

Identification of an Alpha-Tubulin Gene from the Chinese Mitten Crab *Eriocheir sinensis*: Expression Profiles under Immune Challenge and during Larval Development, Autoimmune hepatitis, Immune Cell Therapy, Auto immune Disease

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In this study, an alpha-tubulin gene (EsTUBA, GenBank: KJ509188) was isolated and identified from the Chinese mitten crab *Eriocheir sinensis*. The EsTUBA mRNA expression patterns under larval brachyurization development and immune challenge stress by *Vibrio parahaemolyticus* injection in adult crabs were evaluated. EsTUBA cDNA was 1,695 base pairs (bp) with an open-reading frame of 1,356 bp encoding 451 amino acids (49.95 kDa) and containing GTPase domain and C-terminal Tubulin domain. Sequence alignment showed that EsTUBA shared 95-98% identity and exactly similar structural features with its counterparts reported in other animals. Phylogenetic analysis indicated that EsTUBA clustered into one group together with Tubulins from other crustaceans. Sequence alignment, structure comparison and bioinformatic analyses revealed that EsTUBA is member of the Tubulin family. EsTUBA was fluctuant expressed in all tested larval development stages and various organs, and the levels of EsTUBA mRNA expressed in hepatopancreas, gill and intestine, are significantly expression fluctuated and induced after bacteria *V. parahaemolyticus* injection, while in heart, is almost consistently expressed at 0, 6, 12, 24, 36 and 48 h post-injection compared to control. The molecular cloning of EsTUBA gene is important for further study on the function of this gene.

The cytoskeleton of eukaryotic cells is comprised of microtubules (MTs), intermediate filaments and microfilaments. MTs are hollow cylindrical polymers built through the lateral association of protofilaments composed of longitudinally aligned head-to-tail $\alpha\beta$ - tubulin dimmers. Tubulin is globular protein and consists of several distinct families, including the alpha (α), beta (β), gamma (γ), delta (δ), epsilon (ϵ), zeta (ζ), iota (ι), eta (η), theta (θ) and kappa (κ) tubulin. Tubulins as important component of the MTs in the cytoskeleton have been well known for a long time. The tubulin family builds the MTs that play highly diverse and essential roles in eukaryotic cells, including cell division and elongation, maintaining cell structure, vesicle transport, signal transduction etc. During the last decade, many studies on the structure and function of tubulins and their interaction with other proteins

have been reported. Functional conservation of tubulins across kingdoms is supported by copolymerization of heterologous or chimeric α -tubulin and β -tubulin, either in vitro or in vivo. Study on α -tubulin in Japanese pine sawyer *Monochamus alternatus* indicated that α -tubulin is also very important in the animal development. And in recent years, tubulins have been shown associated with post-transcriptional regulation, mitochondrial metabolism and arrangement, ependymal cells formation and axonic elongation of neuroblasts, knowledge about the role of tubulins have widened.

The Chinese mitten crabs (*Eriocheir sinensis*; family Grapsidae) are limnic and intertidal crabs distributed in Asia and are one of the major groups of brachyuran crabs in the world. They undergo brachyurization metamorphosis during the early physiological stages characterized by a reduced abdomen, folded beneath the cephalothorax, and inserted between the pereopods or in a special cavity. This crab produced in the coastal rivers and estuaries of the Yellow Sea is a traditional savory food, and therefore is one of the most important cultivated aquatic species in China. Due to the rapid development of crab farming, the country's crab production reached about 650,000 tons in 2012. Various diseases caused by viruses, bacteria, etc., have also begun to emerge and have resulted in enormous losses. Up to now, some immune-related genes of *E. sinensis*, such as C-type lectin, Hsp70, Hsp90, and secreted ferritins etc., has been reported. But remarkably little is known about crustacean tubulins and how they involved in innate immune responses. At this time it is still unclear whether the tubulin gene (EsTUBA) expression of the Chinese mitten crab is related to the physiological stages in which brachyurization occurs, and plays role in the immune system of *E. sinensis*. In this study we have cloned the full-length cDNA of EsTUBA from *E. sinensis* and examined expression profiles in association with larval development and immune challenge stress. Due to association of α -tubulin with cell division and immunoprotection, studies on the expression regulation of this gene would be critical to understand its role during the brachyurization development and immune challenge stress in the economically important crabs.