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Characterization and immune function of the thioredoxin-interacting protein (TXNIP) from *Litopenaeus vannamei*

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ABSTRACT

The thioredoxin (Trx) system plays essential roles in maintenance and regulation of the redox state of cysteine residues in cellular proteins. The Trx-interacting protein (TXNIP) is a TRX inhibitory protein that works as a negative regulator in the TRX system. The function of TXNIP in invertebrates, in particular in immunity, remains unclear to date. In the current study, a novel TXNIP from Pacific white shrimp *Litopenaeus vannamei* was identified and characterized and its roles in immune responses was investigated. TXNIP could interact with Trx and inhibit its redox regulatory activity, suggesting that TXNIP was involved in regulation of the cellular redox state in shrimp. The expression of TXNIP was high in the stomach, gill, scape, eyestalk, epithelium, pyloric and muscle and low in the hepatopancreas, intestine, nerve, hemocytes and heart. Stimulations with pathogens white spot syndrome virus (WSSV) and *Vibrio parahaemolyticus* and immune stimulants poly (I:C) and LPS could significantly increase the expression of TXNIP *in vivo*. Silencing of TXNIP using RNAi strategy significantly facilitated the infection of *V. parahaemolyticus* but inhibited the infection of WSSV in shrimp. These indicated that TXNIP could be positively involved in antibacterial responses but negatively involved in antiviral responses in shrimp. Moreover, knockdown of TXNIP *in vivo* exerted opposite effects on expression of antimicrobial peptides anti-lipopolysaccharide factors and penaeidins and enhanced the phagocytic activity of hemocytes against bacteria. These suggested that TXNIP could play a complex role in regulation of humoral and cellular immune responses in shrimp.

1. Introduction

Oxidative stress derived from reactive oxygen-, nitrogen- and sulfur species (ROS, RNS, RSS) can cause cell damage through destruction of cellular vital components such as proteins, lipids and nucleic acids [1–3]. It affects the structure and stability of various proteins through modification of the reversible reduction/oxidation (redox) state of cysteine residues [4,5]. The thioredoxin (TRX) system, consisting of TRX, TRX reductase (TRXR), NADPH and the cellular substrate proteins, is ubiquitously present in organisms from bacteria to human and constitutes an important part of the cellular responses against oxidative stress [6–8]. TRX is a high conserved protein that serves as an electron transport mediator in this important system. TRXR transfers electrons from the electron donor NAPDH to TRX, which further reduces the disulfide bonds in TRX-dependent proteins [9,10]. The thioredoxin-interacting protein (TXNIP), also called thioredoxin-binding protein-2/vitamin D3 upregulated protein 1 (TBP-2/VDUP1), belonging to the arrestin superfamily and inhibiting the disulfide reductase activity of TRX, is a negative regulator of the TRX system [11,12]. Studies in mammals have suggested that TXNIP plays important roles in regulation of cell growth, apoptosis, metabolism, and immune responses [11,13,14]. However, knowledge on the function of TXNIP in invertebrates remains limited to date.

Pacific white shrimp, *Litopenaeus vannamei*, has huge economic value and is one of the major aquaculture crustacean species on Earth [15]. The *L. vannamei* TRX (LvTRX) protein has been identified and

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Table 1
Primers for dsRNA production and real-time PCR.

Primers	Sequences(5′ to 3′)
dsRNA production	
TXNIP-dsR	GTCTTCTCCTCAAGCCATGGTC
TXNIP-dsF	CATGCATGACCCAAGGACCTGTG
TXNIP-dsT7F	GGATCCTAATACGACTCACTATAGGCATGCATGACCCAAGGACCTGTG
TXNIP-dsT7R	GGATCCTAATACGACTCACTATAGGGTCTTCTCCTCAAGCCATGGTC
dsGFP-F	ACGGCAAGCTGACCCTGAAG
dsGFP-R	GACTGGGTGCTCAGGTAGTGG
dsGFP-T7F	GGATCCTAATACGACTCACTATAGGACGGCAAGCTGACCCTGAAG
dsGFP-T7R	GGATCCTAATACGACTCACTATAGGGACTGGGTGCTCAGGTAGTGG
Real-time PCR	
TXNIP-qRTF	GTCTCGGACCGCAGGCAGCAG
TXNIP-qRTR	CTCACTGGCGTCTTCGGACTC
ALF1-qRTF	GGATGTGGTGTCCTGGATGG
ALF1-qRTR	GCGTCGTCCTCCGTGATG
ALF2-qRTF	GCGAACAAACTCACTGGACTG
ALF2-qRTR	ACATGCGACCCTGGAATACAG
ALF3-qRTF	GACCTGTCCAACCCTGAGC
ALF3-qRTR	TCGCCTCCTCCTCCGTTATC
PPO1-qRTF	TCTTCGCCTCACGCATCTC
PPO1-qRTR	TATCCTCACAGTCACCTCCTTC
SOD-qRTF	TTGCCGCTACGAAGAAGTTG
SOD-qRTR	AGAAGATGGTGTGGTTCAAGTG
PEN2-qRTF	CCAAGGCGAAGCGTACAG
PEN2-qRTR	CAATTGCGAGCATCTGAGAC
PEN4-qRTF	GCCCGTTACCCAAACCATC
PEN4-qRTR	AACAATCCCCGTATCTGAAGC
ie1-qRTF	GTTTTCTGTATGTAATGCGTGTAGG
ie1-qRTR	CCCACTCCATGGCCTTCA
Vpa-16s-qRTF	GGTGTAGCGGTGAAATGCGTAG
Vpa-16s-qRTR	CCACAACCTCCAAGTAGACATCG

