

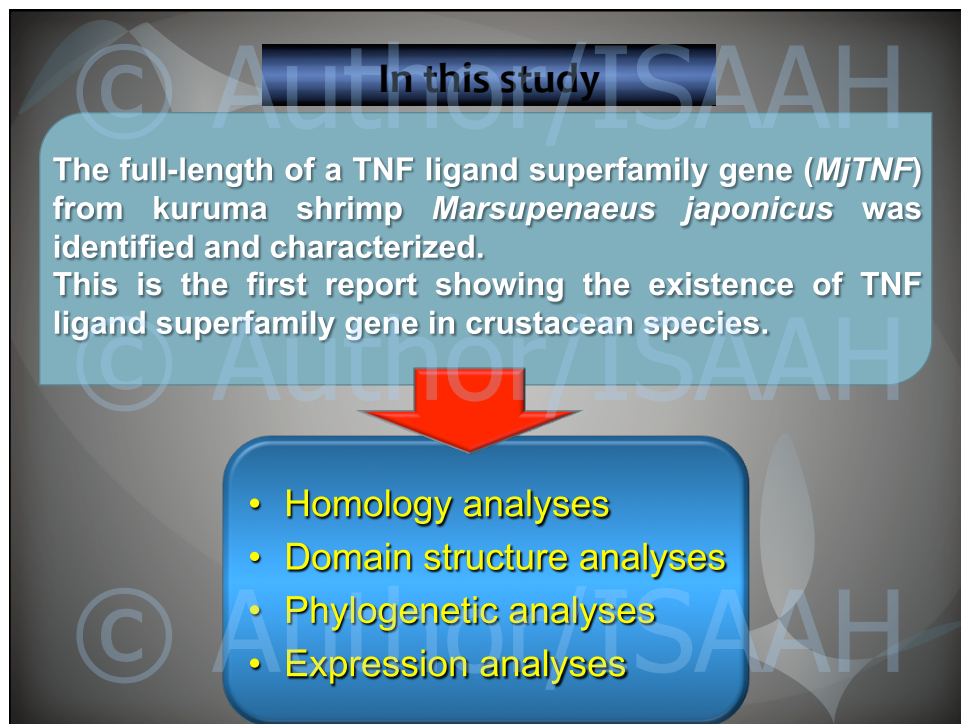
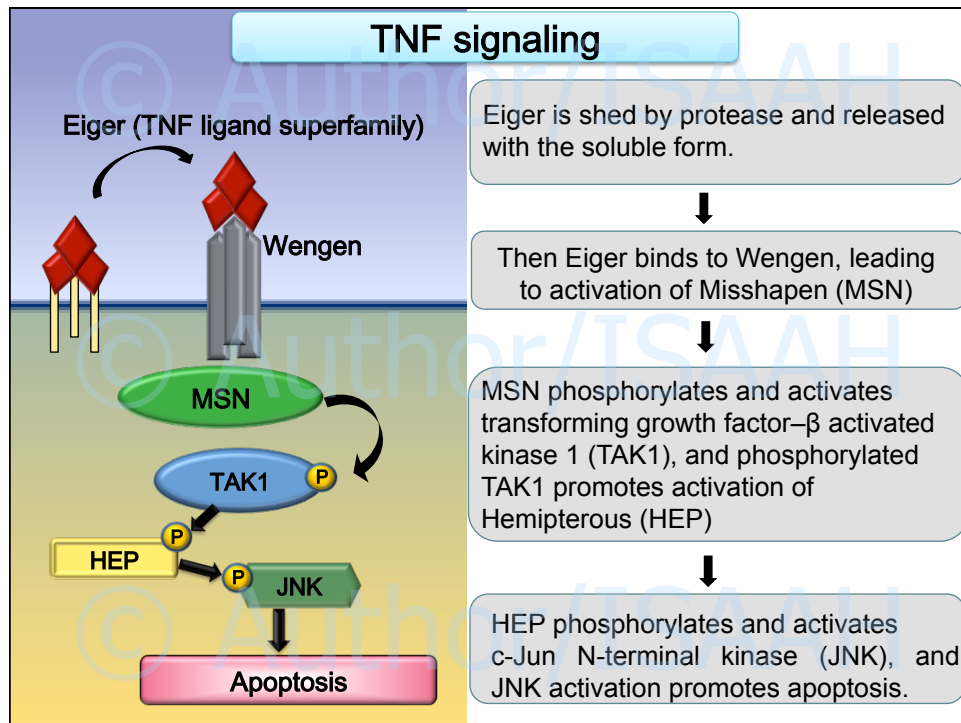
Characterization of tumor necrosis factor ligand superfamily gene in kuruma shrimp *Marsupenaeus japonicus*

