Fish and Shellfish Immunology 92 (2019) 438–449

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Proteomic characterization of the hepatopancreas in the Pacific white shrimp *Litopenaeus vannamei* under cold stress: Revealing the organism homeostasis mechanism

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ARTICLE INFO

Keywords:
Litopenaeus vannamei
Hepatopancreas
Cold stress
iTRAQ

ABSTRACT

To understand the homeostasis mechanism of crustacean hepatopancreas to cold stress, iTRAQ proteomics based on the genome database of *Litopenaeus vannamei* (*L. vannamei*) was applied to investigate proteins changes and variety of the hepatopancreas during cold stress stage in this study. A total of 4062 distinct proteins were identified, 137 differentially expressed proteins (DEPs) including 62 differentially up-regulated proteins (DUPs) and 75 differentially down-regulated proteins (DDPs) were identified in G1 (18 °C) compared with CK (28 °C), 359 DEPs including 131 DUPs and 228 DDPs were identified in G2 (13 °C for 24 h) compared with CK. Based on bioinformatics analysis, the cold tolerance of *L. vannamei* might be related to energy metabolism such as amino acid, carbohydrate, lipid, and oxidative phosphorylation. Moreover, shrimp immunity was declined during cold stress stage. However, *L. vannamei* could cope with cold stress by enhancing the production of ATP and UFA. Notably, arginine kinase, heat shock proteins, and histones may act as positive regulators in *L. vannamei* under cold stress. Ten randomly selected proteins were used for validation using qRT-PCR and the expressions on the transcription level for most of the genes were similar to the results of iTRAQ. These results indicated that *L. vannamei* can maintain the organism homeostasis by a series of orderly regulatory process during cold stress. Furthermore, the results can provide guidance for shrimp farming.

1. Introduction

Stress is physiological disturbance or damage caused to an organism by adverse circumstances from a biological standpoint, and stimuli are responded by multiple systems of the body. In this case, most biological pathways strive to maintain organism equilibrium (or homeostasis) [1]. Environmental stressors continually disrupt this homeostasis, and the organism strives to find the homeostatic point for living. Therefore, the organism needs to equilibrate the fluctuation between far away from homeostasis and back to or near homeostasis [2,3].

Aquatic animals living in water were easy to suffer from physical, environmental, and physiological disturbances. As the tropical origin, the Pacific white shrimp *Litopenaeus vannamei* (*L. vannamei*) were easily affected by temperature [4]. To an organism, temperature (thermal) stress is created by any changes of temperature. There were many reports about the temperature stress in *L. vannamei*, such as gut microbiota profiles response to cold stress [5], survival, growth, and yield influenced by variation of water temperature [6], heat shock proteins (HSPs) [7], microRNAs involved in cold adaptation [8], the mucosal structure and immune response induced by elevated temperature [9], energy metabolic enzyme responses to thermal stress [10], proteomic responses of two contrasting shrimp cultivars [11], TCP-1-eta with cold tolerance [12], dietary taurine, carnitine, and cholesterol supplementation [13]. Although considerable studies have been devoted to the temperature stress in *L. vannamei* from different aspects in recent years, the organism homeostasis mechanism during cold stress stage was still unknown. Especially in the published studies of proteomics, the lack of the genome database of *L. vannamei* limited the discovery of functional proteins.

Proteomics is the large-scale study of proteins in a particular tissue or organism [14]. Meanwhile, it could be used to assess the physiological state among different samples under different condition or stress. In the recent years, the isobaric tag for relative and absolute quantification (iTRAQ) proteomics approach technology is considered to be a

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https://doi.org/10.1016/j.fsi.2019.06.037
Received 23 April 2019; Received in revised form 25 May 2019; Accepted 17 June 2019
Available online 20 June 2019

good technology because of the high labeling efficiency, proteome coverage, automated, and multidimensional [15,16]. With its advantages, iTRAQ-based proteomics has attracted more attention and applied in various studies recently [17–21].

In the present study, an iTRAQ proteomics combined bioinformatics analysis was applied to investigate proteins dynamic change in the hepatopancreas of *L. vannamei* during cold stress stage. Moreover, the published genome sequence of the *L. vannamei* provided support of database for the iTRAQ proteomics [22]. To our knowledge, this study represented the first investigation of the molecular mechanism of cold stress in shrimp using the genome database of *L. vannamei*. The results provided important information on shrimp molecular responses against cold stress and guidance for shrimp farming.

2. Materials and methods

2.1. Shrimps and cold stress

L. vannamei with average weight 5–6 g were obtained from a shrimp farm in Panyu (Guangdong, China). Shrimps were transported to the lab and acclimated in 500 L air-pumped circulating diluted seawater tanks at least one week prior to stress experiment. The water salinity and temperature in tanks were consistent with that of the ponds (salinity 5‰ and temperature 28 ± 1 °C) during the acclimation stage. The shrimps were fed two times per day with commercial feed. Only healthy shrimps in the intermolt stage were used for further experiments.

Seventy-five shrimps were randomly divided into 3 tanks (60 × 40 × 35), 25 individuals per plastic aquarium with exposure chamber and air pump for aeration. An artificial climate incubator (Temperature range 5–50 °C) was used for temperature control and the water temperature was decreased from 28 °C to 13 °C in 2 days at a rate of 2.5 °C/8 h.

2.2. Sample collection

Hepatopancreas of six individuals from each tank was collected as a sample while the water temperature was maintained at 28 °C, 18 °C and 13 °C for 24 h separately during cold stress, three samples as one group. One hepatopancreas was divided into two for the proteomics analysis and expression validation separately. The hepatopancreas was frozen immediately in liquid nitrogen and then stored at −80 °C until protein and RNA extraction.

2.3. Protein extraction

Protein was extracted from hepatopancreas using the method we established before [4]. Protein extraction was performed using 0.01% PBS buffer (135 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, and 8 mM K_2HPO_4, pH7.2). The lysate was centrifuged at 12,000 g and 4 °C for 15min. The supernatant was precipitated with chilled acetone containing 10% TCA and incubated at −20 °C for 2 h and then centrifuged at 12,000 g and 4 °C for 15min. The precipitate was washed by chilled acetone, incubated at −20 °C for 1 h and centrifuged again at 12,000 g and 4 °C for 15min. The washing was repeated two times. After air-dried, the pellet was re-dissolved with the lysis buffer (8 M urea, 2 M thiourea, 2% w/v CHAPS, 20 mM Tris-HCl, 30 mM DTT, and 2% v/v ampholytes). The protein concentration was determined using a BCA protein assay kit. The extracted protein was detected using SDS-PAGE.

