A potential negative regulation of myostatin in muscle growth during the intermolt stage in *Exopalaemon carinicauda*

Jiajia Wang, Jitao Li, Qianqian Ge, Jian Li*

Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, China

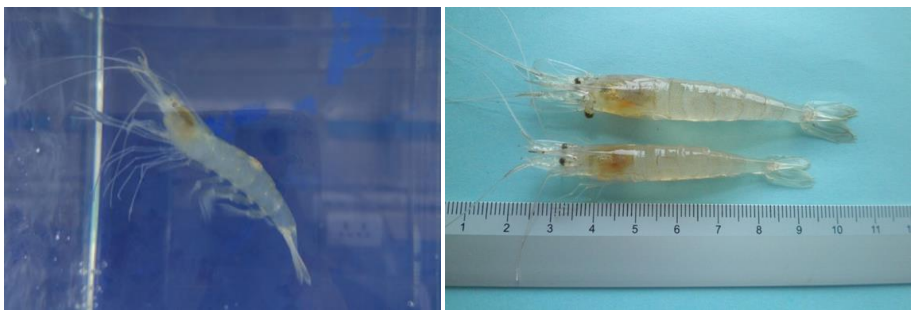
**E-mail: lijian@ysfri.ac.cn*

Introduction

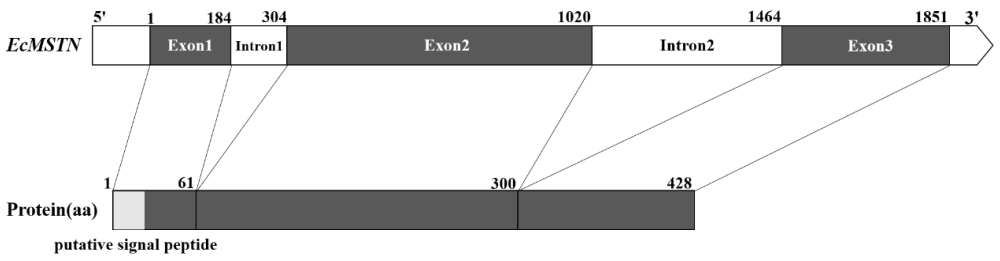
The ridgetail white shrimp *Exopalaemon carinicauda*, belonging to the Palaemonidae family of crustaceans, is a major commercial mariculture species naturally distributed on the coasts of the Yellow Sea and Bohai Sea.

The growth and development of shrimp primarily depend on muscle restoration and net muscle gain during molting.

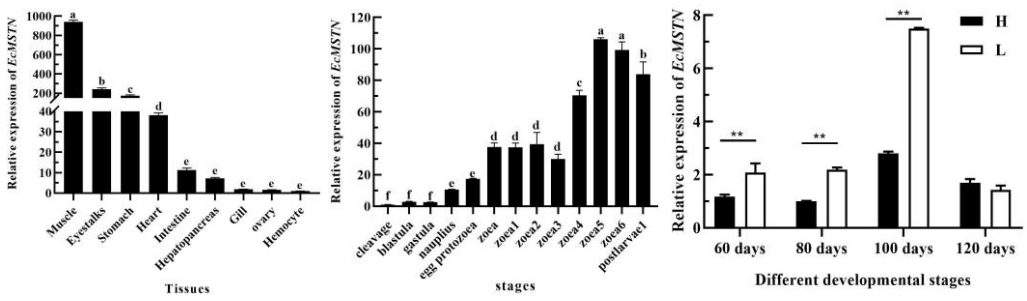
Myostatin (MSTN) plays important roles in regulating embryonic development and maintaining tissue homeostasis in mammals, but until now solid evidence supporting a similar function of MSTN in invertebrates has been lacking.



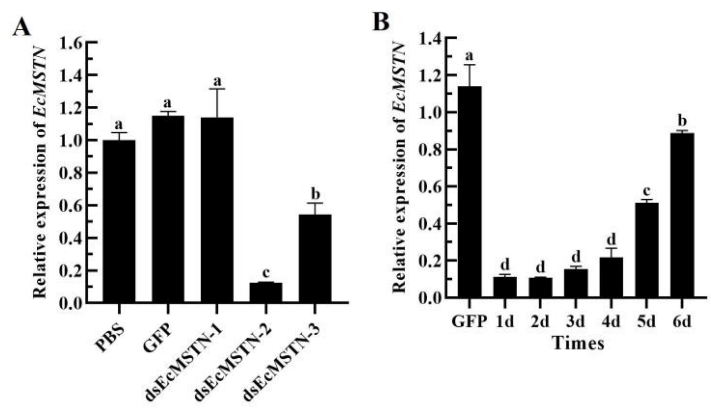
Results



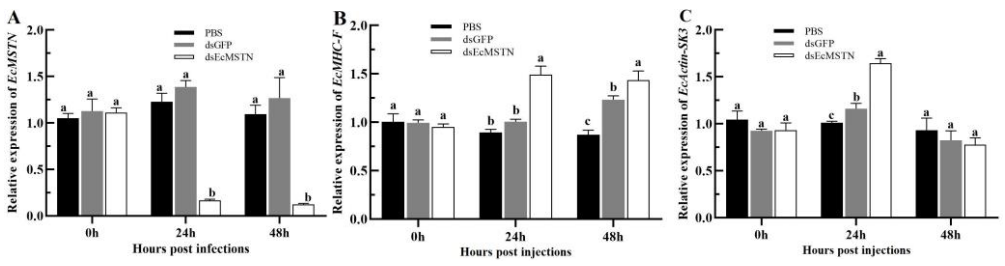
Schematic diagram of *EcMSTN*



Expression level of *EcMSTN* in different tissues and developmental stages of *E. carinicauda*



