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Ribose-phosphate pyrophosphokinase 1 (PRPS1) associated with deltamethrin resistance in *Culex pipiens pallens*

Shanchao Hong,

Department of Pathogen Biology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, People's Republic of China

Dan Zhou,

Department of Pathogen Biology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, People's Republic of China

Chen Chen,

Department of Pathogen Biology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, People's Republic of China

Weijie Wang,

Hebei Medical University, 361 East Zhongshan Road, Shijiazhuang, Hebei 050017, People's Republic of China

Yuan Lv,

Department of Pathogen Biology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, People's Republic of China

Yvting Ye,

Department of Pathogen Biology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, People's Republic of China

Ping Zou,

Department of Pathogen Biology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, People's Republic of China

Jing Yv,

Department of Pathogen Biology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, People's Republic of China

Xuelian Chang,

Department of Pathogen Biology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, People's Republic of China

Xinyou Yv,

Department of Pathogen Biology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, People's Republic of China

Linna Shi,

Department of Pathogen Biology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, People's Republic of China

Lei Ma,

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Correspondence to: Bo Shen, shenbo@njmu.edu.cn; Changliang Zhu, clzhu@njmu.edu.cn.

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Department of Pathogen Biology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, People's Republic of China

Yan Sun,

Department of Pathogen Biology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, People's Republic of China

Donghui Zhang,

Department of Pathogen Biology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, People's Republic of China

Bo Shen, and

Department of Pathogen Biology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, People's Republic of China

Changliang Zhu

Department of Pathogen Biology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, People's Republic of China

Bo Shen: shenbo@njmu.edu.cn; Changliang Zhu: clzhu@njmu.edu.cn

Abstract

Ribose-phosphate pyrophosphokinase 1 (PRPS1) was identified and isolated as a differentially expressed gene between deltamethrin-susceptible (DS) and deltamethrin-resistant (DR) *Culex pipiens pallens* and *Aedes albopictus* C6/36 cell line through microarray and 2D-Gel. An open reading frame of *PRPS1* cloned from *C. pipiens pallens* has 1,011 bp and encodes for a 336 amino acids protein which shares high homology with *Culex quinquefasciatus*. Real-time polymerase chain reaction was used to determine the transcript expression level of *PRPS1* in DS and DR strains. The expression levels of *PRPS1* were higher in DR laboratory strains and natural population JXZ-DR, JXZ-LDR. *PRPS1* was also detected and expressed at all developmental stages of *C. pipiens pallens* and increased expression level in DR3 strain than DS strain in the third and fourth instar larvae, female and male stages. In addition, to further investigate the role of PRPS1 in deltamethrin resistance, PRPS1 was transiently expressed in *A. albopictus* C6/36 cells and detected by western blotting. Cells transfected with PRPS1 had an increased resistance to deltamethrin compared with control cells. These results suggested that the increased expression level of PRPS1 may play roles in the regulation of deltamethrin resistance.

Introduction

Culex pipiens pallens is a predominant mosquito species in northern China. *C. pipiens pallens* is the major vector for lymphatic filariasis and Japanese encephalitis (Hammon et al. 1949; Mattingly 1962; Omori 1958). Efforts on the control of *C. pipiens pallens* have been focused on the use of chemical insecticide. Deltamethrin has been widely used in China since 1970s due to its very low toxicity to humans and rapid killing effect on insects (Barlow et al. 2001; Zhang and Yang 1996). However, frequent use of deltamethrin has resulted in the development of deltamethrin resistance, which has become the major obstacle for vector control (Jinfu 1999; Sun et al. 2007).

Knockdown resistance (kdr) is closely associated with deltamethrin resistance (Burton et al. 2011; Chen et al. 2010). However, recent studies suggested that kdr mutation may not be the only mechanism responsible for the insecticide resistance (Hemingway et al. 2002). Other factors including detoxification enzyme systems based on cytochrome P450 monooxygenases, nonspecific esterases, and glutathione-S-transferases may also play important roles in deltamethrin resistance (Gong et al. 2005; Matambo et al. 2007). To further understand the underlying mechanism, we isolated deltamethrin resistance-related

genes using 2D-Gel and microarray. Ribose-phosphate pyrophosphokinase 1 (PRPS1) was detected by both 2D-Gel and microarray (Table S1).

