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Abstract

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Transferrin, a newly identified iron metabolism protein in tick*

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Haemaphysalis longicornis is one of the most important tick species distributed globally. It transmits various pathogens that cause great damage to human health, livestock production, and wild animals. Knowledge of the character of transferrin (Tsf) in insects has grown rapidly during the last decades. However, Tsf in ticks is still poorly understood. Here, we identified for the first time a new iron metabolism-related gene, namely, *Tsf*, in ticks. The open reading frame (ORF) of the *Tsf* gene in *H. longicornis* contained 2,412 bp, which encoded a protein of 803 amino acids. The molecular weight of the Tsf protein was predicted to be 88.9 kDa. The dynamic changes of the *Tsf* gene in *H. longicornis* at different developmental stages and tissues were detected by using real time quantitative polymerase chain reaction. The *Tsf* gene was expressed at all stages (larva, nymph and adult) and in different tissues (midgut, salivary gland, malpighian tubals and ovary). By constructing the prokaryotic expression vector and optimizing the expression conditions, recombinant Tsf of *H. longicornis* (rHlTsf) with high purity was obtained. The results showed that the maximum iron-chelating activity of rHlTsf was 95.3% at 0.5 μg/μL concentration. The maximum antioxidant activity of rHlTsf was 77.6% at 0.5 μg/μL concentration. Culturing *Escherichia coli* with rHlTsf protein for 4–5 hours inhibited its proliferation. There was clear evidence of some hemolysis: there was no hemolysis within the concentration range of 1 μg/μL; the hemolysis rate was 2.2% at the concentration of 1 μg/μL; the hemolysis rate was 35.1% at the concentration of 2 μg/μL. *Tsf* expression in *H. longicornis* was interfered by RNA interference (RNAi) to detect the protein's function *in vivo*. The results demonstrated that silencing the *Tsf* gene by RNAi decreased engorgement weight, oviposition, and ovary weight at repletion in female ticks and had adverse impacts on hatching rate and incubation period. These results provide an important scientific basis for comprehensively revealing the mechanism of Tsf protein in *H. longicornis*, and finding new targets for control of the tick and damage prevention, which has important scientific significance and potential application.

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Keywords: transferrin, *Haemaphysalis longicornis*, iron metabolism

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