functionally studied, which has antioxidant activity and is essentially involved in various biological processes [16–18]. The crystallographic structure of LvTRX has also been finely analyzed using X-ray crystallographic method, which provides a structural foundation for in-depth understanding of the TRX system [19,20]. However, the function of the invertebrate TRX system is still poorly understood. In this study, a novel TXNIP gene from *L. vannamei* was identified and its roles in humoral and cellular immune responses were investigated, which could help to further establish the functional roles of the TRX system in invertebrate immunity.

2. Materials and methods

2.1. Animals and pathogens

Pacific white shrimp (~10 g) were obtained from an aquaculture farm in Zhuhai, Guangdong Province and were randomly sampled to ensure to be free of white spot syndrome virus (WSSV) and *Vibrio parahaemolyticus* by PCR following previously described methods [21,22]. Shrimp were acclimated at ~28 °C for over one week in air-pumped seawater (1.0% salinity) in a recirculating water tank system before experiments. The stocks of *V. parahaemolyticus* and WSSV were prepared as previously described [23].

2.2. Cloning of the LvTXNIP gene

The expressed sequence tag (EST) homologous to TXNIP was retrieved from a previously reported *L. vannamei* transcriptome library [24]. The full open reading frame (ORF) of LvTXNIP mRNA was obtained by rapid amplification of cDNA ends (RACE) amplification using a SMARTer RACE cDNA Amplification kit (Clontech, Japan).

2.3. Bioinformatics analysis

The amino acid sequences of many TXNIP genes were obtained from the National Center for Biotechnology Information (NCBI) databases. Sequence alignments were made by Clustal W 1.8 and phylogenetic tree was generated by the neighbor-joining (NJ) method using MEGA 5.0 software with parameters as previously described [25].

2.4. Co-immunoprecipitation (Co-IP)

The open reading frames (ORFs) of *L. vannamei* TXNIP and TRX were cloned into the pAc5.1/V5-His A plasmid (Invitrogen, USA) to generate Flag- and V5-tagged expression vectors, respectively, and co-transfected into *Drosophila* Schneider 2 (S2) cells. A V5-tagged green fluorescent protein (GFP) expression vector was used as control. After 48 h, cells were harvested and lysed in NP-40 lysis buffer with a protease inhibitor cocktail (Sigma, USA). Co-immunoprecipitation was performed using anti-V5 affinity gel (Sigma, USA) and western-blot was performed with a rabbit anti-Flag primary antibody and a horse radish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (Sigma, USA).

2.5. Intracellular superoxide assay

The coding region of *L. vannamei* TXNIP, TRX and a maltose binding protein (MBP, as control) were cloned into the pAc5.1/V5-His A plasmid (Invitrogen, USA) to generate expression vectors and transfected into *Drosophila* S2 cells. At 48 h post infection, the intracellular superoxide level was measured with a Superoxide Assay Kit (Beyotime, China) according to the manufacturer's protocol. Cells were treated with 50 μM H_2O_2 for 4 h, triply washed with serum-free medium, and treated with serum-free medium containing Dihydroethidium (5 μM) for 30 min at 28 °C in the dark. The fluorescence intensity was measured using a BD Accuri C6 Flow Cytometer (USA) (300 nm excitation, 610 nm emission).

2.6. Real-time PCR

For examination of the distribution of LvTXNIP mRNA, various tissues were sampled from healthy *L. vannamei* as previously described [26]. For challenge experiments, shrimp were intramuscularly injected at the second abdominal segment with WSSV (10^4 copies), *V. parahaemolyticus* (10^6 CFU), LPS (5 μg), Poly I:C (5 μg), and PBS buffer (as control). Hemocytes and gill were randomly sampled from 9 shrimp at 0, 4, 12, 24, 48, 72 and 96 h post injection. RNA extraction, cDNA synthesis and real-time PCR were performed following the methods as previously described [26]. The EF-1α gene (Genbank accession No. GU136229) was used as internal control. Sequences of primers used in this study were listed in Table 1.

2.7. RNA interference

Double stranded RNAs (dsRNAs) specific to TXNIP (dsTXNIP) and green fluorescent protein (dsGFP, as control) were prepared by in vitro transcription using a T7 RiboMAX™ Express RNAi System (Promega, USA) following a previously described method [26]. Shrimp were injected with 50 μl PBS containing 5 μg LvTXNIP and GFP dsRNA (as control). The RNA interference (RNAi) efficiency was determined using real-time PCR. At 48 h post injection, nine shrimp were sampled and the RNA interference (RNAi) efficiency and the expression of many immune related genes in gill and hemocytes were measured using real-time PCR. The primers used were listed in Table 1. In parallel experiments, the dsRNA treated shrimp were further injected with 10^6 copies of *V. parahaemolyticus* and WSSV. At 48 h and 72 h post infection, the copy numbers of WSSV in muscle and *V. parahaemolyticus* in gill were determined using relative quantitative real-time PCR as previously described [27,28].