PRPS1, which catalyses the phosphoribosylation of ribose 5-phosphate to 5-phosphoribosyl-1-pyrophosphate (prpp), is essential for the de novo pathway of purine, pyridine histidine, and tryptophan biosynthesis (Roessler et al. 1990). Currently, there was no report on the role of PRPS1 in insecticide resistance. This paper was designed to investigate the relationship between PRPS1 and deltamethrin resistance.

In this experiment, we detected the expression level of *PRPS1* in deltamethrin-susceptible (DS) and deltamethrin-resistant (DR) *C. pipiens pallens* strains from laboratory and natural populations by real-time polymerase chain reaction (PCR). The expression pattern of *PRPS1* in different developmental stages of two mosquito strains was also characterized by real-time PCR. In addition, we cloned full-length *PRPS1* from *C. pipiens pallens*. PRPS1 expression plasmid was transiently transfected into C6/36 cells, and the viability of transfected cells was analyzed. Through this experiment, we tried to determine whether overexpression of *PRPS1* could change the deltamethrin sensitivity.

Materials and methods

C. pipiens pallens strains

Four laboratory strains of *C. pipiens pallens* were used in this study. DS strain and DR (DR1, DR2, and DR3) strains of *C. pipiens pallens* were obtained from the Shanghai insect institute of the Chinese Academy of Sciences and maintained in our laboratory. These strains were reared at 28–30 °C in a 16-h light/8-h dark photoperiod with 70–80 % humidity. DS strain is a susceptible strain that has been reared in the laboratory without exposure to deltamethrin. DR strains were selected with deltamethrin from DS strain for generations to induce deltamethrin resistance. Fifty percent larval lethal concentrations (LC₅₀) of DS strain and DR1, DR2, DR3 strains used in this research are 0.040, 0.5, 1.3, and 16.12 mg/l.

JXZ *C. pipiens pallens* mosquitoes had been collected from Jiang Xin Zhou county, Nanjing, Jiangsu, in 2011. JXZ mosquitoes were directly reared from field-collected larvae. Non-blood-fed female JXZ *C. pipiens pallens*, 2 to 3 days post-adults emergence, were tested by WHO bottle bioassay with the 0.05 % deltamethrin-treated papers for insecticide susceptibility. Twenty-five female mosquitoes for each population were used. After 15 min exposure to diagnostic concentration of deltamethrin (0.05 %), the number of dead and surviving mosquitoes was recorded. The surviving mosquitoes resistant to deltamethrin were called JXZ-DR strain, and the dead mosquitoes relatively susceptible to deltamethrin were called JXZ-DS strain. JXZ fourth-instar mosquito larvae were tested by bioassay, and the LC₅₀ was 0.02 ppm. JXZ fourth-instar surviving and dead mosquitoes in the 0.02 ppm were collected. The surviving mosquitoes were called JXZ-LDR strain, and the dead ones were called JXZ-LDS strain.

RNA extraction and cDNA synthesis

Total RNA was extracted from 30 mg adult *C. pipiens pallens* using TRIzol (Invitrogen, USA), and contaminant genomic DNA was removed by DNase I treatment. cDNA was synthesized from 500 ng total RNA with PrimeScript™RT Reagent Kit (TaKaRa, Japan) according to the manufacturer's protocol.

