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Discovery of immune molecules and their crucial functions in shrimp immunity

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ABSTRACT

Several immune-related molecules in penaeid shrimps have been discovered, most of these via the analysis of expressed sequence tag libraries, microarray studies and proteomic approaches. These immune molecules include antimicrobial peptides, serine proteinases and inhibitors, phenoloxidases, oxidative enzymes, clottable protein, pattern recognition proteins, lectins, Toll receptors, and other humoral factors that might participate in the innate immune system of shrimps. These molecules have mainly been found in the hemolymph and hemocytes, which are the main sites where immune reactions take place, while some are found in other immune organs/tissues, such as the lymphoid organs, gills and intestines. Although the participation of some of these immune molecules in the shrimp innate immune defense against invading pathogens has been demonstrated, the functions of many molecules remain unclear. This review summarizes the current status of our knowledge concerning the discovery and functional characterization of the immune molecules in penaeid shrimps.

1. Introduction

Penaeid shrimps include some economically important and aquacultured marine species, such as the Pacific white shrimp *Litopenaeus vannamei* and the black tiger shrimp *Penaeus monodon* that are currently the two main successfully species cultured worldwide. However, disease outbreaks have caused massive mortality and a great loss to the shrimp cultivation industry, and particularly from the outbreaks caused by the major shrimp pathogens of white spot syndrome virus (WSSV), yellow head virus (YHV), infectious myonecrosis virus (IMNV), and bacteria in the genus *Vibrio* [1,2]. Understanding the innate immune responses of shrimps against invading microbes provides essential information for the establishment of effective methods to control these and, potentially, those of related emerging infectious diseases.

Lacking an adaptive immune system, shrimps rely on their effective cellular and humoral innate immune responses to combat invading microbes [3]. The cellular immune reactions include phagocytosis, nodulation and encapsulation, whereas the humoral responses involve the synthesis and release of several immune proteins, such as antimicrobial peptides (AMPs), proteinase inhibitors, cytokine-like factors, etc. In crustaceans, including shrimps, major immune reactions take place in hemolymph, which contains three different principal types of hemocytes that are defined as the hyaline, granular and semigranular hemocytes [4]. Several immune molecules are produced and stored in the granules of hemocytes before being released into the hemolymph upon activation by bacterial and/or fungal cell wall components, such as peptidoglycan (PG), lipopolysaccharides (LPS) and β-glucans (BGs) [5]. Pattern recognition proteins (PRPs) or pattern recognition receptors (PRRs) recognize and bind the microbial cell wall components and activate various immune responses [6–8].

In this review, we describe the discovery of immune-related molecules by the high throughput technologies of genomic and proteomic analyses and the characterization of these immune molecules that participate in the major immune reactions against invading pathogens in shrimp.

2. Discovery of immune genes/proteins by high throughput genomic/proteomic approaches

2.1. Expressed sequence tag (EST) analysis of immune genes in penaeid shrimp

Shrimp have a relatively large genome size of approximately 2×10^9 bp [9], which in part reflects the high percentage of repetitive sequences [10]. The whole genome sequencing of *L. vannamei* is now in progress but the information is not yet

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publically available. Rather, to date the information on the shrimp genome has largely been obtained from the analysis of expressed sequence tags (ESTs) sequences. This has led to several tissue-specific transcripts being identified as well as candidate genes that may be implicated in the shrimp immune responses (Table 1). Nevertheless, the function of these immune genes and proteins are poorly understood and almost all require further studies to unveil their function in the shrimp immune system.

The analysis of EST libraries that has been generated from various cells or tissues provides information on the tissue-specific profiles of gene expression and relative transcript abundance that is likely to reflect the function of those cells or tissues. Likewise, the comparison of EST libraries between different pathogen infected or control tissues at various times after infection can provide information on genes whose transcript expression levels are significantly changed over time as a result of the infection.

