

Role and post-transcriptional regulation of Kunitz-type serine protease inhibitor from the black tiger shrimp *Penaeus monodon*

Chantaka Wongdontri¹ and Kunlaya Somboonwiwat^{1*}

¹ Center of Excellence for Molecular Biology and Genomics of Shrimp, Department of Biochemistry, Faculty of Science Chulalongkorn University; boobee@live.com

* Correspondence: kunlaya.s@chula.ac.th; Tel.: 02-2185413

Abstract: Kunitz type serine protease inhibitor (KuSPI) plays an important role in immune response. We previously reported that PmKunitz gene, a predicted target mRNA of pmo-miR-bantam in *Penaeus monodon*, was highly expressed in WSSV-infected shrimp. Herein, we aim to functionally characterize the PmKunitz and to confirm its post-transcriptional regulation by pmo-miR-bantam. First, we found that the recombinant protein PmKunitz (rPmKunitz) exhibited the inhibitory activity on the protease from hepatopancreas extract of WSSV-infected *P. monodon*. Then the function of PmKunitz was examined in shrimp (1-2 months old) in vivo by RNAi technique. The PmKunitz gene silencing did not affect neither prophenoloxidase activating system nor apoptosis but decreased the number of total hemocyte cell. Moreover, the PmKunitz silencing delayed the WSSV-infected shrimp mortality and significantly reduced both number of total hemocyte and WSSV copy. This evidence is positively correlated to what observed in the pmo-miR-bantam-overexpressed shrimp. The transcriptional inhibition of PmKunitz by pmo-miR-bantam mimic also decreased the level of PmKunitz transcripts and reduced the number of WSSV. Taken together, we conclude that PmKunitz was a multifunctional proteinase inhibitor that are post-transcriptionally regulated by pmo-miR-bantam and plays roles in maintaining hemocyte homeostasis and innate immune response against WSSV infection in shrimp.

Keywords: kunitz-type serine protease inhibitor, miR-bantam, WSSV infection, *Penaeus monodon*, innate immunity



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