Cloning and sequencing

Cloning and sequencing the ORF of *PRPS1*. To clone the full length of *PRPS1* gene, we used the primer designed based on the ORF of *Culex quinquefasciatus* (NCBI reference

sequence: XM_001846240.1). The sequence of the oligonucleotide primers was *PRPS1*-F1, 5'-ATGCCCAA CATCAAGGTCTTC-3'; *PRPS1*-R1, 5'-TTAGTAAG GAACGTTAGAGAACAGA-3'. PCR conditions were initial denaturation for 5 min at 94 °C, followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min, with 10 min extension at 72 °C. 3'RACE special primer, 5'-CGCCATTCTGACCCACGGCATCTT-3', was based on the cloned ORF fragment, and the 3' adaptor primers, 5'-CTGATCTAGAGGTACCGGATCC-3, were provided by TaKaRa 3'-full race core set. Oligo dT adaptor primer was used in reversed transcription from the RNA of DR3 strain. PCR was performed with Advantage 2 Polymerase Mix (Clontech, USA) in 50 µL volume. The PCR condition was initial denaturation for 1 min at 95 °C, followed by 35 cycles of 95 °C for 30 s, 68 °C for 1 min, and finally extended at 68 °C for 2 min. 5'RACE special primer, 5'-AAGATGCCGTGGGTCAGAATGGCG TAGA-3', was based on the ORF, and the universal primer, 5'-CTAATACGACTCACTATAGGGCAAGCAGTGGTATC AACGCAGAGT-3', was provided by SMARTTM RACE cDNA amplification kit (BD, USA). 5'CDS-Primer was used in reverse transcription from the RNA of DR strain. PCR was also performed with Advantage 2 Polymerase Mix (Clontech, USA) in 50 µL volume. PCR conditions were initial denaturation for 1 min at 95 °C, followed by 35 cycles of 95 °C for 30 s, and 68 °C for 1 min, with final 2 min extension at 68 °C, 50 µl volume.

PCR products were separated by 1 % agarose gel electrophoresis and purified using a QIAquick Gel Extraction Kit (Qiagen, Germany). Products were then cloned into the PCR 2.1 plasmid (Invitrogen, USA) overnight at 16 °C. This ligated mixture was transformed into *Escherichia coli* TOP10 competent cells and cultured in LB plate containing ampicillin (70 µg/mL). Positive clones were validated and sent for sequencing at BGI. The sequences of these three fragments were assembled to generate a putative full-length cDNA.

Sequence alignment and phylogenetic tree

The standard protein/protein BLAST sequence comparison programs (<http://beta.uniprot.org/?tab=blast>) were used to search for sequences in the SWISSPROT databases with similarities to the translated sequences of *PRPS1*. Similar amino acid sequences were aligned using the ClustalW2 computer program (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>). The phylogenetic tree was constructed by the neighbor-joining method using the Clustalx1.83 program.

Quantitative RT-PCR analysis

Quantitative PCR was performed on the ABI Prism 7300 HT Sequence Detection system (Applied Biosystem, CA, USA) using FastStart® SYBR Green (Roche) according to the manufacturer's protocol. The sequences of forward and reverse primers used for *PRPS1* were *PRPS1*-F2, 5'-CAG CCTCGGCAAACATCAT-3' and *PRPS1*-R2, 5'-ATTCTGACCCACGGCATCTT-3', and the product size was 159 bp. Another pair of primers was used for β -*actin*: 5-AGCGT GAACTGACGGCTCTTG-3' and 5-ACTCGTCGTA CTCTGCTTGG-3, with a products size for 153 bp. The following program was employed: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min. A melting curve program was run immediately after the PCR reaction, and the data were analyzed with 7300 System SDS Software v1.2.1 (Applied Biosystems). The raw threshold cycle (Ct) values were normalized against β -*actin* standard to obtain normalized Ct values, which were then used to calculate relative expression levels in the samples using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). The expression levels of *PRPS1* in DR and DS strains, in JXZ-DR, JXZ-DS strains, and in JXZ-LDR, JXZ-LDS strains of *C. pipiens pallens* were detected. The expression level of *PRPS1* in DS, JXZ-DS, or JXZ-LDS strain was considered as background level. Developmental stage-specific expression of *PRPS1*

was determined by quantitative PCR. The samples include eggs, larvae, pupae, and adults from laboratory DS strain and DR3 strain. The expression level of *PRPS1* in susceptible strain was considered as background. The real-time quantitative PCR analysis was performed three times using independent purified RNA samples with three replicates for each sample.