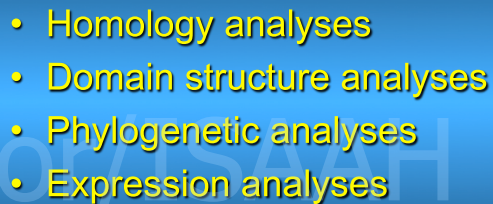
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Objectives

- Tumor necrosis factor (TNF) is a cytokine which plays an important role in the host defense mechanism, and have been identified widely from mammals to fishes.
- In mammals, the structure and functions of more than 19 genes of the TNF ligand superfamily members have been well-defined. In invertebrate, TNF ligand superfamily member named Eiger (ectodysplasin-like cell death trigger) was identified in fruit fly *Drosophila melanogaster*.
- TNF ligand superfamily gene in shrimp can be identified using information derived from insects.





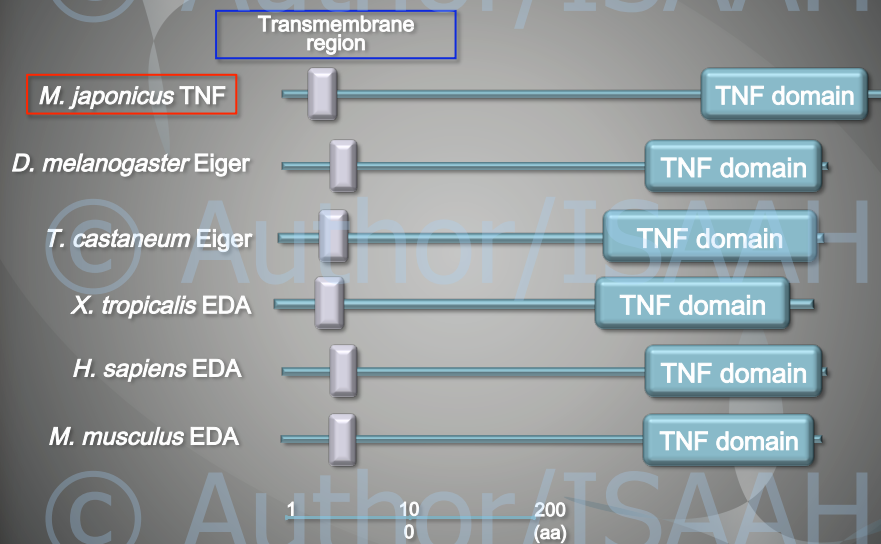
1,386 bp
462 amino acids
51 kDa

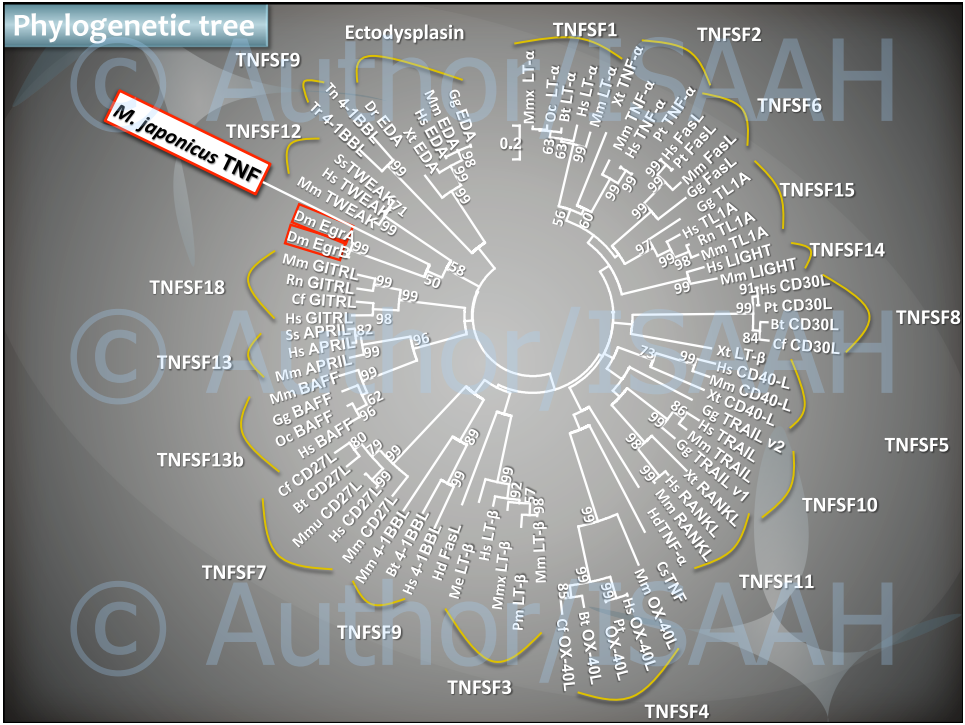
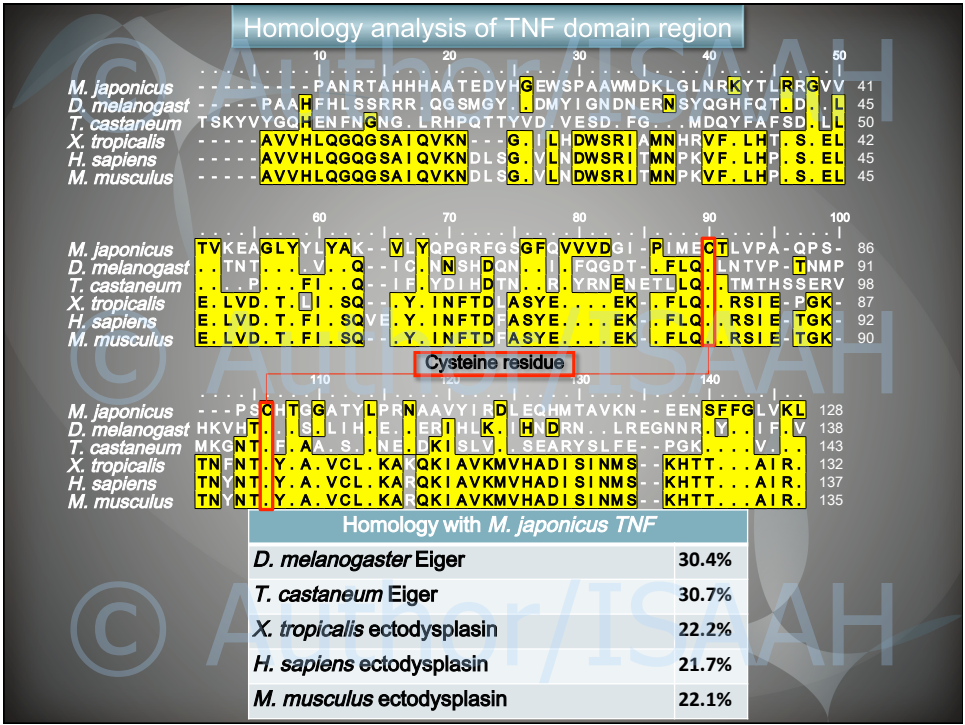
Analysis of amino acid identity and similarity of *MjTNF*

	1	2	3	4	5	6
1. <i>MjTNF</i>	shrimp	20.2	19.7	19.1	19.3	21.0
2. <i>DmEiger</i>	37.7	fly	22.8	18.9	19.0	19.1
3. <i>TcEiger</i>	40.8	42.4	flour beetle	20.2	20.5	20.0
4. <i>XtEDA</i>	34.9	35.9	35.5	flog	65.9	66.8
5. <i>HsEDA</i>	34.9	35.4	36.9	73.9	human	94.1
6. <i>MmEDA</i>	34.5	37.1	36.2	74.8	94.4	mouse

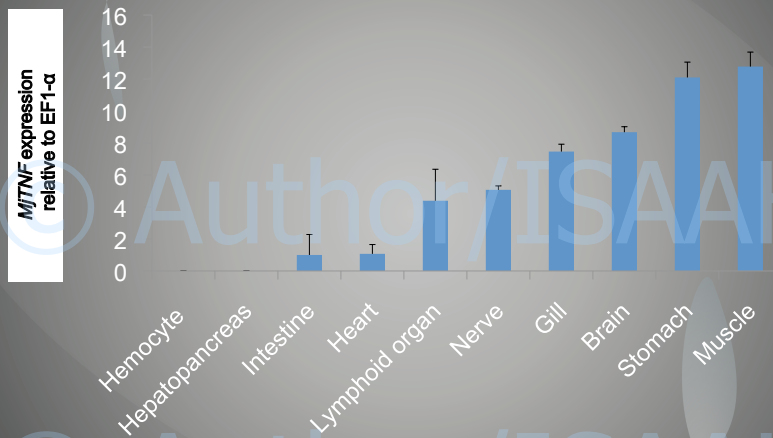
Upper triangle: identity, lower triangle: similarity.