2.4. Protein digestion and iTRAQ labeling

Protein digestion method was according to the method established by Wisniewski JR et al. [23]. Briefly, total protein (100 μg) for each sample was digested using trypsin (Promega, USA) at 1:50 trypsin-to-protein mass ratio at 37 °C for 16 h. Then peptides were reconstituted in 0.2 M TEAB and processed according to the manufacturer's protocol for 8-plex iTRAQ reagent (AB SCIEX, Framingham, MA, USA). Two biological replicates of the control group (CK-1 and CK-2 for the temperature at 28 °C) were labeled with 113 and 114 isobaric tags, respectively. The peptides with three biological replicates of 18 °C -treated group (G1-1, G1-2, and G1-3) were labeled with 115, 116 and 117 isobaric tags, respectively. The peptides with three biological replicates of 13 °C for 24 h treated group (G2-1, G2-2, and G2-3) were labeled with 118, 119 and 121 isobaric tags, respectively. Samples were placed at room temperature for 2 h, 100 μl deionized water was added into each tube, and three groups of labeled samples were mixed and dried by Speed-vacuum concentrator (savant DNA 120, Thermo Scientific).

2.5. High pH reversed-phase chromatography and NanoLC-ESI-MS/MS analysis

The peptides mixtures were subjected to the first-dimensional fractionation procedure using high pH reversed-phase chromatography column (Agilent, ZORBAX Extended-C 18 2.1). A total of 16 fractions were finally collected and dried for the following LC-MS analysis. The lyophilized peptide fractions were re-suspended in 2% acetonitrile containing 0.1% formic acid, and loaded into a ChromXP C18 (3 μm, 120 Å) trap column. The online Chromatography separation was performed on the Ekspert nanoLC 415 system (SCIEX, Concord, ON). The trapping and desalting procedures were carried out at a flow rate of 4 μL/min for 5 min with 100% solvent A (0.1% formic acid, 2% acetonitrile and 98% water). Then, an elution gradient of 8–38% solvent B (0.1% formic acid, 98% acetonitrile and 2% water) in 70 min was used on an analytical column (75 μm × 15 cm C18-CL, 3 μm 120 Å, ChromXP, Eksigent). IDA (information-dependent acquisition) mass spectrum technique was used to acquire tandem MS data on a Triple TOF 6600 tandem mass spectrometer (Sciex, Concord, Ontario, Canada) fitted with a Nanospray III ion source. Data were acquired using an ion spray voltage of 2.4 kV, curtain gas of 35 PSI, nebulizer gas of 12 PSI and an interface heater temperature of 150 °C. The MS was operated with TOF-MS scans. For IDA, survey scans were acquired in 250 ms and up to 40 product ion scans (60 ms) were collected if exceeding a threshold of 260 cps with a charge state of 2–4. A rolling collision energy setting was applied to all precursor ions for collision-induced dissociation. Dynamic exclusion was set for 16 s.

2.6. Database searching and protein quantification

The MS/MS data were analyzed using IPeak and IQuant software to obtain the protein identification and quantification [24,25], and NCBI *Penaeus vannamei* genome (33273 entries) [22], the following settings were selected: Enzyme: Trypsin; Variable modifications: Oxidation (M), iTRAQ8plex (Y); Fixed modifications: Carbamidomethyl (C), iTRAQ8-plex (N-term), iTRAQ8plex (K); Peptide mass tolerance: 20 ppm; Fragment mass tolerance: 0.05Da. Only proteins identified at global FDR ≤1% with ≥ 1 peptide were considered for protein lists and further downstream analysis. To determine a differentially expressed protein, it must be identified and quantified with at least 1 significant peptide and the p-values of proteins quantitation should be less than 0.05 and fold change ≥1.5.

2.7. Bioinformatics analysis

Functional annotation was performed using Gene Ontology (GO) (http://www.geneontology.org). The Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/or http://www.kegg.jp/) was used to predict the main metabolic pathways [26]. The Clusters of Orthologous Groups (COG) of proteins (http://www.ncbi.nlm.nih.gov/COG/) database was used for the functional classification. The eggNOG was also used for the functional classification (http://eggnogdb.embl.de). The eggNOG database was created in 2007 [27] and updated to version 4.5.1 in 2016 [28].

Table 1
The information of genes used for qRT-PCR.

Name	Sequence (5'→3')	Length (bp)	Accession Number
TMABA-DH-F	GCCGTCCTTACAGATTGCCGAGATG	204	XP_027225104.1
TMABA-DH-R	AGACAGTTCCTGCTTCAATGGCGTTAG		
EIF1AY-F	CACATCCGTGGGAAACTCCGAAAGA	179	XP_027212903.1
EIF1AY-R	AGCATTCTCGTTCACCTTGACTGATTCT		
NPA1-F	CACACTTTAGACCTACTGTTCGC	163	XP_027224970.1
NPA1-R	TCACCTTCTCTTTCAATTGATCCC		
WDR17-F	CATAGACGAGACAGATCAAGGC	247	XP_027227450.1
WDR17-R	CATGATTGATGAAGTGTAGTTTGGC		
TRET1-F	CCTTGTTCGCCTTCTTCCTCGTG	108	XP_027226333.1
TRET1-R	CGGTGCTGATCTTGCGGTTCC		
CRA2-F	ACAACGCCTATCAGCCATTCACTC	84	XP_027238674.1
CRA2-R	CTGTGGTCACCTTGAAGCCGTAG		
SERCA-F	TCCTTCGTGTTGGCTTGCTTCG	135	XP_027212602.1
SERCA-R	GATGGCGGACTCGGCATTGC		
AK-F	GGTTTCCTCTACCCTTTCTAGCC	194	XP_027224228.1
AK-R	TGTTGTCGTTGTGGTAGATGCC		
CEP76-F	TTGGTTTGGATGCCTGGGTG	119	XP_027221964.1
CEP76-R	ACGGCATCCTGTTGAGGACT		
ZC3H13–F	CAGCATCTCACACCAAGCAAGGAG	114	XP_027234855.1
ZC3H13-R	CATTGACATCACTGCCGACAGACC		