2.8. Phagocytic activity analysis

At 48 h post dsRNA injection, the phagocytic activity of hemocytes was measured following a previously described method [29]. Briefly, hemocytes were washed with 2× Leibovitz's L-15 medium (Gibco, USA) triply and mixed with fluorescein isothiocyanate (FITC)-labled *V. parahaemolyticus* at a 1:100 ratio of cells/bacteria. After incubation at 28 °C for 1 h, hemocytes was detected using cytometry for the signals of FITC and the forward scatter (FSC) values of cells. A FSC threshold was determined through detection of free FITC-labeled *V. parahaemolyticus* to eliminate cell debris and bacteria, and the fluorescence boundary was set based on detection of the self-fluorescence of untreated hemocytes. A total of 150,000 events were detected for each sample.

2.9. Statistical analysis

The statistical procedures were carried out using a Graphpad Prism software. The mean and standard deviation (SD) from three detections was calculated. Student's t-test was used to compare the two means. The Kaplan-Meier plot (log-rank $\chi 2$ test) was used to analyze the mortalities between different groups.

3. Results

3.1. Cloning and bioinformatics analysis of LvTXNIP

The ORF of *L. vannamei* TXNIP is 1044 bp encoding a protein of 347 amino acids with a calculated molecular weight of 39.95 kDa and a theoretical isoelectric point of 8.60 (Genbank accession No. MH551173) (Fig. 1A). The 8-160- and 184-315- residues regions of TXNIP match Arrestin N and C domains with expect scores of 3.3e-21 and 6.55e-24, respectively. Multiple-sequence alignment showed that *L vannamei* TXNIP shared identities of 75.4% with TXNIP from the crustacean cladoceran *Daphnia mana*, 76.1% with the insect dampwood termite *Zootermopsis nevadensis*, 21.0% with zebrafish *Danio rerio*, and 21.3% and 21.3% with mammals *Mus musculus* and *Homo sapiens*, respectively (Fig. 1B). In the constructed phylogenetic tree, the analyzed TXNIP genes could be categorized into two clades of vertebrates and invertebrates, and the *L. vannamei* TXNIP was closest clustered with the *D. mana* TXNIP in the invertebrates clade (Fig. 1C).

3.2. Properties of the TXNIP protein

The interaction of TXNIP with the previous reported *L. vannamei* TRX protein was analyzed using Co-IP (Fig. 2A). The Flag-tagged TXNIP protein could be co-precipitated with the V5-tagged TRX but not with the GFP protein, confirming the interaction between TRX and the novel identified TXNIP. The influence of TXNIP on the intracellular superoxide level of S2 cells transfected with expression vectors and treated with H_2O_2 was further investigated using flow cytometry (Fig. 2B). The results demonstrated that compared with the control, TRX significantly decreased the superoxide level in cells induced by H_2O_2 while TXNIP exerted an opposite effect. Furthermore, co-expression of TXNIP could attenuated the effect of TRX on the intracellular superoxide level. These suggested that TXNIP could function as an inhibitor of TRX in *L. vannamei*.

3.3. Tissue distribution and expression profile of TXNIP

The examination of the TXNIP mRNA distribution using real-time PCR demonstrated that TXNIP was expressed in all the detected tissues. Hepatopancreas expressed the lowest level of TXNIP (Fig. 3A). The highest expression of TXNIP was detected in stomach, which was 553.3-fold higher than that in the hepatopancreas. The expression of TXNIP in muscle, pyloric, epithelium, eyestalk, scape and gill were 78.6- to 276.6-fold higher than that in hepatopancreas. In contrast, intestine, nerve, hemocytes and heart expressed low levels of TXNIP, which were only 1.1- to 9.2-fold higher than that in hepatopancreas. To explore the role of TXNIP in immune responses, the expression of TXNIP in hemocytes and gill after immune stimulations was further detected (Fig. 3A and B). The results demonstrated that stimulation with both the pathogens and immune stimulants could increase the mRNA level of TXNIP in these two tissues. In hemocytes, the TXNIP expression was periodically increased after WSSV infection with two peaks of 3.9- and 3.2-fold increases at 24 and 72 h, respectively. This may suggest a complex regulatory mechanism underlying the expression of TXNIP after virus infection, which requires further exploration. The up-regulated levels of TXNIP expression were 3.8-fold at 24 h post *V. parahaemolyticus* infection, and only 1.0-fold at 24 h post ploy (I:C) stimulation and 1.2-fold at 24 h post LPS stimulation. In gills, the 3.8- and 2.2-fold increases of TXNIP expression at 24 h post WSSV infection and 72 h post *V. parahaemolyticus* infection were observed, respectively, while 1.5- to 1.9-fold up-regulations were detected at the early stage of ploy (I:C) and LPS stimulations, respectively. Obviously, the effects of the pathogens WSSV and *V. parahaemolyticus* on the TXNIP expression were more pronounced than those of ploy (I:C) and LPS.