Construction of the eukaryotic expression plasmid

The ORF of *PRPS1* was constructed into eukaryotic vector pIB/V5-His expression vector. The pair of specific primers was as follows: forward primer, 5'-GGACTAGTGAGATG GAAATGCCCAACATCA AGGTCTTC-3'; reverse primer, 5'-CCCTCGAGGTAAGGAACGTTAGAGAACAGA TACG-3'. Kozark sequence GAGATGG was added before starting code ATG, and two additional AA were added after it to avoid frameshift mutation which were translated to GLU without affecting the function (Kozak 1986). The stop codon TAA was removed from the sequence. The forward primer had a SpeI recognition site (ACTAGT), and the reverse primer also had an XhoI recognition site (CTCGAG). The pIB/V5-His expression vector and the PCR product were all digested by SpeI and XhoI and then purified. The two purified objective bands were ligated with T4 DNA ligase (Takara, Japan) overnight and transformed to TOP10 competent cells. Bacteria liquid was subjected to PCR with OPIE2 forward and RPP-R1 reverse sequencing primers, and the positive bacteria liquid was sequenced at BGI.

Cell culture and transient transfection

Aedes albopictus C6/36 cells were obtained from China Center for Type Culture Collection (CCTCC, China). The cells were maintained in Eagle's minimum essential medium (EMEM, Invitrogen, USA) supplemented with 10 % (v/v) fetal bovine serum (FBS, Sijiqing, China), 100 IU/mL penicillin, and 100 µg/mL streptomycin (P/S, Gibco, Invitrogen) and cultured at 28 °C in a humidified atmosphere with 5 % CO₂. Transfection was performed using FuGENE[®] HD transfection reagent (Roche, USA) according to the manufacturer's protocol. Cells, 4×10⁵, were plated in 2 mL of complete growth medium in ever 6-well plate overnight, and at the time of transfection, each well achieved the desire density of 80 % confluency. In each well, 2 µg plasmid DNA and 4 µL transfection reagent were pipetted into the tube and vigorously tapped for 2 s to mix the contents, and the transfection complex was incubated for 20 min at room temperature. Then, the complex was added to the cells below the surface of the medium and swirled to ensure distribution over the plate surface. Seventy-two hours post-transfection, the cells were collected and used for Western blot analysis.

Western blot analysis

Cells were washed in PBS and incubated with pre-chilled RIPA lysis buffer (Beyotime) containing protease inhibitor PMSF on ice for 30 min. The cell lysates were clarified by centrifugation at 12,000×g for 15 min, and the supernatants were collected. Protein concentration was determined, and 40 µg proteins were loaded on 10 % polyacrylamide SDS gels. Samples were transferred electrophoretically to PVDF membranes (Millipore), blocked with 5 % non-fat dry milk for 2 h at room temperature, and incubated with His-Tag[®] Monoclonal Antibody anti-His antibody (1:500, NovaGen, USA) at 4 °C overnight. After incubation with HRP-conjugated secondary antibody for 2 h at room temperature, chemiluminescence was detected by an ECL detection system (Bioworld Technology, Minneapolis, MN, USA). Tubulin (Bioworld Technology, Minneapolis, MN, USA) was used as an internal control.

Cytotoxicity assay

Cell Counting Kit-8 (CCK-8, Dojindo, Japan) was used to determine the deltamethrin resistance of pIB/V5-His-PRPS1 transient C6/36 transfectants under deltamethrin treatments. Cells suspension was distributed (20,000 cells/well) in a 96-well plate, and plates were pre-incubated overnight in a 5 % CO₂-humidified incubator at 28 °C, and then each well was added with 50 ng pIB/V5-His-PRPS1 and 0.25 µL transfect reagent according to the manufacturer's instruction. Cells were incubated with the transfection mixture for 4 h, and the transfection medium was replaced with 2 mL normal medium. After additional 24 h incubation, wells were then treated with various concentrations of deltamethrin (final concentrations, 0, 10^{0.5}, 10^{1.0}, 10^{1.5}, and 10^{2.0} µg/mL; Sun et al. 2011). After another 48 h, 10 µL of CCK-8 solution were added to each well, and the plates were detected at 450 nm using a microplate reader after additional 4 h incubation. C6/36 cells transiently transfected with pIB/V5-His were used as control.