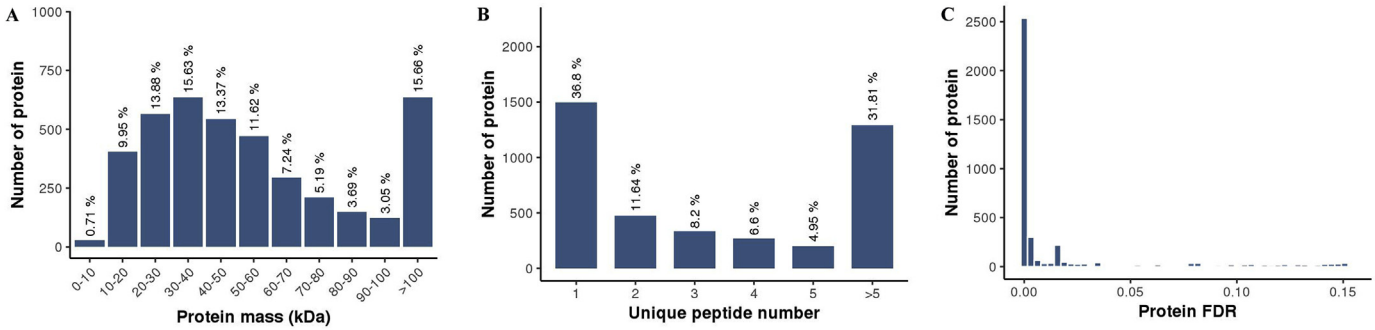

Fig. 1. Distribution of all identified proteins among different molecular weights (A); Distribution of proteins containing the different number of identified unique peptides (B); Distribution of the reliable protein (C).

2.8. Quantitative real-time PCR (qRT-PCR) analysis

Total RNA was extracted from shrimp hepatopancreas using Trizol reagent (Takara Bio, Otsu, Japan) following the manufacturer's protocol. The RNA quality was assessed by electrophoresis using 1.0% agarose gel. Total RNA was purified and first-strand cDNA was synthesized using PrimeScript® RT Reagent Kit With gDNA Eraser (Takara Bio Inc., Shiga, Japan) according to the instructions of the manufacturer.

Based on the identified differentially expressed proteins, 10 randomly selected proteins were used to analyze the transcription level under cold stress using qRT-PCR with β-actin as the internal control. The qRT-PCR assay was carried out on a CFX Connect™ Real-Time System (Bio-Rad, Hercules, CA, USA) using THUNDERBIRD® SYBR® qPCR Mix (TOYOBO, Osaka, Japan). The qRT-PCR was carried out with 95 °C for 1min followed by 40 cycles of 95 °C for 15s, 60 °C for 15s (WDR17,TRET1,CRA2, and CEP76 were used 58 °C for 30s) and 72 °C for 45s, and then a step at 95 °C for 15s, finally followed by a step at 60 °C for 15s. Gene expression was presented using the comparative Ct method ($2^{-\Delta\Delta Ct}$) method [29]. The information of the primers was presented in Table 1.

2.9. Statistical analysis

The experiments were performed with three biological replicates per treatment. All the data were expressed as mean ± SD of triplicates. Data were statistically analyzed by SPSS 21.0 (IBM, USA) with One-way ANOVA and Duncan's method at $P < 0.05$ level.

3. Results

3.1. Protein profiling from quantitative proteomics analysis

Using the iTRAQ LC-MS/MS proteomic analysis, a total of 481268 spectra were obtained. After data filtering to eliminate labeling free and low-scoring spectra, a total of 131323 identified spectra were matched to 25616 peptides. Then, proteins were assembled and identified with the false discovery rate (FDR) of peptides was < 0.01, and 4062 proteins were identified based on NCBI *Penaeus vannamei* genome reference. Considering the statistical significance of protein mass distribution, the number of identified proteins accounted for most (84.34%) of the total identified proteins in groups with molecular weight 0–10 kDa (0.71%), 10–20 kDa (9.95%), 20–30 kDa (13.88%), 30–40 kDa (15.63%), 40–50 kDa (13.37%), 50–60 kDa (11.62%), 60–70 kDa (7.24%), 70–80 kDa (5.19%), 80–90 kDa (3.69%) and 90–100 kDa (3.03%) (Fig. 1A). Among all the identified proteins, 63.2% were covered with 2 peptides or more (Fig. 1B), indicating a good sequence coverage of identified proteins. In terms of estimating incorrect peptide and protein identifications, the target-decoy search strategy was used with FDR < 1%, and the results showed the reliability of protein identification (Fig. 1C).

3.2. Identified protein functional annotation

The putative functions of identified proteins were annotated based on the GO, KEGG, COG and eggNOG database. Among the 4062 identified proteins, a total of 1618 had GO annotations, a proportion of