3.4. Involvement of TXNIP in immunity

To investigate the role of TXNIP in antiviral and antibacterial responses, shrimp were injected with TXNIP-specific dsRNA to knockdown the expression of TXNIP and further challenged with pathogens. Compared with the control, silencing of TXNIP significantly increased the mortality of *V. parahaemolyticus*-infected shrimp at the later stage of infection and significantly up-regulated the bacterial load in gill, suggesting that TXNIP could play a positive role in antibacterial responses (Fig. 4A and C). However, the mortality of WSSV-infected shrimp was significantly decreased and the viral load in muscle was also down-regulated, indicating that TXNIP could facilitate the infection of WSSV in shrimp (Fig. 4B and D).

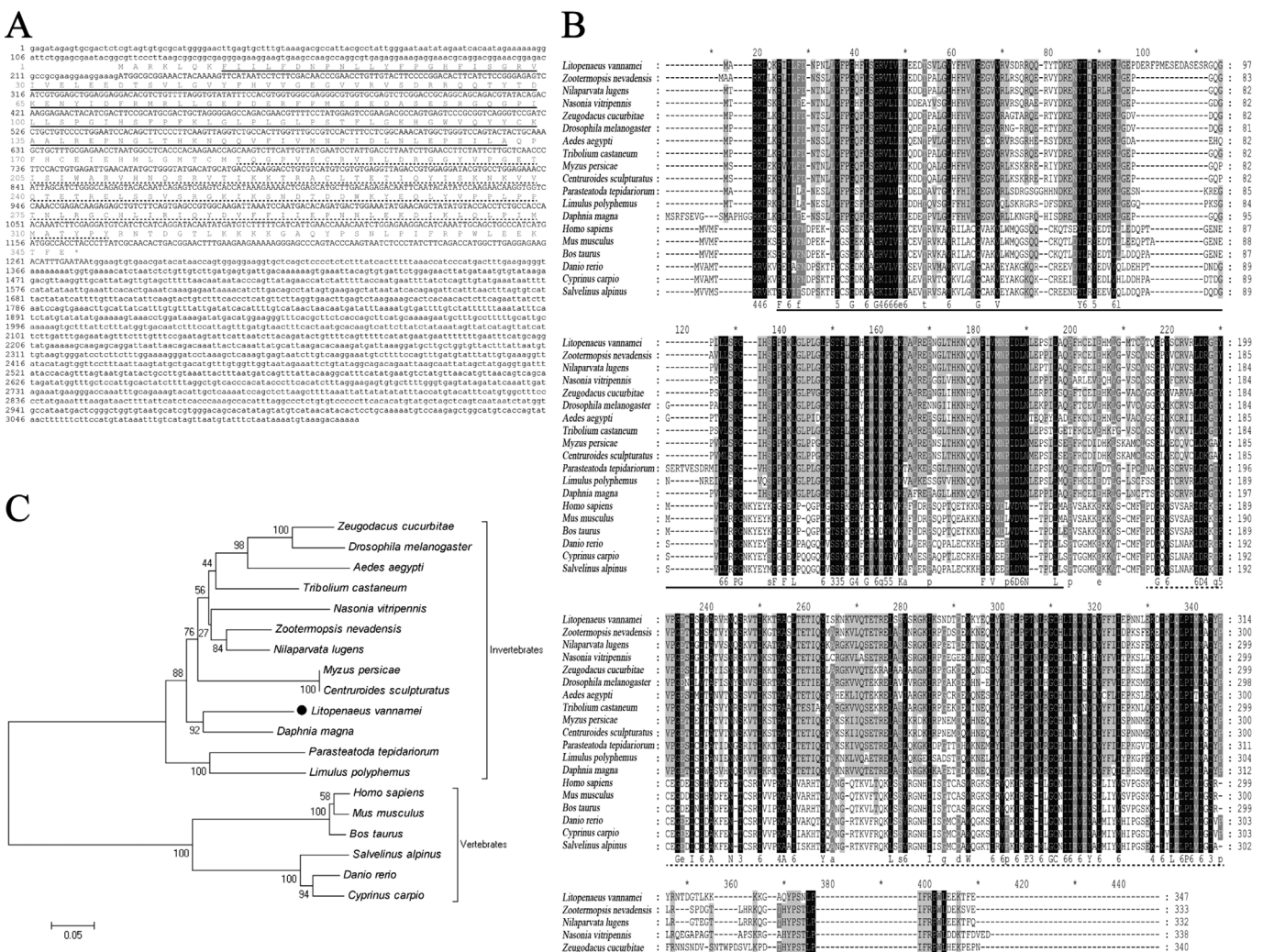

Fig. 1. Sequence analyses of *L. vannamei* TXNIP. (A) Nucleotide and deduced amino acid sequences of the TXNIP mRNA. The deduced amino acid sequence was shown above nucleotide sequence (lower case) and numbered on the left. The arrestin N and C domains were underlined with solid and dotted lines, respectively. (B) Multiple-sequence alignment of the deduced amino acid sequence of *L. vannamei* TXNIP with TXNIP proteins from other species. The identical amino acid residues were shaded in black and the similar residues in gray and the arrestin N and C domains were underlined with solid and dotted lines, respectively. (C) Neighbor joining phylogenetic tree analysis of the amino acid sequences of TXNIPs. The *L. vannamei* TXNIP was marked with black triangle. Proteins analyzed include: *Homo sapiens* (NM_006472.5), *Mus musculus* (NM_001009935.2), *Bos Taurus* (NM_001101875.2), *Danio rerio* (BC067376.1), *Cyprinus carpio* (XP_018951332.1), *Salvelinus alpinus* (XP_023830764.1), *Daphnia magna* (KZS06009.1), *Zootermopsis nevadensis* (XP_021930686.1), *Nilaparvata lugens* (XP_022194018.1), *Parasteatoda tepidariorum* (XP_015928754.1), *Myzus persicae* (XP_022178427.1), *Centruroides sculpturatus* (XP_015371360.1), *Limulus polyphemus* (XP_013787968.1), *Nasonia vitripennis* (XP_001604921.2), *Tribolium castaneum* (XP_972691.1), *Zeugodacus cucurbitae* (XP_011180640.1), *Aedes aegypti* (XP_001652563.1), *Drosophila melanogaster* (NP_612004.2).

To explore the underlying mechanism of the immune role of TXNIP, the expression of many immune related genes in gill and hepatopancreas of TXNIP-silenced shrimp at 48 h post dsRNA injection was examined using real-time PCR (Fig. 5A and B). Compared with the control, the expression of antimicrobial peptides anti-lipopolysaccharide factor 1 (ALF1), ALF2 and ALF3 and immune effector proteins polyphenoloxidase 1 (PPO1) and cytosolic manganese superoxide dismutase (C-MnSOD) was significantly down-regulated, while the expression of antimicrobial peptides penaeidin 2 (PEN2) and PEN4 was significantly up-regulated. Furthermore, the effect of TXNIP on the phagocytic function of shrimp hemocytes was investigated using flow cytometry (Fig. 5C). Compared with the control, silencing of TXNIP could significantly improve the phagocytic activity of hemocytes against *V. parahaemolyticus*, suggesting that TXNIP could negatively regulate the cellular immune responses in shrimp.

4. Discussion

The Trx system is a major player that is integral to maintaining the cellular redox homeostasis and antioxidant defenses [30]. As the central