The immune system of invertebrates has been well studied in insects, including the outstanding model of the fruit fly *Drosophila melanogaster*, for which a large number of deposited EST sequences (821,005 ESTs as of May, 2012) are available in public databases and backed up by extensive gene characterization studies. Moreover, several immune-related genes from other insects have now been identified and characterized. In contrast, much less information is currently available for crustaceans including shrimps. For penaeid shrimps, there are only 216,436 EST (0.3% of the total number of ESTs) sequences deposited in GenBank, and are comprised of 161,241 ESTs from *L. vannamei*, 39,397 ESTs from *P. monodon*, 10,446 ESTs from *Fenneropenaeus chinensis* and 5352 ESTs from other penaeid shrimps. To gain more information on the genomics of shrimps, several ESTs have been constructed from various tissues of shrimps that were reared under normal, stressed or pathogen-challenged conditions, depending on their purposes [11–13]. In 2011, Leu et al. [14] have constructed a shrimp transcriptome database based on EST libraries of four major penaeid shrimps including *L. vannamei*, *P. monodon*, *F. chinensis* and *Marsupenaeus japonicus*. To isolate the shrimp immune-related genes, the large-scale EST libraries were established from three shrimp species, *L. vannamei*, *P. monodon* and *F. chinensis* [11–13]. O'Leary et al. [12], generated cDNA libraries derived from multiple tissues of *L. vannamei* and these derived cDNA libraries depleted of the redundant transcripts. From the total of 13,656 EST clones, 7896 EST clones were randomly sequenced from six non-normalized cDNA libraries that were derived from the hemocyte, hepatopancreas, gill, lymphoid organ, eyestalk and ventral nerve cord, respectively. A further 5760 EST clones were randomly selected from 34 different suppression subtractive hybridization (SSH) derived cDNA libraries generated from the hemocyte, gill and hepatopancreas, respectively, as they were predicted to be immune-related organs [15]. Analysis of these sequences identified 7466 unique sequences represented by 1981 contigs and 5485 singletons, and 38% of the unique genes were homologs of genes deposited in the GenBank database. Nearly 40% of the EST clones were derived from hemocytes, which are likely to be the primary immune cells of shrimps. From these analyses, several potential immune genes were identified, including AMPs, proteinase inhibitors, clottable protein and heat shock proteins (HSPs). Tassanakajon et al. [11] reported the EST analysis of 15 cDNA libraries from *P. monodon*, comprised of 13 standard libraries and two normalized libraries. For the standard libraries, six different tissues (hemocyte, hepatopancreas, lymphoid organ, eyestalk, hematopoietic tissue and ovary) from healthy and microbial-challenged or heat-stressed shrimps were used to potentially identify candidate genes involved in the immune defense, growth and sex differentiation. Additionally, two normalized cDNA libraries were generated from the hepatopancreas and lymphoid organ. The total of 10,100 EST clones were

Table 1
Immune-related genes of penaeid shrimps initially identified by expressed sequence tag (EST), suppression subtractive hybridization (SSH) and microarray as well as their characterized functions.

Immune-related genes	Tissue distribution[a]	Stress response[b]	Function
1. Antimicrobial peptides			
Antilipopolysaccharide factors	G, Hc, Lo	dsRNA, H, N, V, W, Y	Antimicrobial activity, antiviral activity, antifungal activity
Bactinecin	Hc	W	ND
Crustins	Ep, G, Hc	AA, H, N, P, W, V, Y	Antimicrobialactivity
Lysozymes	G, Hc, Hp, Ht, Lo	dsRNA, N, W, V, H, P	Antimicrobial activity
Penaeidins	Hc	AA, H, N, V, W, Y	Antimicrobial activity, antifungal activity
Single whey acidic protein domain-containing peptides	Hc	N, P, W	Antimicrobial activity
2. ProPO system			
Masquerade-like serine proteinase-like protein	Hc	W, Y, V, P	Mediates hemocyte adhesion, binding to bacterial cell wall, antimicrobial activity
Prophenoloxidase	Hc	AA, H, N, P, V, W, Y	Clearing the bacteria from circulation after infection
Prophenoloxidase activating factor	Hc, Lo	H, N, V, W, Y	Clearing the bacteria from circulation after infection
3. Oxidative stress			
Copper chaperone	Hc	N	ND
Copper/zinc superoxide dismutase	Hc	W, Y	ND
Cytosolic MnSOD	Hc, Hp	H, N, V, W	ND
Death associated protein diphenol oxidase	Hc	N	ND
Glutathione peroxidase	Hc	H	ND
Glutathione-S-transferase	G, Hc, Hp	N, V, W, Y	ND
Heme peroxidase	Lo	N, V, W	ND
Peroxiredoxin	Hp	N	Antioxidant activity
Peroxisomal antioxidant enzyme	Hc	N	ND
Thioredoxin	C, G, Hp	N, W	Antioxidant activity
Thioredoxin reductase	G, Hp	W	ND
4. Proteinases/proteinase inhibitors			
Alpha-2-macroglobulin	Hc	AA, P, W, Y	Inhibit fibrinolytic activity of bacteria
Aminopeptidase	G, Hc	N, W	ND
Antileukoprotease	Hc	W	ND
Astacin protease	Hp	W	ND
Caspase	Hp	W	Protease, WSSV-induced apoptosis
Cathepsin A	Hc, Lo	N, V, W	ND
Cathepsin C	Lo	N, V	ND
Cathepsin D	Hp, Lo	N, W	Muscle proteases
Cathepsin L	G, Hc, Hp, Lo,	AA, dsRNA, N, V, W, Y	ND
Cathepsis B	Hc, Lo	N, V	Protease
Chelonianin	Hc	W	Antimicrobial activity
Cubilin protease	G	W	ND
Cystein protease caspase-2	Lo	N, V, W	ND
Double whey acidic domain-containing peptide	Hc		Proteinase inhibitor, antimicrobial activity
Elastase inhibitor	Hc	W	ND
Gene MAC25 protein	Hc	H, N, V	ND
Kunitz-type inhibitor	Hc	W	Inhibit the activity of trypsin
Leucocyte elastase inhibitor	G, Hc, Hp, Lo	V	ND
Lysosomal caboxypeptidase	Hc	W	ND