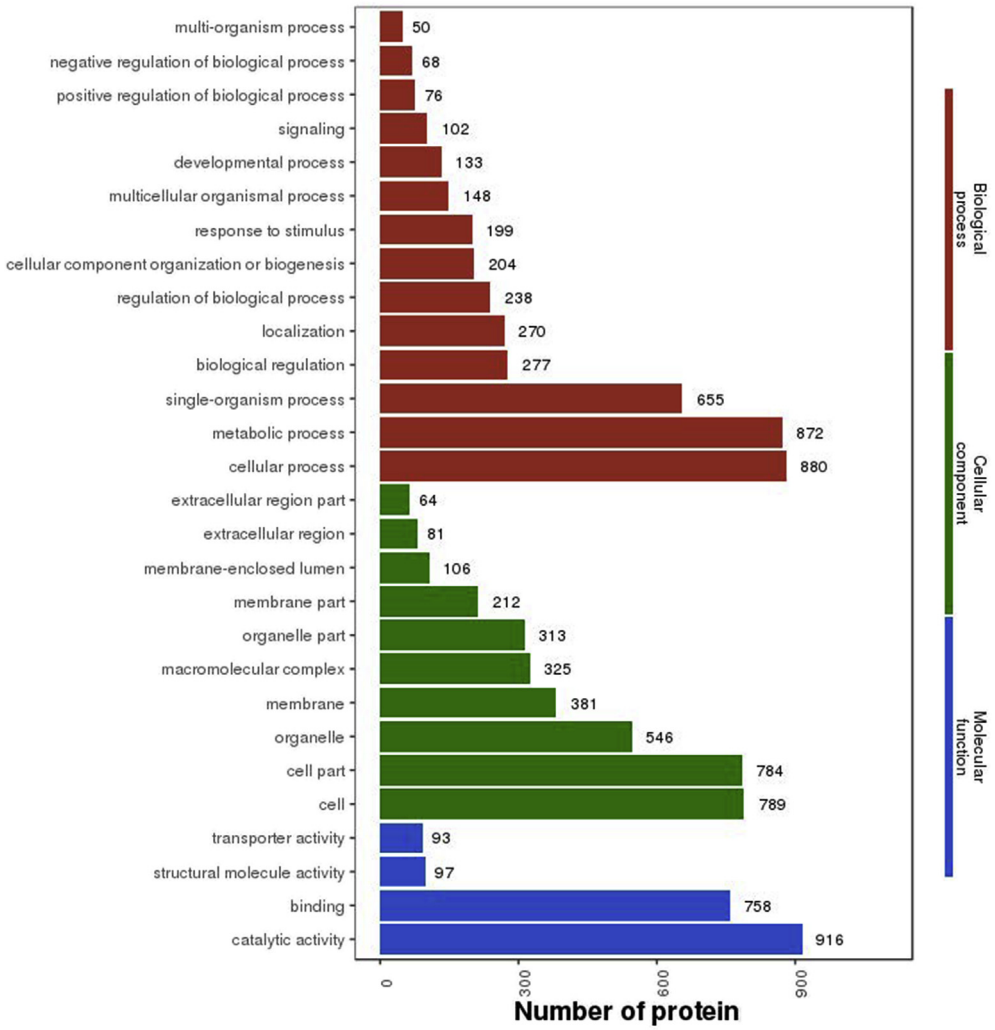

Fig. 2. GO categorization of all identified proteins. Most proteins divided into the three major categories, including biological process, cellular component and molecular function.

39.83% of all proteins; A total of 3218 had KEGG annotations, a proportion of 79.22% of all proteins; A total of 1755 had COG annotations, which took a proportion of 43.21% of all proteins; Finally, a total of 3813 had eggNOG annotations, a proportion of 93.87% of all proteins.

According to GO terms, 1618 proteins were classified into three major functional categories, containing biological progress, cellular component, and molecular function (Fig. 2). The functional enrichment analysis of GO annotations for biological processes showed that proteins were mostly involved in cellular process (880), metabolic process (872), and single-organism process (655). GO annotations for molecular component showed that proteins were mainly involved in cell (789), cell part (784), and organelle (546). Proteins with the molecular function classification were mainly involved in the catalytic activity (916) and binding (758).

According to KEGG database, 3218 proteins were classified into 318 biological pathways (Fig. 3). All the pathways were mapped to metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems, and human diseases. The most abundant proteins were involved in signal transduction (29 pathways) which were belonging to the environmental information processing, followed by the immune system (20 pathways) and endocrine system (18 pathways) which were belonging to organismal systems. In addition, proteins were mainly involved in lipid, carbohydrate and amino acid metabolism which was belonging to metabolism.

The COG classification was vital for functional annotation and evolutionary study. A total of 1755 proteins were finally mapped to 24 different COG categories (Fig.S1). Based on the number of unique proteins identified in each functional category, the most frequently detected functional category was “posttranslational modification, protein turnover, chaperones”, including 203 members and representing more than 11.57% of all the identified proteins. The following categories were “translation, ribosomal structure and biogenesis” (179 members), “general function prediction only” (175 members), “Carbohydrate transport and metabolism” (166 members), “Lipid transport and metabolism” (154 members) and “Amino acid transport and metabolism” (147 members).

According to eggNOG, 3813 proteins were classified into five categories at level 1, cellular processes and signaling, energy production and conversion, information storage and processing, metabolism, and poorly characterized. At level 2, proteins were mostly involved in posttranslational modification, protein turnover, chaperones (624) except function unknown (1563), followed by signal transduction mechanisms (572), intracellular trafficking, secretion, and vesicular transport (374), carbohydrate transport and metabolism (302), translation, ribosomal structure and biogenesis (296), lipid transport and metabolism (280) and amino acid transport and metabolism (271) (Fig.S2).

The subcellular location of a protein was closely connected with its function. Newly synthesized proteins in the cytoplasm were targeted to the correct subcellular compartments and played their biological roles.