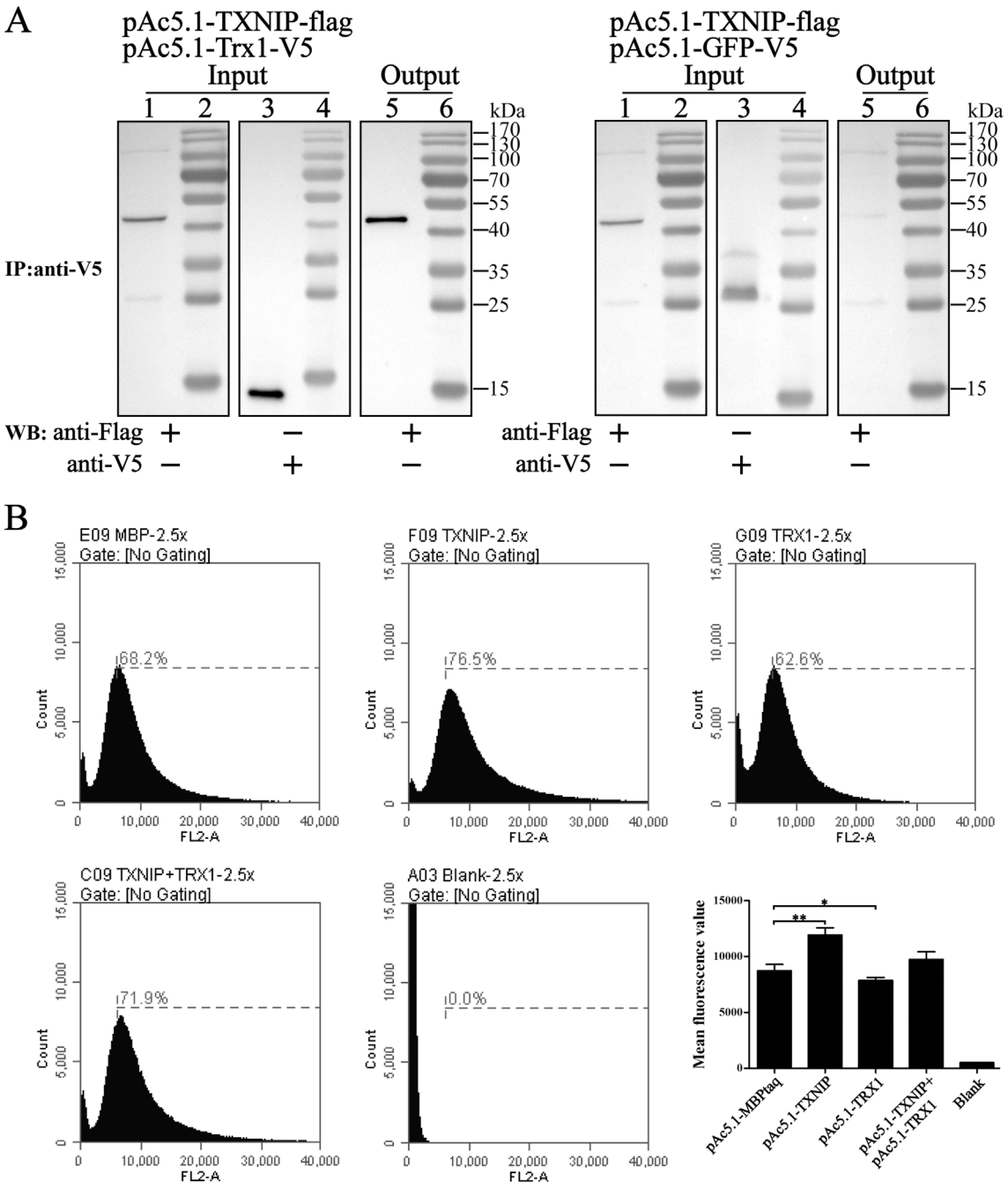

Fig. 2. Effects of *L. vannamei* TXNIP on activity of TRX. (A) Co-immunoprecipitation analysis of the interactions of flag-tagged TXNIP with V5-tagged TRX1 and GFP (as control). (B) Intracellular reactive oxygen species (ROS) assays. The intracellular superoxide level in S2 cells expressing MBP (as control), TXNIP, and TRX1, and co-expressing TXNIP and TRX, was measured using flow cytometry after treatment with 50 μM H_2O_2. The untreated cells were analyzed as a blank control. The histograms representing one of the three flow cytometric detections were shown and the data were provided in the lower right panel.

component of the Trx system, Trx has antioxidant activity and functions to defense against oxidative stress through regulating protein dithiol/disulfide balance [31]. The TRX system has been known to be implicated in immunity. In mammals, Trx is involved in regulation of various immune processes through inducing or inhibiting the expression of a series of cytokines, including IFN-γ, TNF, IL-1, IL-6 and IL-8, and MIF [32–34]. In *L. vannamei*, Trx can affect the transcription of inhibitor of nuclear factor κB kinase (IKK), p38 mitogen-activated protein kinase (LvP38) and signal transducer and activator of transcription (STAT) in the hemocytes and hepatopancreas, thus participating in regulation of their corresponding signaling pathways [18]. Recently, a novel member of the Trx superfamily, thioredoxin-related protein of 14 kDa (LvTRP14), also called the thioredoxin domain containing 17 protein (TXNDC17), was identified from *L. vannamei* [35]. LvTRP14 was found to be involved in antiviral and antibacterial immune responses through regulating the activity of NF-κB family members Relish and Dorsal as well as the expression of multiple antimicrobial peptides. These indicated that the cellular redox homeostasis controlled by the Trx system could be important for regulation of the immune system in both vertebrates and invertebrates. As a Trx-binding protein, TXNIP negatively regulates the activity of the Trx system to avoid over-tilting of the cellular redox balance to the reduction side [11]. Therefore, it is not surprising that TXNIP could be also involved in immunity. Accumulating evidence has established the important role of