Statistics

All data are presented as mean±SEM. Comparison between groups was made using the Student's *t* test. The statistical significance level was set at *P*<0.05. All experiments were performed in triplicate on at least two separate occasions.

Results

Cloning the full-length cDNA of *PRPS1* gene from *C. pipiens pallens*

The full-length cDNA of *PRPS1* was amplified from *C. pipiens pallens* by PCR with 3'-RACE and 5'-RACE. One fragment of 1,542 bp was obtained from 3'-RACE and another 943 bp obtained from 5'-RACE, and they were assembled. The ORF of *PRPS1* has 1,011 bp and encodes a 336 amino acids protein. Start codon ATG is found to be nucleotides (63–65) of the gene, and a stop codon TAA is 1071–1073 with tailing signal sequence AATAAA presented at the 3'-untranslated region, which proves it is the full-length cDNA sequence of *PRPS1* (Fig. 1). Amino acid sequence alignment between *C. pipiens pallens PRPS1* and other *PRPS1* from insects by ClustalW2 software showed high amino acid similarity, indicating that *PRPS1* are conserved between species (Fig. 2a). Phylogenetic relationships showed that *PRPS1* of *C. pipiens pallens* has the highest homology with *C. quinquefasciatus* (Fig. 2b). Structure analysis (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) showed that the protein contains phosphoribosyltransferase (PRT) domain, which can catalyze the displacement of the alpha-1'-pyrophosphate of 5-phosphoribosyl-alpha1-pyrpphosphate (PRPP) by a nitrogen-containing nucleophile.

PRPS1 expression level

Real-time quantitative RT-PCR was used to detect the expression level of *PRPS1* in DR strains and DS strain of *C. pipiens pallens*. *β-actin* was used as a normalization control. *PRPS1* expression level was 2.2-, 3.0-, and 7.2-fold higher in the DR1, DR2, and DR3 strains compared with the DS strain (Fig. 3a). These results suggested that *PRPS1* expression level was upregulated in the DR strains, and it was gradiently increased with the DR level. The expression of the *PRPS1* gene in different *C. pipiens pallens* developmental stages was analyzed by real-time PCR. As shown in Fig. 3b, *PRPS1* gene is expressed at all developmental stages. Comparison between DS strain and DR3 strain indicated that the expression of *PRPS1* was higher in resistant strain than susceptible strain in the third-instar larvae and fourth-instar larvae, female and male stages (*P*<0.05).

To further detect the expression level of *PRPS1* in the natural *C. pipiens pallens* population, JXZ-DR, JXZ-DS strains and JXZ-LDR, JXZ-LDS strains were used in our experiment. As

shown in Fig. 3c, d, *PRPS1* expression levels in JXZ-DR, JXZ-LDR strains were 7.9-fold, 2.9-fold higher compared to JXZ-DS, JXZ-LDS strains.

Cytotoxicity assay

To investigate the role of increased *PRPS1* in deltamethrin resistance, C6/36 cell line transiently transfected with pIB/V5-His-PRPS1 and pIB/V5-His were used for cytotoxicity assay. To confirm the exogenous fusion protein PRPS1-His was successfully expressed in C6/36 cells, Western blot analysis using anti-His antibodies identified a protein around 40 kDa (Fig. 4a). A Cell Counting Kit-8 was used to measure cytotoxicity assay of the transiently transfected cells. The dose-response of cell viability over a wide range of concentrations (0, $10^{0.5}$, $10^{1.0}$, $10^{1.5}$, and $10^{2.0}$ $\mu\text{g/ml}$) of deltamethrin was measured. The proliferation of transiently transfected cells decreased with the increasing of deltamethrin concentration as expected. As shown in Fig. 4b, the significantly increased viability in C6/36-PRPS1 cells under high dosage deltamethrin treatment ($10^{1.0}$, $10^{1.5}$, and $10^{2.0}$ $\mu\text{g/ml}$) suggested that *PRPS1* overexpression might strongly be associated with deltamethrin resistance.

