

The Sea Star Ig kappa Gene and New Concept

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ABSTRACT

Next to the sea star T and B lymphocytes, the preservation of the Ig kappa gene for so extended a period of evolution in organisms as distinctively different as sea star, fish, mammal, indicates that it plays an essential role in the survival of organisms role in the regulation of immune response, in Asterids. The presence of Fc receptor gene, Fab gene in *Asterias rubens*, MHC genes corroborate these data.

Keywords: Invertebrates; Asterids; Ig kappa genes

INTRODUCTION

The purpose of this work is to draw attention to the mass of Ig kappa genes that has accumulated on the sea star Immune system since 2011. From this year, genomes of immunized and non-immunized sea stars to (Horse-Radish Peroxidase) HRP have been studied [1]. Although IgKappa gene has been isolated [2] and found in mouse, this gene has also been detected in fish (Zebra fish and *Larimichthys crocea*) and mammals. In this paper we will mainly review information on a fish: *Larimichthys crocea* and a mammal: *Tupaia chinensis*, to attempt to evoke Ig kappa gene evolutionary considerations.

ABOUT THE STUDY

Immunized and non-immunized *Asterias rubens* sea stars to HRP were used [1]. The axial organs were removed; RNA was extracted using Trizol (Invitrogen) according to manufacturer instructions from immunized sea stars (Horse-Radish Peroxidase) HRP and (Controls) C.

cDNA was normalized using double strand specific nuclease essentially as described by Zhulidov et al. [3]. cDNA was fragmented using DNA Fragmentase (New England Biolabs), according to the manufacturer's instructions. After ligation of adapters for Illumina's GSII sequencing system, the cDNA was sequenced on the Illumina GSII platform sequencing 100 bp from one side of the approximately 200 bp fragments. Sequences were assembled using Velvet Zerbino et al. [4]. Assembled nodes were used for further assembly including *Beta vulgaris* EST-Data from NCBI in MIRA [5,6].

RESULTS

First result concern non-immunized animals that is controls and contig to *Larimichthys crocea* (fish)

One Contig (Contig3054|m.205) could be annotated via BLASTX to *Larimichthys crocea* "IgKappa chain C region" from the TREMBL database, with an e-value of 3.415e-09. On an aligned region of 102 amino acids, 46 positive and 29 identical amino acids were found.

Second one is obtained from immunized sea stars to HRP

Another Contig (Contig12275|m.10416) could be annotated via BLASTX to *Larimichthys crocea* "IgKappa chain C region" from the TREMBL database, with an e-value of 2.538e-09. On an aligned region of 90 amino acids, 42 positive and 24 identical amino acids were found.

At last, we discover transcriptomes of Contigs via BLASTX to the mammal: *Tupaia chinensis*.

• Controls

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5'CCGCTGAGTTTTTGAACATATTGCCAAGTTAAAATAT
CTCATACCCTAATAATACACCATGTAATGTATTGCTTTA
ACATTGTAAAGATCAATGTGTGTACTACTGTAGTTAATA
TATCAGATCTCTCTGAAGTTAACACGTGTATCATACTTC
ATGGAGGCATGCACCTCAGCCTTG
GTTATCCCTTGGGAAAAGTTCTGTAAGAGTAGAATTGT
GTACCAGTGGGACTAAACATAA
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Received: December 31, 2020; **Accepted:** January 14, 2021; **Published:** January 21, 2021

Citation: Leclerc M (2021) The Sea Star Ig kappa Gene and New Concept. Intern Med. 11:327.

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TTGTGTGCATCTGTTTGGATAATATAAAAATAATATTTT
 ACAATGTGAAAGTATTTGTCA
 AGTGCATGGTGTAGGAAAATTAAGACTACATTTG
 TTTTCTGTTTTGTTACCTTTG
 CAATAAGTGTATGACACTGTTGGAAACAATAAAATGT
 TCAAATTTTGTATA3'

• **Second in Immunized sea stars to HRP**

One Contig (Contig12017|m.10218) could be annotated via BLASTX to *Tupaia chinensis* "Ig kappa chain V-III region HAH" from the TREMBL database, with an e-value of 3.11e-09. On an aligned region of 68 amino acids, 43 positive and 25 identical amino acids were found.