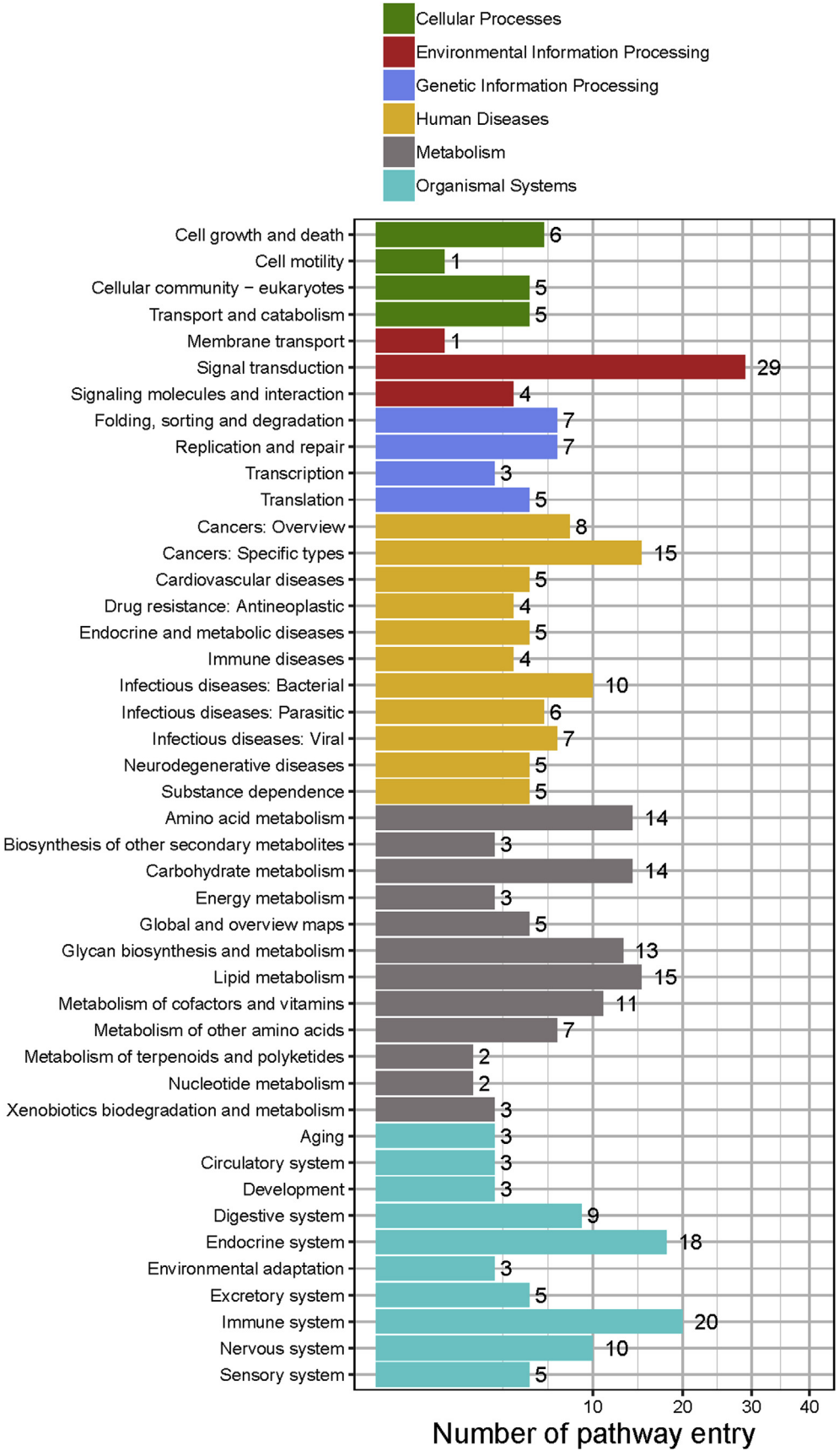

Fig. 3. KEGG pathway of all identified proteins.

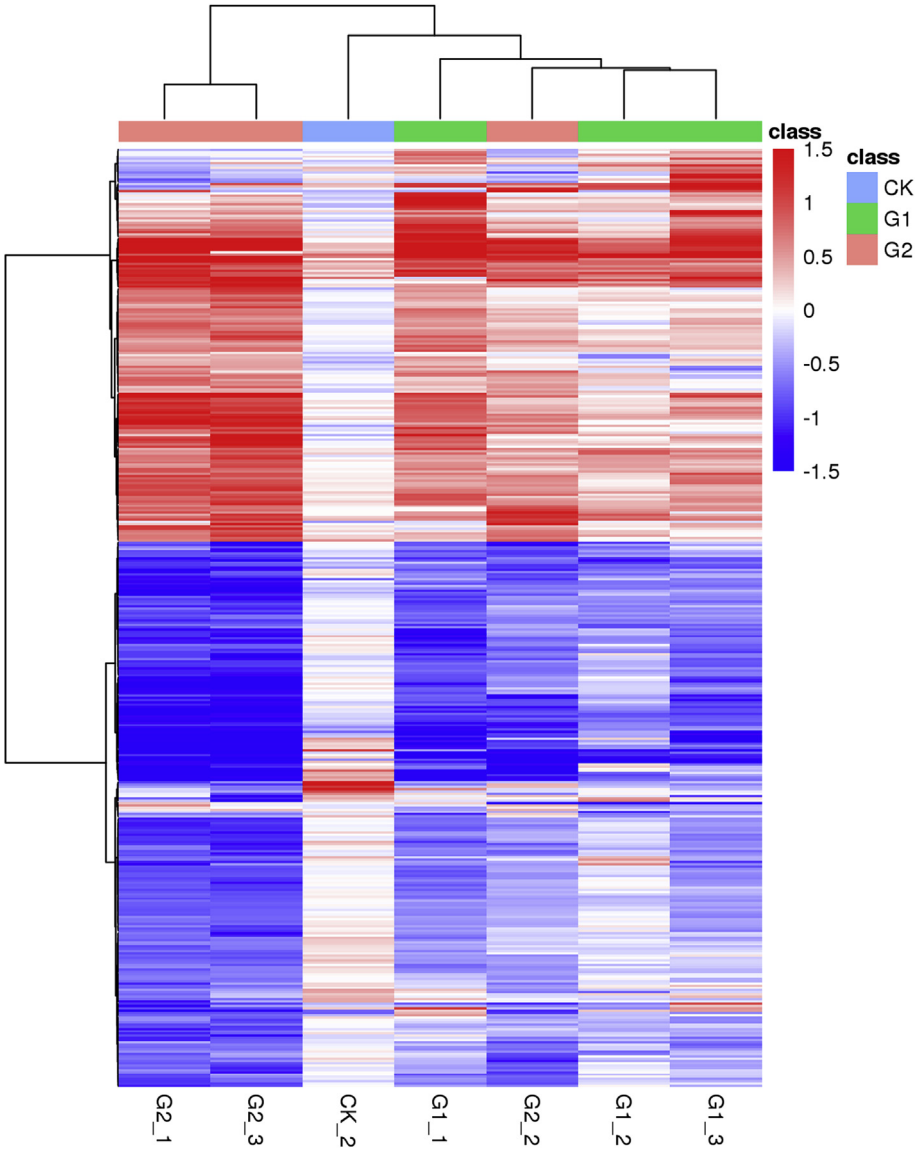

Fig. 4. The hierarchical clustering for DEPs among the three comparable groups (CK, G1, and G2). CK-1 as the reference of other samples (no need to show in figure). The red color showed the high expression, and the blue color represented the down expression. The color from red to blue represented the ratio from large to small. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

In our study, a total of 4061 proteins were finally mapped to 10 different subcellular locations. Most of the proteins were located in the cytoplasm (1416), followed by nucleus (810), extracellular (673), mitochondria (542) and plasma membrane (402) (Fig.S3).