Discussion

Deltamethrin, a type II pyrethroid, has been intensively used for several decades (Vais et al. 2001). Prolonged and intensive use of deltamethrin has led to the development of resistance in many insect species (Foster et al. 2010; Yoon et al. 2008). Knockdown resistance (*kdr*) has now been studied in many important pest insects and considered to be causative of the resistance phenotype to pyrethroid and DDT (Soderlund and Knipple 2003). However, recent discoveries suggested that *kdr* mutation alone may not produce an entire insecticide resistance phenotype, and other possible mechanisms including detoxification enzyme systems and behavioral changes may participate in the development of pyrethroid resistance (Montella et al. 2007; Pedra et al. 2004; Vontas et al. 2005). Recently, new techniques were used to investigate insecticide resistance-associated genes. Montella et al. (2007) found some differentially expressed ESTs between insecticide-resistant and -susceptible mosquitoes using 454 Pyrosequencing. Our previous study also identified several deltamethrin resistance-related genes using microarray (Yang et al. 2008; Zhang et al. 2011). Microarray and the proteomics results showed that PRPS1 was increased in DR *C. pipiens pallens* and *A. albopictus* C6/36 cells.

PRPS1 as a member of PRPP synthetases is essential for de novo synthesis of purine and pyrimidine. N-terminal domain of PRPS1 we cloned is frequently found in the ribose-phosphate pyrophosphokinase. PRT-type I domain of PRPS1 catalyzes the displacement of the α -1'-pyrophosphate of PRPP by a nitrogen-containing nucleophile. These features and the amino acid identity of PRPS1 suggest that it encodes a functional PRPS1 protein.

We detected PRPS1 expression level increase coordinated with increased deltamethrin resistance, and this result indicated that PRPS1 might play roles in the development of deltamethrin resistance. *PRPS1* was expressed in all developmental stages of *C. pipiens pallens*, and the expression level increased dramatically in adult stage compared to DS strain. This result suggested that *PRPS1* gene was involved in resistance to deltamethrin of *C. pipiens pallens*, especially in adults. We cloned the full-length cDNA of PRPS1 and confirmed this gene through alignment in *C. pipiens pallens*, *C. quinquefasciatus*, and other insects. To determine the association between PRPS1 and deltamethrin resistance, PRPS1 was transiently transfected to C6/36 cells, and cytotoxicity assay was performed on cells. The result suggested that C6/36 cells transfected with PRPS1 were more resistant to deltamethrin. We hypothesized overexpressed PRPS1 could enhance the synthesis of purine nucleotide and pyrimidine nucleotide. Carboxylesterases, P450 monooxygenases, and GST

were considered as an evolutionary response to insecticide, and their transcriptional upregulation underlies many instances of insect resistance (Hemingway et al. 2004; Pasteur and Raymond 1996). Transcriptional upregulation needs more purine and pyrimidine nucleotide, and overexpressed PRPS1 could provide enough nucleotide for this process.

It was very interesting to detect PRPS1 expression levels were higher in surviving mosquitoes than the dead after deltamethrin treatment from field population. This result combined with previous study let us assume that *C. pipiens pallens* with high PRPS1 expression level may be more likely to survive under deltamethrin treatment. Our study on the relationship between PRPS1 and deltamethrin resistance is still in a preliminary stage because RNAi in vivo might provide more sufficient evidence, and in the model we studied, *C. pipiens pallens*, deltamethrin resistance is *A. albopictus* cell line C6/36. So, further experiments should be done to explore the relationship between PRPS1 and deltamethrin resistance, and the mechanism of PRPS1 is involved in deltamethrin resistance.

