interestingly, with the over-expression of LvTAK1 in S2 cell, the promoter activity of Metchnikowin (Mtk), a main target gene of Toll/Dif pathway, was up-regulated over 30 times, suggesting that LvTAK1 may also take part in signal transduction of the Toll pathway. In conclusion, we provided some evidences that the involvement of LvTAK1 in the regulation of both Toll and IMD pathways, as well as innate immune against bacterial infection in shrimp.

1. Introduction

Transforming growth factor β-activated kinase 1 (TAK1), a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family, functions as a key signaling molecule in innate immune signaling pathways. TAK1 can be activated by various stimuli including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), B and T cell receptor (BCR and TCR) ligands and Toll-like receptor (TLR) ligands [1,2]. The activated TAK1 then regulates cell viability, necroptosis and inflammation through activating several downstream signaling events including mitogen-activated protein kinases (MAPKs) and NF-κB pathways, as well as NF-κB independent pathways such as oxidative stress and receptor-interacting protein kinase 1 (RIPK1) kinase activity-dependent pathway [2,3].

In mammals, after stimulated by IL-1β and TLR ligands, the adaptor molecular myeloid differentiation primary response gene

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88 (MyD88) activates IRAK1 and IRAK4, resulting in the recruitment of E3 ubiquitin ligase tumor necrosis factor (TNF)-receptor-associated factor 6 (TRAF6) and its accessory factors ubiquitin-conjugating enzyme 13 (Ubc13) and ubiquitin E2 variant 1a (Uev1A) [4]. On the other hand, with the stimulation of TNF-α, TRAF2 and TRAF5, instead of TRAF6, are recruited by another adaptor molecular receptor-interacting protein 1 (RIP1) in a MyD88-independent manner [5,6]. In conjunction with Ubc13/Uev1A, TRAF2/5/6 can catalyze lysine (K)-63 polyubiquitination (Ubi) of the TAK1 protein [7]. The polyubiquitin of TAK1 is bound to its accessory protein TAB1 and the homologs TAB2 or TAB3 so that TAK1 can exist as a heterotrimeric complex composed of TAK1—TAB1—TAB2 or TAK1—TAB1—TAB3 [7–9]. The TAK1 complex is then able to phosphorylate and activate mitogen-activated protein kinases (MAPKs) and IκB kinases (IKK), thereby terminating in the activation of activator protein 1 (AP1) and NF-κB respectively, both of which enhance the transcriptional expression of pro- or anti-inflammatory factors such as IL-6, TNF-α and IL-1β [2].

The role of TAK1 in innate immunity is evolutionarily conversed from flies to mammals [10]. In *Drosophila*, homologs of TAK1 and TAB2 have been identified as essential members for immune deficiency (IMD) pathway against microbial pathogens [11,12]. When challenged with Gram-negative bacteria, IMD pathway is activated through the binding of *meso*-diaminopimelic acid (DAP)-type peptidoglycan (PGN) to specific peptidylglycan recognition proteins (PGRPs), which results in the recruitment of a signaling complex consisting of the IMD, a death domain protein homologous to mammalian RIP1, an adaptor protein FADD, and a caspase-8 homolog Dredd [13]. IMD, after cleaved by Dredd, then recruits and activates TAK1/TAB2 complex that leads to the cleavage and activation of Relish [14,15]. The N-terminal fragment of Relish then migrates to the nucleus to trigger the expression of a battery of antimicrobial peptides including Cecropins, Drosocin, Attacins and Diptericin, among which the Diptericin (Dpt) is commonly used as a read-out of the activation of IMD pathway [16,17].