3.3. Differential expressed proteins during cold stress stage

Using a cutoff 1.5-fold change and a p-value less than 0.05, 137 differentially expressed proteins (DEPs), including 62 differentially up-regulated proteins (DUPs) (Tables 1) and 75 differentially down-regulated proteins (DDPs) (Table S2), were identified in G1 compared with CK; 359 DEPs, including 131 DUPs (Table S3) and 228 DDPs (Table S4), were identified in G2 compared with CK. The total DEPs were further estimated by a hierarchical cluster analysis among the three comparison groups. The hierarchical clustering of the DEPs provided a visualized mode to display the clustering patterns of the DEPs among the three groups, which showed the ratio's absolute value of DEPs among all groups is G2 > G1 > CK (Fig. 4). Moreover, the clustering patterns of the DEPs PCA analysis also showed a similar cluster result according to the hierarchical clustering (Fig.S4).

3.4. Functional enrichment analysis of DEPs

The DEPs were annotated based on the GO, KEGG, COG and eggNOG database. A total of 20 significantly changed GO terms (P-value < 0.05) were obtained in the DEPs of G1-VS-CK (Fig.S5A), containing 8, 6 and 6 terms for the categories of biological progress, cellular component, and molecular functions, respectively. A total of 20 significantly changed GO terms (P-value < 0.05) were obtained in the DEPs of G2-VS-CK (protein number > 10) (Fig.S5B), containing 11, 1, and 8 terms for the categories of biological progress, cellular component, and molecular functions, respectively. Moreover, the enrichment factor analysis which reflected the degree and significance of enrichment was carried out (Fig.S5C and Fig.S5D).

In the enrichment analysis of KEGG pathway for the DEPs, a total of 38 KEGG pathways were detected in the G1-VS-CK group (Fig. 5A), with 8 significantly changed KEGG pathways (P values < 0.05) were detected (Fig. 5C). Moreover, a total of 43 KEGG pathways were detected in the G2-VS-CK group (Fig. 5B), with 16 significantly changed KEGG pathways (P values < 0.05) were detected (Fig. 5D). The number of DEPs involved in the enrichment of KEGG pathway increased

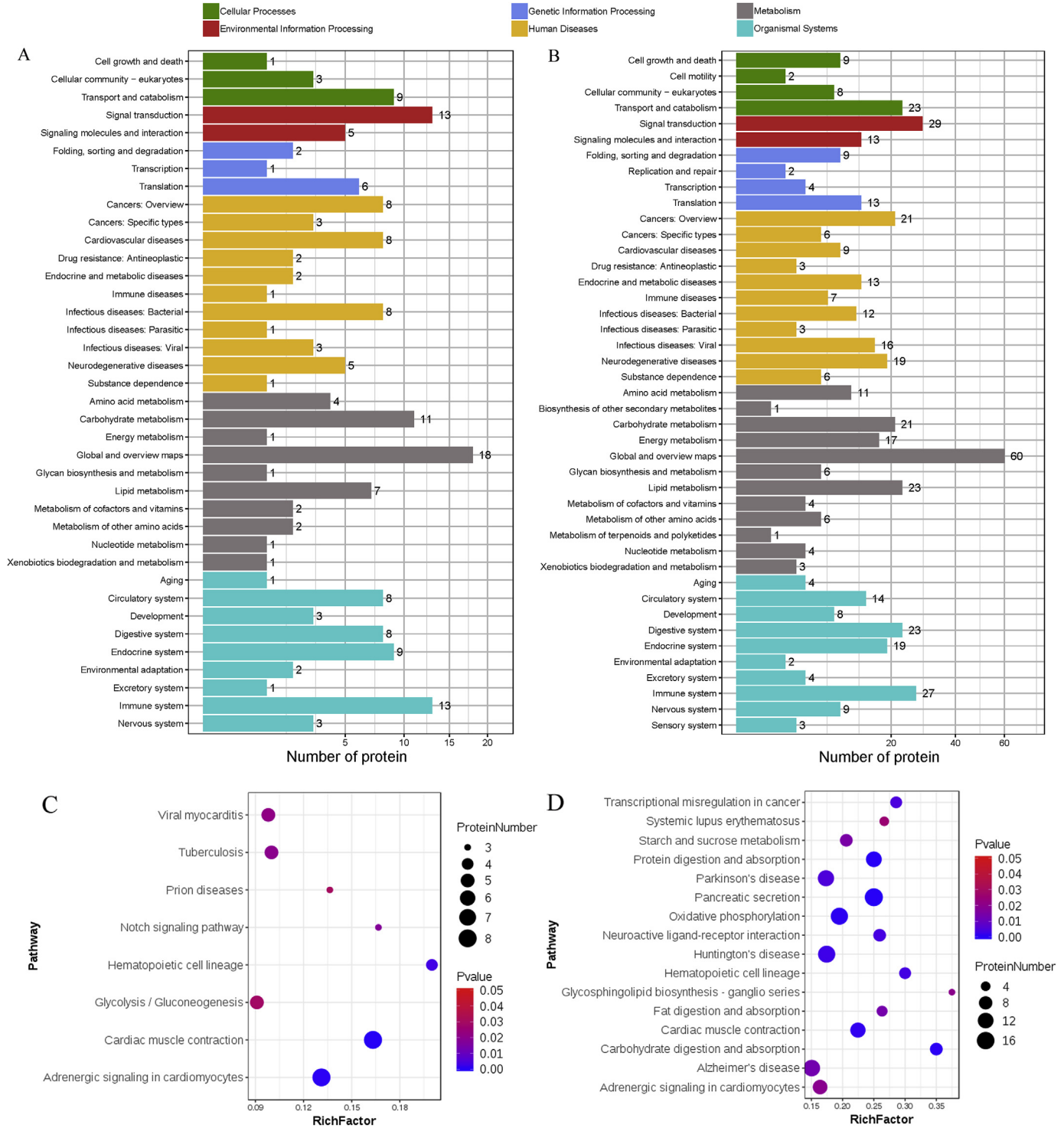

Fig. 5. Enrichment analysis of KEGG pathway for the DEPs in G1-VS-CK (A) and G2-VS-CK (B). The enrichment factor analysis of KEGG pathway for the DEPs in G1-VS-CK (C) and G2-VS-CK (D).

according to the cold stress intensity (from normal temperature 28 °C–18 °C and finally 13 °C for 24 h). The COG, EggNOG annotations and subcellular location categorization of the DEPs were shown in Figure S6.