In summary, we have cloned the PRPS1 gene from *C. pipiens pallens*. Overexpression of PRPS1 in C6/36 cells could enhance deltamethrin resistance. Our study suggests that PRPS1 may be a candidate gene for insecticide resistance. The mechanism of PRPS1 participating in deltamethrin resistance needs to be further investigated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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1 ACATGGGGAGTCAAGCCGACAGTAGCCGACAGCAGCAGCCTCTCTGCACTCCAGA
 63 ATGCCAATCAAGGCTTTCTCCGTTCTCCATCCGGATCGGCTCGGCATCGTGACCGCTCGGAATCGACCTCGGAAGGTG
 H P N I K U F S G S S H P D L A S R I U D R L G I D L G K U
 153 GTCACGAAAAGTTCAAGCAATCTGGAACATCGCTCGAGATCGGCGAGTCGGTCCGCGGAGGACGTGTACATCGTGCAGTCCGGCTCC
 U T K K F S N L E T C U E I G E S U R G E D U Y I U Q S G S
 243 GGTGAGATCAAGCAACCTGATGGAGATGCTGATCATGATCAACGCGTGCAGATCGCCTCGGCGTCCGCGTCAAGCTGTGATACCC
 G E I N D N L M E M L I M I N A C K I A S A S R U T A U I P
 333 TGGTTCCTACGCCCCGACAGACAAGACAAGACAGCCAGCATAAGCTGCGCGCTTGATGAAGACCAATGAGTGAAGTTTCGG
 D K L A A L M K T N E W K F R C F P Y A R Q D K K D K T G D
 423 AGCGTGGCCCAATCTCCGCAAGCTGCTGGCAACATGCTGCTGGTGGCGGAGCGATCACATCATGATCTGCAGCCCTCA
 S R A P I S A K L U A N M L S U A G A D H I I T M D L H A S
 513 CAGATTCAGGCTTTCTCGACATCCGCTGGACACCTGTACCGGAACCGGCGTGTCAAGTGGATCGGCGAAGCATTGCCAGTGG
 Q I Q G F F D I P U D N L Y A E P A U L K W I R E N I A E W
 603 CGGAATCGATCATGCTGCTCGCGACGCGGCGGTGCCAAGCTGTACGTCGATCGCCGATCGGTGAAGCTGAGTTTCCGCTGATC
 R N S I I U S P D A G G A K R U T S I A D R L N U E F A L I
 693 CACAAGGACGCGAAGGCGCAACGAGTCCGCTCGATGCTGCTGGTGGTGAAGGACCGCTGGCCATCTGCTGGACGATATG
 H K E R K K A N E U A S M U L U G D U K D R U A I L U D D M
 783 GCGGACAGTGGCGACCATTTGCCAGCGCGCGACAGCTGCTGAGGCGGGCGCCACGAGGTCTACGCCATTCTGACCCACGGCATC
 A D T C G T I C H A A D K L U E A G A T R U Y A I L T H G I
 873 TTCAGTGGCGCGCATTTCCGGATAAATACGCTGCTTCGAGGCTGTGCTGTCGACGACACATCCCCAGGATGGCCACATGAAG
 F S G P A I S R I N N A C F E A U U U T N T I P Q D G H M K
 963 GACTGTCCCAAGATTCAAGTCGATCGAGTTTCCATGATGTTTCCGAGGCTGTCCGCGGACCCACAACGCGGAAGTGTGCTATCTG
 D C P K I Q C I D U S M M F A E A U R R T H N G E S U S Y L
 1053 TTCTCTAAGCTTCCTTAAGTAA TAA
 F S N U P Y
 1074 GACGCCACCGCCAGGCACTACACCGGACCGGGAATGATGACAAACGGGAATACGACCGCTCAGCCCGAAGTGAACCGTTTTT
 1164 TTTAACTATACATTATATCATTGTTTATTCAAGCTGACAAATTTTGCCTTCACGGTAACTATTGCCATAGTAAGTAAATGAAGAA
 1254 GACTCATACAAACAGCTCAAGCATCTACAGAAAACAAAGCAAAACGTTATAATCGGTATGTTTAATTAAAGTTTAAAGTTTCTCCA
 1344 TTTATCCGATTTCCGGACGCGCTTCTTCTATTTCCATATTAAGTAACACTAAACCAAGTTTATATTAGCTAACGAGAGAGAGAGC
 1434 TAAACCTTAGAGAGSAAACGTTAGAGTTGTTACGCTTAGTACCGGCTTTCTTTATAAAGAGTTGAACAATAAACATAGACACACAA
 1524 AGCCCAATAGTCTGTATTTTCATGCCAGCATAGATCCATTAGTTGAGAAACCCACCGAAGAGAGTGTGACACTGTTTTCG
 1614 TAGTAAGATAGATTGTTACAGCTGCTCCTGTTAGATTGTTTGCATTACACTTACAGTACACCGATACAAACAGGACATCCAA
 1704 ACCACAACACACACAGGCAATTATAAATTAGATCAAACTTATTGTAGGTATCACTGAACAACTTTTAAATTTACGCTAATTG
 1794 ATATGGAAGTAAAGTCAATAAACTTAAAAAATAGTAAGAAAAA