As for shrimp, Toll, IMD and JAK/STAT pathways are regarded as the main pathways regulating the innate immune response [18]. Unlike the Toll and the JAK/STAT pathways that have been studied clearly, only a few components of IMD pathway have been identified in shrimp [19,20]. Thus, the knowledge on the role of IMD pathway in the immune response of shrimp is very limited. LvTAB2, an important component of IMD pathway, has been reported recently to play a role in bacterial and viral infection [21]. However, until now, no homolog of TAK1 has been characterized in Crustacea and thus this arouses our interest. In this study, LvTAK1, the first homolog of TAK1 in shrimp (*Litopenaeus vannamei*), is cloned for the first time. In addition, our results demonstrated that LvTAK1 could bind with its binding protein LvTAB2 and played a protective role against Vibrio *parahemolyticus* infection.

2. Materials and methods

2.1. Cloning of full length of LvTAK1 cDNA

Using the SMARTer PCR cDNA Synthesis Kit (Clontech) and the rapid amplification cDNA ends (RACE) method, we cloned the full

Table 1
Summary of primers in this study.

Name	Sequence (5′–3′)
RACE	
LvTAK1-5RACE1	GTAAAAGGCATTGACCTCTGATTCCG
LvTAK1-5RACE2	CTCCGAATGATCCTTTTCCAATACTCTC
LvTAK1-3RACE1	CCTCCACCTCCCATCCTGCCAAC
LvTAK1-3RACE2	CCGCATGTACGGGGACATGGTTC
Real-time RT-PCR	
LvEF-1α-F	TATGCTCCTTTTGGACGTTTTGC
LvEF-1α-R	CCTTTTCTGCGGCCTTGGTAG
LvTAK1-F	ATGGAATATGCTGATGGTGGATC
LvTAK1-R	TGGCTGTATGGAGTGGAGGTAG
LvLYZ1-F	TACGCGACCGATTACTGGCTAC
LvLYZ1-R	AGTCTTTGCTGCGACCACATTC
LvLYZ2-F	CCCATGTTCCGATCTGATGTC
LvLYZ2-R	CACTTGCTGTTGTAAGCCACC
LvLYZ4-F	ACGATGGAAGGGCAAAGGAG
LvLYZ4-R	AATAGGCAACACTTGATACTGAATGG
RT-PCR	
LvEF-1α-F	TCGCTTCAAGGAAATCCACAAG
LvEF-1α-R	AAGGTCTCCACGCACATAGGC
LvTAK1-F	ATGGAATATGCTGATGGTGGATC
LvTAK1- R	CAACTGCCACTGTTATTGGTGTCTA
Protein expression	
LvTAK1-F	GGGGTACCATGCACATATCTGCCATGGATA
LvTAK1-R	TTGGGCCCGAGGGGGGTCAGGGAGAGGT
RFP-F	GGTTCGAAATGGCCTCCTCCGAGAACGTC
RFP-R	GGGTTTAAACTTACAGGAACAGGTGGTGGCGGCCCTCGGTGCGCTCGTACTGCTCCAC
Dual-luciferase reporter assay	
PGL3-DmDpt-F	TTGGTACCAGCTATGGTCAGTTAGTACACG
PGL3-DmDpt-R	GGAGATCTCAAAGTAGAAGCGATTGCGCAGC
DsRNA templates amplification	
dsRNA-LvTAK1-T7-F	GGATCCTAATACGACTCACTATAGGATGGAATATGCTGATGGTGGATC
dsLvTAK1-R	CAACTGCCACTGTTATTGGTGTCTA
dsLvTAK1-F	ATGGAATATGCTGATGGTGGATC
dsRNA-LvTAK1-T7-R	GGATCCTAATACGACTCACTATAGGCAACTGCCACTGTTATTGGTGTCTA
dsRNA-GFP-T7-F	GGATCCTAATACGACTCACTATAGGCGACGTAAACGGCCACAAGTT
dsRNA-GFP-R	ATGGGGGTGTTCTGCTGGTAG
dsRNA-GFP-F	CGACGTAAACGGCCACAAGTT
dsRNA-GFP-T7-R	GGATCCTAATACGACTCACTATAGGATGGGGGTGTTCTGCTGGTAG