3.5. Multigroup analysis of the DEPs

Multigroup analysis of DEPs was performed to explore the relationship between G1-VS-CK and G2-VS-CK. The intersection analysis of the DEPs between G1-VS-CK and G2-VS-CK was shown with a Venn diagram (Fig. 6A). 100 proteins were shared by G1-VS-CK and G2-VS-CK. Using Mfuzz, the DEPs ratios of multigroup were clustered (Fig. 6B). Mfuzz clustering on the mean expression values resulted in nine clusters. The protein expression almost declined to the lowest value in Cluster 3 (15 DEPs), while the protein expression raised to the highest value in Cluster 7 (24 DEPs). Moreover, the clustered proteins were shown in the heat map (Fig. 6C) and pathway analysis of DEPs was analyzed (Fig. 6D). The most DEPs in G2-VS-CK group participated

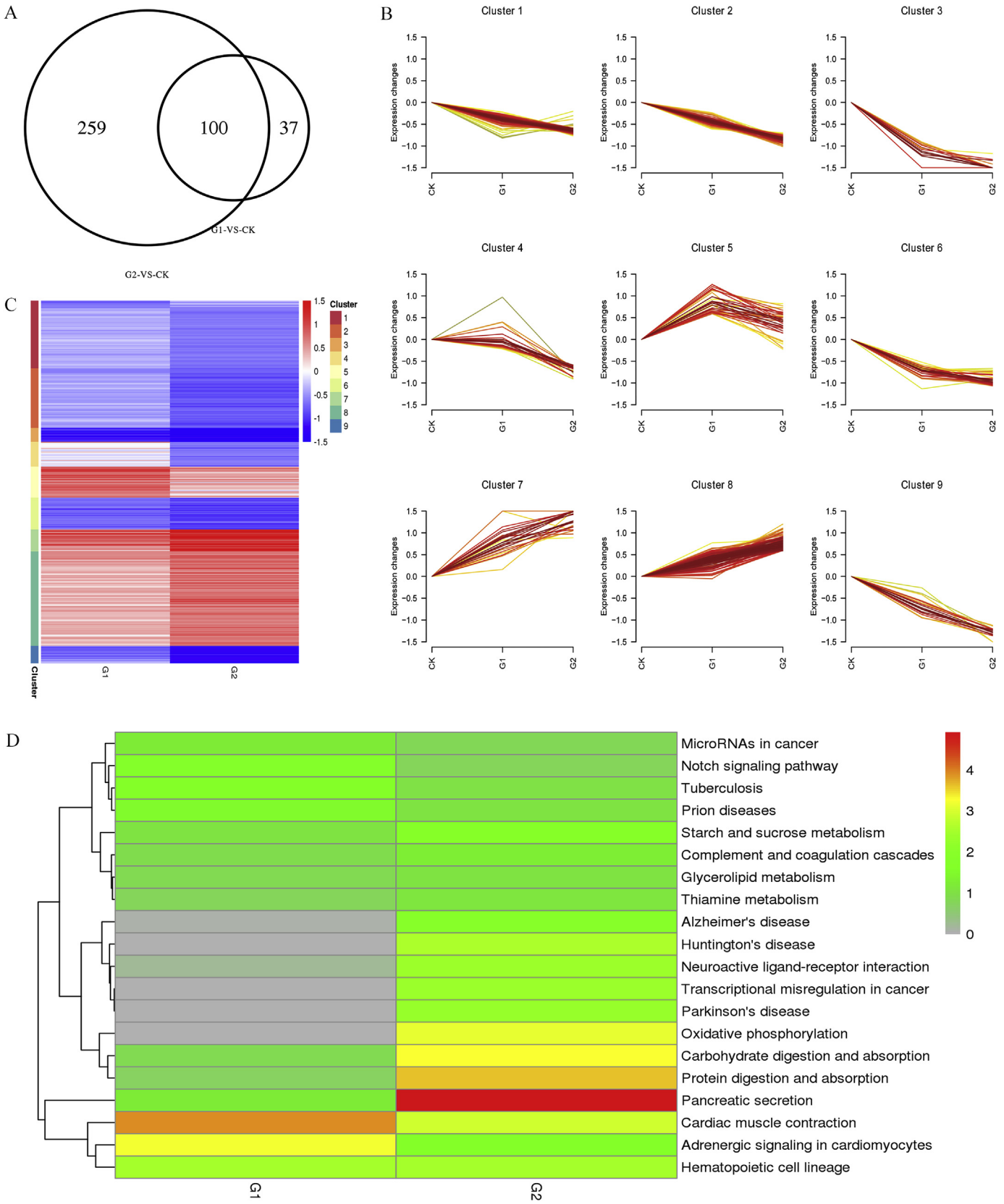

Fig. 6. Multigroup analysis of the DEPs between G1-VS-CK and G2-VS-CK. Venn diagram (A), Mfuzz clustering (B), heat map (C) and pathway analysis (D).