Fig. 1.

The nucleotide and deduced amino acid sequence of *Culex pipiens pallens* PRPS1. The deduced amino acid sequence is presented *below* the nucleotide sequence in *single letter code*. The putative polyadenylation signals of the 3'-untranslated region are *underlined by red line*. The initial code "ATG" and the termination code "TAA" are also *boxed*. N-terminal domain is frequently found to the ribose-phosphate pyrophosphokinase is *underlined by black line*. Phosphoribosyltransferase (PRT)-type I domain which catalyzes the displacement of the alpha-1'-pyrophosphate of 5-phosphoribosyl-alpha1-pyrpphosphate (PRPP) by a nitrogen-containing nucleophile is *underlined by a green line*

Fig. 2.

a Amino acid sequence alignment of *Culex pipiens pallens* PRPS1. Asterisks indicate identical amino acid, and dots indicate similar amino acids. **b** Phylogenetic relationships of PRPS1 among *C. pipiens pallens* and some other species. Species name and GenBank Accession No.: *C. pipiens pallens*, JQ478426; *Aedes aegypti*, XP_001648571.1; *Culex quinquefasciatus*, XP_001846292.1; *Acromyrmex echinator*, EGI65763.1; *Harpegnathos saltator*, EFN85070.1; *Tribolium castaneum*, EFA00168.1; *Glossina morsitans morsitans*, ADD19462.1; *Drosophila melanogaster*, XP_002007996.1

Fig. 3.

Relative mRNA expression levels of *Culex pipiens pallens* PRPS1. **a** PRPS1 relative expression levels in DS strain and DR1, DR2, and DR3 strains. **b** PRPS1 transcripts at different developmental stages. **c** PRPS1 relative expression levels in JXZ-DS strain and JXZ-DR strain. **d** PRPS1 relative expression levels in JXZ-LDS strain and JXZ-LDR strain. Results are normalized against the internal control actin. Results are expressed as “mean $\pm$ SD” of three independent experiments. * $P < 0.05$

Fig. 4.

a Western blot analysis of PRPS1 expression in transiently transfected cells. 1, C6/36-control (vector control); 2, C6/36-PRPS1. Tubulin was used as an internal control. Three independent experiments were repeated. **b** Overexpression of PRPS1 enhances deltamethrin resistance in C6/36 cells. Transiently transfected cells C6/36-control and C6/36-PRPS1 were treated with deltamethrin at the indicated concentrations, and cell viability was measured after 48 h treatment. The percentage of viable cells is shown relative to the 0-μg/ml concentration. Results are expressed as mean±SD, and three independent experiments were done. **P*<0.05 compared to C6/36-control