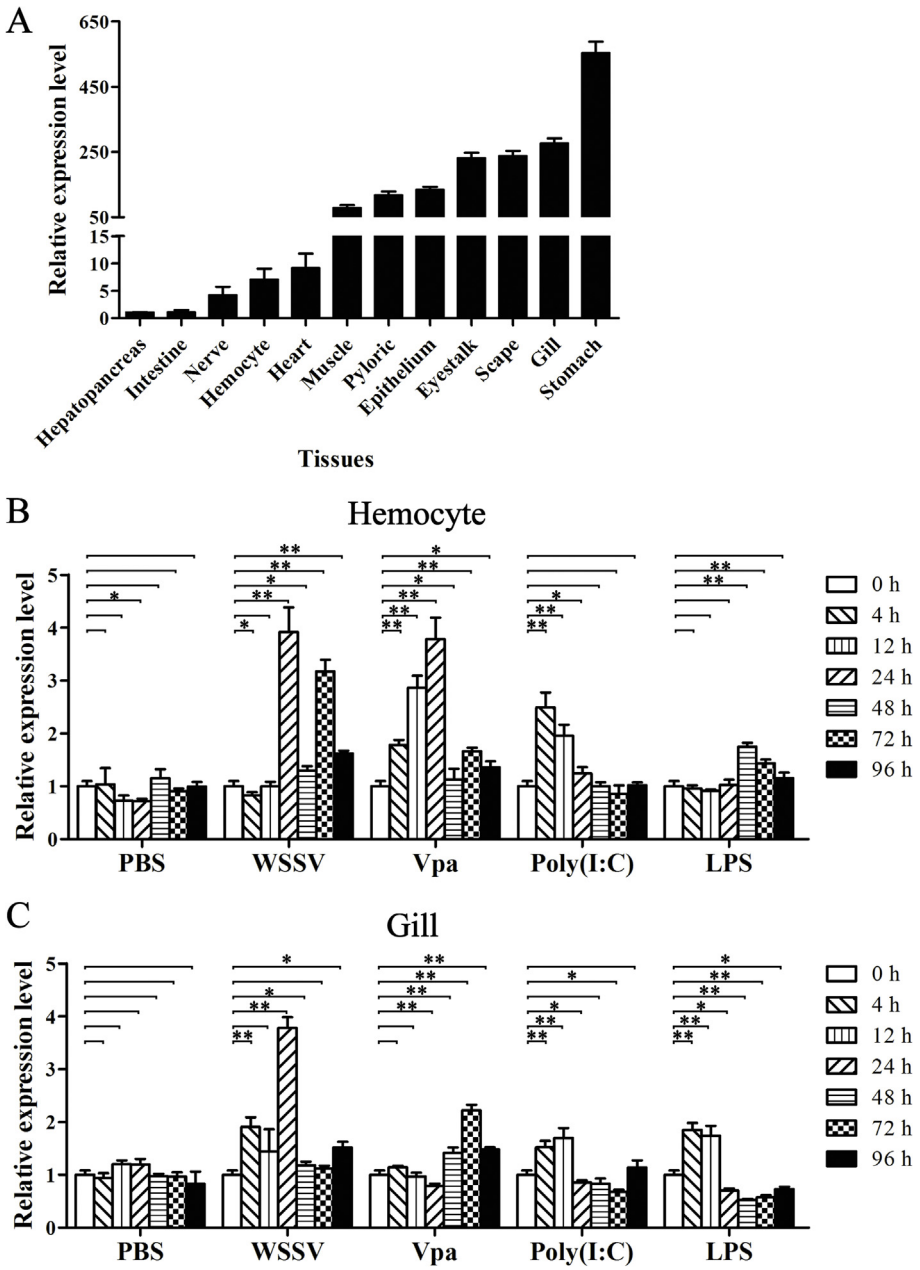

Fig. 3. Expression of TXNIP measured using real-time PCR. Each bar represents the mean ± SD of the three detections. (A) The distribution of TXNIP mRNA in tissues of healthy *L. vannamei*. The mRNA levels were calculated relative to that in hepatopancreas (set as 1.0). (B) and (C) The expression profiles of TXNIP in hemocytes and gill of LPS, *V. parahemolyticus*, poly (I:C), and WSSV-challenged shrimp. The statistical significance was calculated using Student's *t*-test. *, $p < 0.05$, **, $p < 0.01$.

TXNIP in the relationship between innate host defense mechanisms and oxidative stress in mammals [36,37]. However, the involvement of TXNIP in invertebrate immunity has not been concerned so far. The current study explored the immune function of the LvTXNIP gene from *L. vannamei* and demonstrated that LvTXNIP was essentially involved in antiviral and antibacterial responses, which may provide important information to fill the research gap in this area.

The hepatopancreas, intestine, nerve, hemocytes and heart expressed low levels of TXNIP, while the stomach, gill, scape, eyestalk, epithelium, pyloric and muscle expressed high levels, which were up to hundreds times higher than that in hepatopancreas. The great differences of the expression level among the detected tissues may indicate the different roles of TXNIP in the function of these tissues. The mRNA level of TXNIP in hemocytes and gill was up-regulated after immune stimulations, indicating the involvement of TXNIP in immunity. However, the