Comparison of TNF domain structure





Transcriptional analysis of *MjTNF* in various organs



In vitro expression of *MjTNF* from lymphoid organ cells treated with immunostimulants

Lymphoid organs from 15 individual kuruma shrimp were collected and pooled together (average weight :12g)



Lymphoid organ cells were cultured in 2x L15 Leibovitz medium by incubating at 22°C in an incubator.



In vitro stimulation using lipopolysaccharide (LPS), peptidoglycan (PG) and polycytidylic acid (PolyI:C) was performed at a final concentration of 10 ng/ml.



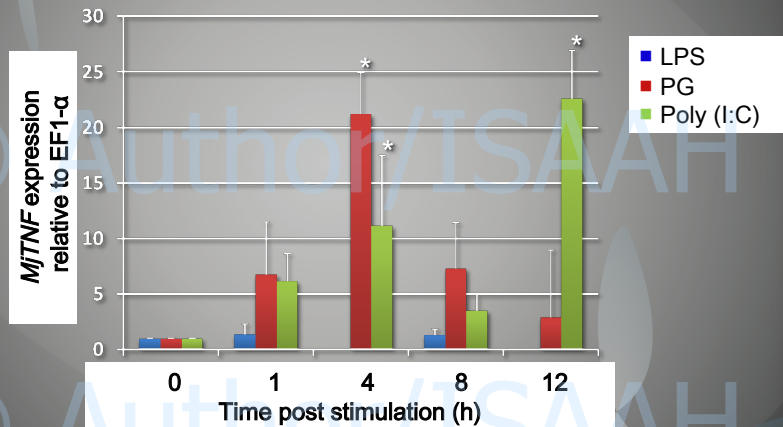
Different hours (1, 4, 8 and 12) sampling were performed post-stimulation



Total RNA was extracted from each sample and cDNA was constructed



In vitro expression analysis of *MjTNF* in lymphoid organ cells stimulated with LPS, PG and Poly I:C



In vivo expression of *MjTNF* following the injection with LPS and *V. penaeicida*

Kuruma shrimp was injected with LPS (200 µg/shrimp) and heat-killed *V. penaeicida* (1×10^5 cfu /ml)



Different hour sampling was performed at 1, 2, 4, 6, 8, 12 and 24 hours post stimulation

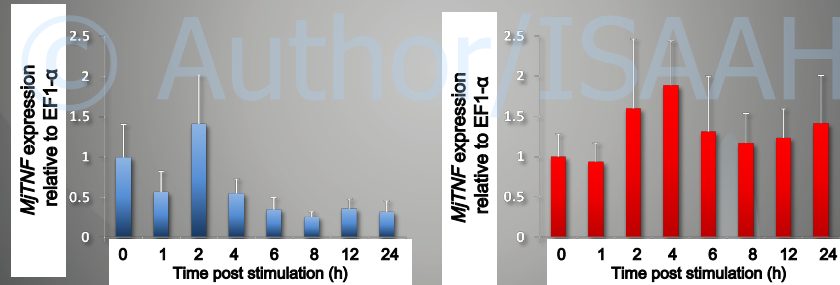


Total RNA was extracted from gill tissues of each sample and cDNA was constructed for *MjTNF* expression analysis

In vivo expression analysis of *MjTNF* with LPS and *V. penaeicida* injection at different time interval

LPS-injected group

V. penaeicida -injected group



Conclusion

- ◆ We have isolated and characterized a TNF ligand superfamily gene from kuruma shrimp (*MjTNF*) for the first time in crustacean species.
- ◆ Phylogenetic analysis revealed that the *MjTNF* was found in the cluster along with *DmEiger* genes.
- ◆ *MjTNF* was constitutively expressed in the muscle, stomach, brain, gill, nerve, lymphoid organ, heart, and intestine. In contrast, the *MjTNF* expression in the hemocytes and hepatopancreas was not detected.
- ◆ In *in vitro* experiment, expression level of *MjTNF* in PG-stimulated cells showed 21.2 folds higher at 4h post-stimulation. Higher levels of *MjTNF* expression was observed in Poly I:C stimulated cells at 4h and 12h, which are 11.1 and 22.6 folds higher, respectively.
- ◆ In *in vivo* experiment, higher levels of *MjTNF* expression was observed at 2h and 4h post stimulation in LPS and *V. penaeicida* treated groups. However, statistical differences were not observed among the samples.