5'TGATGAATCTCTTAAAATTATATTTAAAATTACAAATT
 AAAAATTATTTGATATTTTGTCTGGCTCAAACCTTATT
 GTATTTGTGTTGTATCAAGACTATGTGCCTGGACTTG
 GTTTGGGATCTTGCACCCCTAGGGTGGTTCTGTGGGG
 AACCGTGACAAGTGTCTGGAGGAACTTTTGTGAGAA
 TTGTAGAAGAACAAGTGAACCTCATGAACAAAGCAA
 ACACCCACTTTGTGAGAGATAGATTATCCTGTTCAAA
 ATATCACAGTTATGCAGGTGTTTTTGTTTTTT
 TTCAATCTTTGTCTTTTTTCAGACATTTATGGCAATGCAG
 TCCAAGTATGCACAACCAATG
 TTTGTTTGTGGTAAATCTTTGTATGAAAATCTATGTGTTT
 ATTCACACTGTGATATCTACT
 TAGTAAATTCATTCAATTTTCAGGGTTGATGCTTTGTAA
 ACTTTGCTTTTTGTATAAAAAT
 AAGGAAACATAAATGGAATGTGAGGTAAAACAAAGTCA
 ACAATGTACATAAATGTGGCCAAGTCACACTAATGGGT
 TAAAAGATAACTTTGTAAATGAGGCGTGAGACAAATGT
 AACTTTTTTGTGCGCAGTCTTTTCTGTACATTCAAAG
 CTGTTTCATGATTTTTTCATTGCAAAAATA
 AATAAATTGACCTTAAGAAGTTACAAGGTCATATATTAC
 TACAAAACCACGTTCCCTCA
 TATGTTACTCTTTTGTGCACATCAGTGTAGAACCACCC
 ACATATGTATATTGGCCACTG
 ACCTATGACATTTTGTATGAATGCAATCGATGTGTAACA
 CTTGTGGAATATTGAAGTGTGT
 GTAGTACAATGGCACATTGTCCGTGTTTTGTATAAAAA
 TAGGAAATAAATGGTACACCA
 CT3'

There is evidence for the presence of Ig kappa genes through the animal kingdom. These results are summarized in Table 1 and Table 2:

Control	Genes
Contig3054 m.205	tr A0A0F8CMX3 A0A0F8CMX3_LARCR Ig kappa chain C region OS= <i>Larimichthys crocea</i> GN=EH28_01288 PE=4 SV=1
Contig3376 m.6635	tr L9JQR9 L9JQR9_TUPCH Ig kappa chain V-III region HAH OS= <i>Tupaia chinensis</i> GN=TREES_T100021693 PE=4 SV=1

Contig15579 m.12525	tr L8HL23 L8HL23_9CETA Ig kappa chain V-III region SIE (Fragment) OS= <i>Bos mutus</i> GN=M91_06423 PE=4 SV=1
TR38397 c0_g1_i1 m.2857	tr A0A0F8C094 A0A0F8C094_LARCR Ig kappa chain V-I region Wes OS= <i>Larimichthys crocea</i> GN=EH28_01990 PE=4 SV=1
TR48242 c0_g3_i1 m.3430	tr A0A091ENA6 A0A091ENA6_FUKDA Ig kappa chain V-VI region NQ2-6.1 (Fragment) OS= <i>Fukomys damarensis</i> GN=H920_01478 PE=4 SV=1

Table 1: The Ig kappa genes in controls.

And now, what about immunized sea stars to HRP?

Control	Genes
Contig12017 m.10218	tr L9JQR9 L9JQR9_TUPCH Ig kappa chain V-III region HAH OS= <i>Tupaia chinensis</i> GN=TREES_T100021693 PE=4 SV=1(similar protein)
Contig12275 m.10416	tr A0A0F8CMX3 A0A0F8CMX3_LARCR Ig kappa chain C region OS= <i>Larimichthys crocea</i> GN=EH28_01288 PE=4 SV=1
Contig8150 m.7973	tr A0A0F8CMX3 A0A0F8CMX3_LARCR Ig kappa chain C region OS= <i>Larimichthys crocea</i> GN=EH28_01288 PE=4 SV=1
Contig18903 m.13528	tr L8HL23 L8HL23_9CETA Ig kappa chain V-III region SIE (Fragment) OS= <i>Bos mutus</i> GN=M91_06423 PE=4 SV=1
Contig12300 m.10433	tr A0A091DDJ6 A0A091DDJ6_FUKDA Ig kappa chain V-V region T1 OS= <i>Fukomys damarensis</i> GN=H920_10033 PE=4 SV=1
Contig11501 m.9857	sp P01841 KAC5_RABIT Ig kappa-b5 chain C region OS= <i>Oryctolagus cuniculus</i>

Table 2: The Ig kappa genes in HRP (from immunized sea stars to HRP).

CONCLUSION

The sea star Ig kappa gene is clearly the oldest Ig kappa gene of the immune system of animals. It shows already two Ig sites! The

forms of Ig kappa genes are all found in vertebrates, they share many details with the sea star, including the presence of Ig sites. The preservation of the Ig kappa gene in immunized and non-immunized sea stars is an excellent opportunity for further experiments. It is important to notice that the Ig kappa chain V-III region HAH of *Tupaia chinensis* is situated (in the assumptions behind the theory of evolution) between the Ig kappa chain precursor V-II region (RPMI/133) and Ig kappa chain precursor V-IV region/121. The preservation of the IgKappa gene for so extended a period of evolution in organisms as distinctively different as sea star, fish, rodent, mammal, indicates that it plays an essential role in the survival of the organisms, role in the regulation of the immune response. Additionally, the existence of members of the IgKappa gene family with conserved functional characters, indicate that the sea star Ig kappa gene has evolved prior to the evolutionary divergence between Invertebrate and Vertebrates: It must be claimed. On the other hand, the discovery of a Fc receptor gene, of a Fab gene, MHC genes, in *Asterias rubens* genome,

corroborate the presence of the Invertebrate Primitive Antibody (IPA) in Asterids.

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