expression of TXNIP in these tissues also showed different trends and profiles. For example, after *V. parahaemolyticus* infection, expression of TXNIP mainly changed at the early stage of infection in hemocytes but not at the later stage in gill. These indicated that the regulatory mechanism of the TXNIP expression could be different in various tissues of shrimp during immune responses, which needs further investigation.

Silencing of TXNIP *in vivo* could significantly increase the mortality of *V. parahaemolyticus*-infected shrimp but the effects were mainly exhibited after 32 h post infection. Also, the change of the bacterial load at 48 hpi was more obvious than that at 24 hpi. The ALF family and the PEN family antimicrobial peptides have a broad spectrum of antimicrobial activities against bacterial infection in *L. vannamei* [38,39]. The PPO and MnSOD are important components of the reactive oxygen system in crustaceans and play crucial roles in defense against bacterial

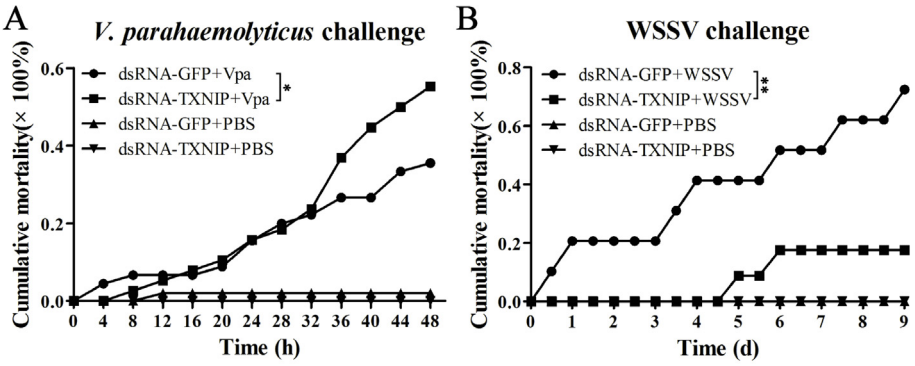

Fig. 4. Immune function of TXNIP. (A and B) Mortalities of TXNIP- and GFP-dsRNA treated *L. vannamei* ($n = 50$) infected by *V. parahemolyticus* and WSSV. Cumulative mortalities were recorded every 4 h. Differences in cumulative mortality levels between treatments were analyzed by Kaplan-Meier log-rank χ^2 tests (**$p < 0.01$). (C and D) The relative bacterial load in gill and the viral load in muscle analyzed by quantitative real-time PCR. Each bar represents the mean ± SD of three samples (**$p < 0.01$).

