Executive summary

RNA interference (RNAi) plays an important role in an antiviral defense in penaeid shrimp. However, the mechanism of shrimp RNAi pathway remains largely elusive, especially the role of the RNAi machineries. In this study, a gene encoding a RISC-associated factor, tudor staphylococcal nuclease (*PmTSN*) was identified from *Penaeus monodon* and its role in shrimp RNAi pathway was investigated. The full-length cDNA of PmTSN was 2897 bp with an open reading frame encoding a putative protein of 889 amino acids. Phylogenetic analysis and domain structure comparison revealed that PmTSN was more closely related to vertebrate TSN by sharing 57% amino acid sequence identity with *Danio rerio* TSN. PmTSN represented a new type of TSN protein by exhibiting the four tandem repeat of staphylococcal nuclease-like domain (SN1-4) followed by a Tudor and a partially truncated C-terminal SN5 domain. Knockdown of PmTSN partially diminished the activity of dsRNA-mediated gene silencing in either silencing of the endogenous *PmRab7* or inhibition of YHV replication, suggesting the involvement of PmTSN in shrimp RNAi pathway. The interaction between PmTSN and three shrimp Argonaute proteins (PmAgo) were characterized by yeast two-hybrid and *in vitro* pull-down assays. The results demonstrated that PmTSN interacted with PmAgo1 but not with PmAgo2 or PmAgo3, suggesting that PmTSN is a component of PmAgo1-RISC. The interaction between PmAgo1 and PmTSN was mediated through the N-terminal domain of PmAgo1 and the SN1-2 domains of PmTSN. Knockdown of *PmAgo1* substantially diminished the activity of dsRNA-mediated gene silencing when compared with the result which was observed in *PmTSN* knockdown, suggesting that PmAgo1 plays a crucial role in shrimp RNAi pathway and PmTSN is a minor component of PmAgo1-RISC.

Microarray analysis was used to identify the transcripts which were regulated by *Marsupenaeus japonicus* Ago1 and TSN. The results showed that 294 transcripts were up-regulated upon *MjAgo1* knockdown. In addition, a common set of 58 transcripts were found to be up-regulated in both *MjAgo1*- and *MjTSN*-knockdown shrimps. Functional classification of these up-regulated transcripts indicated that with the exception of unknown genes, the greatest number of the up-regulated genes was related to enzyme and metabolism (22.4%), while the lowest was related to cell structure and cellular compartment organization (1.7%) and cell death (1.7%). These up-regulated genes represented the potential targets of RNAi. The microarray results implied that a

variety of biological processes in shrimp cells are regulated by RNAi via Ago1-RISC. Moreover, a number of transcripts (310 genes) were down-regulated in *MjTSN*-kd shrimps. The transcripts are linked to the functions of TSN in the elongation step of the transcriptional process, signaling pathway, and mRNA splicing in shrimp. Further studies are required to clarify these scenarios better.

Yeast two-hybrid screening was performed to identify the interacting proteins of PmTSN. White spot syndrome virus (WSSV)-infected shrimp hemocyte cDNA library was used to screen by mating approach. By screening 1.38 million clones (6.57% mating efficiency), 10 diploids showed the positive results observed on the quadruple dropout medium. After sequence analysis by using Blastx, the results showed that five clones were Laminin receptor (40S Ribosomal protein SA or Lamr), one was histone H2A, and four were unknown. Previous studies showed that Lamr was identified as a binding protein for capsid protein (VP1) of taura syndrome virus (TSV) and envelope protein gp116 of yellow head virus (YHV). To study the involvement of the interaction between PmTSN and Lamr upon YHV and TSV infection, the interaction of PmTSN and Lamr during YHV and TSV infection was investigated by an *in vitro* pull down assay. The interaction of PmTSN and PmLamr cannot form a complex with viral binding proteins gp116 of YHV or VP1 of TSV. These results suggested that the effect of the interaction between PmTSN and Lamr may not play a significant role during viral infection.

In summary, this study provided the first description of the RISC-associated protein, PmTSN and the interaction between PmTSN and Lamr is possibly involved in other biological processes (not viral infection) in shrimp. Furthermore, the fraction of transcriptome which regulated by the RNAi mechanisms was revealed. These findings would be helpful to reveal the molecular events in the penaeid shrimp RNAi pathway.