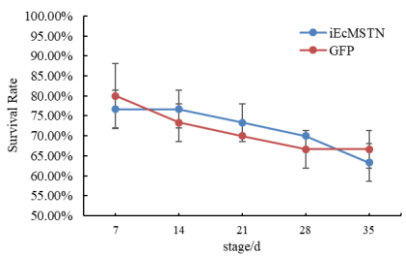
Expression analysis of *EcMSTN* after dsEcMSTN injection at 24 h (A) and after dsEcMSTN-2 injection at different days (B)



Expression analysis of *EcMSTN*(A), *EcMHC-F* (B) and *EcActin* (C) after dsEcMSTN injection

Groups	1 d	7 d	14 d	21 d	28 d	35 d
iEcMSTN	0.70±0.15	0.76±0.16	0.85±0.14	0.93±0.17	1.00±0.19	1.11±0.20
GFP	0.68±0.17	0.71±0.17	0.77±0.15	0.83±0.16	0.87±0.17*	0.93±0.16*

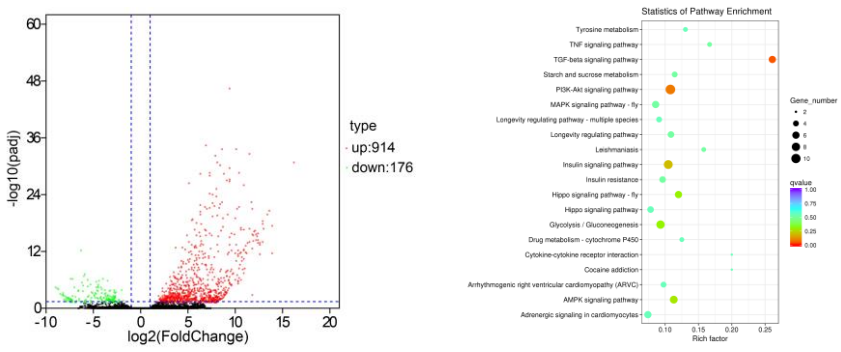
Comparison of body weight between iEcMSTN and GFP groups



The survival rate

SNP name	Genotype	Frequency	Body length (mm)	Body weight (g)
g.Mstn220	AA	0.28	54.97 ± 2.12^{a}	2.54 ± 0.29^{a}
	GG	0.53	52.33 ± 1.75^{ab}	2.27 ± 0.27^{ab}
	AG	0.19	49.13 ± 3.87^{b}	1.79 ± 0.40^{b}
g.Mstn567	TT	0.16	49.68 ± 3.88^{b}	1.80 ± 0.44^{b}
	CT	0.59	50.48 ± 3.68^{b}	1.99 ± 0.43^{b}
	CC	0.25	54.65 ± 2.39^{a}	2.49 ± 0.36^{a}

Association analysis



Differentially expressed genes

Statistics of pathway enrichment

基因ID	log2	功能注释	分类
Cluster-23185.5689	2.45	肌球蛋白重链	肌肉组成
Cluster-23185.6307	11.72	肌球蛋白轻链2	
Cluster-23185.10331	3.99	肌动蛋白	
Cluster-23185.7178	8.27	骨骼肌肌动蛋白	
Cluster-23185.6028	3.56	肌钙蛋白C	
Cluster-21974.0	4.09	血蓝蛋白	生长发育
Cluster-23373.0	6.54	热休克蛋白70	
Cluster-23185.11303	3.64	cAMP反应元件结合蛋白	
Cluster-23185.130	3.57	整联蛋白	
Cluster-17297.0	2.47	p38/MAPK	MAPK
Cluster-23185.1441	1.37	RAS	
Cluster-23185.7243	2.78	核糖体蛋白S6激酶	
Cluster-510.0	4.25	PI3K	PI3K/AKT
Cluster-17574.0	5.70	丝氨酸苏氨酸蛋白激酶	
Cluster-16032.1	1.17	TAK1	
Cluster-23185.1737	1.43	Smad3	TGF-β
Cluster-23453.0	1.30	Smad1	

Conclusions

In conclusion, we successfully identified and characterized *EcMSTN* from *E. carinicauda*. The tissue and embryonic developmental expression patterns suggested that it may be involved in muscle differentiation and growth. Further results of RNAi indicated its negative function in myogenesis and growth traits. *EcMSTN* may regulate growth trait by regulating MAPK and PI3K-Akt signaling pathways. Moreover, association analysis identified two SNP loci in *EcMSTN*. This study would contribute to clarify the negative role of MSTN in crustaceans, suggesting that it may have great potential and economic benefits for crustacean breeding programs.

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