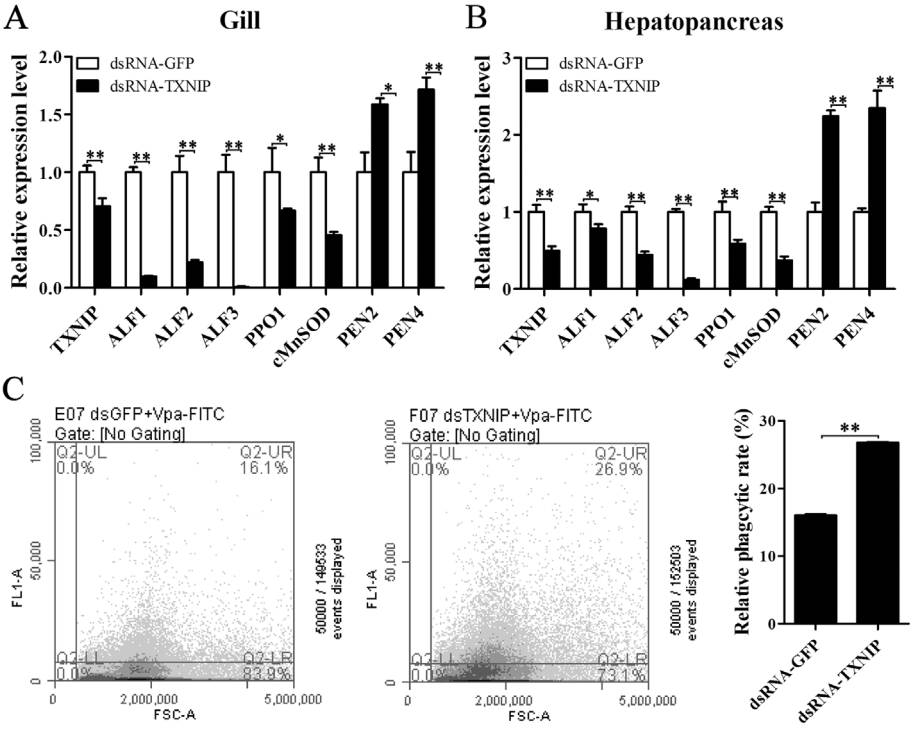

Fig. 5. Involvement of TXNIP in immune regulation. (A) and (B), expression of various immune related genes in hemocytes and gill of TXNIP-silencing shrimp analyzed by real-time PCR. Each bar represents the mean ± SD of three samples. (C) The phagocytic activity of hemocytes from TXNIP- and GFP-dsRNA treated shrimp against FITC-labeled *V. parahaemolyticus* detected by flow cytometry. The result shown is a representative of three independently performed experiments. The scatter plots representing one of the three flow cytometric detections were shown in the left panel and the data were provided in the right panel. Cells were examined by forward scatter (FSC, x-axis) and the phagocytosis of FITC-labeled *V. alginolyticus* was indicated by intracellular green fluorescence (y-axis). **$p < 0.01$.

infection [40,41]. Down-regulation of their expression could contribute to the increased mortality of *V. parahaemolyticus*-infected shrimp after silencing of TXNIP. However, the expression of antimicrobial peptides PEN2 and PEN4 was up-regulated in TXNIP-silenced shrimp, suggesting that TXNIP could inhibit their expression *in vivo*. Moreover, knockdown of TXNIP significantly increased the phagocytic activity of hemocytes, which is an important part of the cellular immune responses in shrimp. These could be negative factors affecting the antibacterial function of TXNIP. Therefore, the role of TXNIP in antibacterial immunity could be a complex result compromising its contradictory effects on both the humoral and cellular immune responses, which is worthy of further studies.

Previous studies have demonstrated that TRX could facilitate the infection of WSSV in shrimp through rescuing the DNA binding ability of WSSV immediate early gene (*ie1*) and enhancing the expression of WSSV structural protein VP28 [18]. Moreover, shrimp TRX could also directly interact with the viral VP362 and TK-TMK proteins and increase the viral load of WSSV in tissues [18]. The current study showed that the novel identified TXNIP could interact with TRX to inhibit its redox regulatory activity, which was consistent with the results of

previous studies in other organisms. However, knockdown of TXNIP *in vivo* significantly decreased the mortality of WSSV-infected shrimp, suggesting that TXNIP could also facilitate the infection of WSSV. These may indicate that the WSSV infection requires a certain level of redox state in cells. As important components of the TRX system, TXNIP and TRX balance each other and both contribute to the maintenance of the cellular redox state the WSSV infection and replication require. Silencing of TXNIP could upset the redox balance in cells and affect the infection of WSSV. Further studies are needed to verify this hypothesis.

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