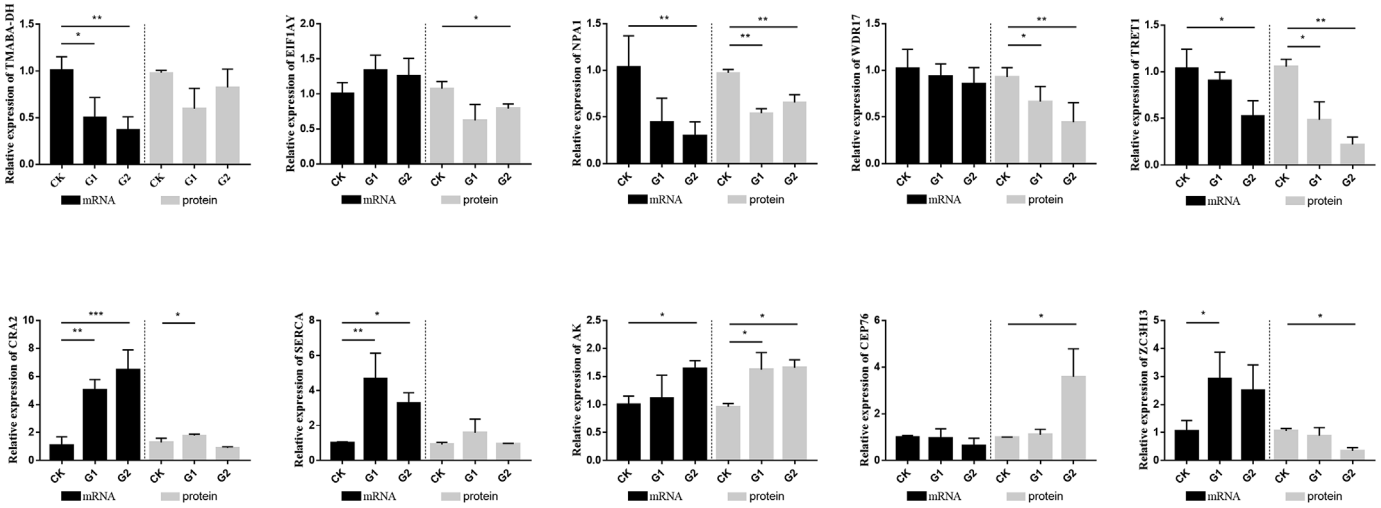

Fig. 7. Verification of the genes on the transcription level using qRT-PCR compared with the translation level. The transcription level of 10 randomly selected genes during cold stress stage was determined using the beta-actin gene as an internal control. Data were shown as the mean ± SD of triplicates, * stands for $0.01 < P \leq 0.05$, ** stands for $0.001 < P \leq 0.01$ and ***stands for $P \leq 0.001$.

in the pancreatic secretion pathway, while cardiac muscle contraction pathway in G1-VS-CK group.

3.6. Verification of the DEPs

The proteome data were verified on the transcription level to confirm if the level of transcription and translation were correlated. qRT-PCR results showed that the expressions on transcription level for most of the genes were similar to the results of iTRAQ in shrimp during cold stress stage except EIF1AY, CEP76, and ZC3H13 among the 10 randomly selected proteins (Fig. 7).

4. Discussion

L. vannamei living in water was easy to suffer from temperature stress. Protein as a basic substance of life performed a vital function. In this study, iTRAQ technology was used instead of the traditional 2-DE gel-based proteomic method to investigate the protein expression dynamic changes in the hepatopancreas of *L. vannamei* during cold stress stage. Protein changes, protein modes of action, and potential biomarkers responding to cold stress were investigated during cold stress stage. In total, 4062 proteins were identified and quantified under cold stress based on the genome of *L. vannamei*. To our knowledge, this study is the first report on the proteomics in Pacific white shrimp using the published genome database of *L. vannamei*. The putative functions of all identified proteins were annotated based on the GO, KEGG, COG and eggNOG database.

In the present study, most proteins were annotated with “post-translational modification, protein turnover, chaperones” function, meaning that most proteins involved in protein chemical modification after translation, synthesis and degradation, folding, maintenance, intracellular transport, degradation as well as in facilitating cell signaling based on the COG and eggNOG database except function unknown. The results of KEGG confirmed that signal transduction is the most impacted pathway. Meanwhile, most proteins were annotated with “catalytic activity and binding” based on the GO database, which means the annotation of proteins in the different database was similar. Moreover, more proteins were identified to involve in amino acid, carbohydrate and lipid transport and metabolism pathway in the four databases, indicating that it is of greatest importance to assess the relationship between the cold tolerance and energy metabolism of *L. vannamei*.

4.1. DEPs related to metabolism

The DEPs related to metabolism in G1-VS-CK and G2-VS-CK groups were involved in energy metabolism, coupled with substance biosynthesis, biodegradation and metabolism, such as amino acid, carbohydrate, glycan, lipid, cofactors and vitamins, terpenoids and polyketides, nucleotide, xenobiotics and secondary metabolites.

In the amino acid metabolism, most DEPs were up-regulated, indicating that amino acid was the main energy resource for the shrimp in response to cold stress. We focused our interesting in arginine kinase (AK, EC 2.7.3.3, accession number: XP_027224228.1), an up-regulated DEP related to amino acid metabolism in G1-VS-CK and G2-VS-CK groups. AK as a phosphagen kinase played a pivotal role in energy metabolism in invertebrates. It was also demonstrated that shrimp AK can cause an allergic reaction in hypersensitive individuals [30]. It has been reported that AK responded to laminarin stimulation in the haemolymph of Chinese shrimp *Fenneropenaeus chinensis* [31]. AK expression responded to many stresses no matter environment or pathogen, such as salinity [32,33], hypoxia [34,35], Vibrio alginolyticus [36], LPS [37]. Our result confirmed that AK was gradually up-regulated during cold stress stage, indicating that AK played a vital regulation role in response to cold stress.

In the carbohydrate metabolism, most DEPs were up-regulated at 18 °C but down-regulated at 13 °C for 24 h, meaning that carbohydrate can be used as an energy resource for shrimp during the initial stage of cold stress but carbohydrate metabolism was hard to cope with cold stress along with the stress strengthening. Only the DEPs related to pathways of propanoate metabolism and pentose and glucuronate interconversions were always up-regulated during the cold stress stage. The previous study has reported that propanoate metabolism pathway was significantly changed in response to acute low salinity stress of *L. vannamei* [38]. Moreover, pathways analysis of the gills in the red swamp crayfish *Procambarus clarkii* also showed that propanoate metabolism pathway was a differentially expressed pathway infected with WSSV [39]. Additionally, most of the DEPs related to carbohydrate metabolism were enzymes, such as phosphoglycerate kinase, alpha-amylase, xylose isomerase, and hydroxybutyrate dehydrogenase.

In the lipid metabolism, most DEPs related to glycerolipid metabolism, synthesis and degradation of ketone bodies, sphingolipid metabolism, steroid hormone biosynthesis, glycerophospholipid, steroid biosynthesis, and ether lipid metabolism were down-regulated during cold stress stage. However, we paid more attention to the DEPs related to biosynthesis of unsaturated fatty acids (UFA), which were up-

regulated according to the cold stress. UFA is the key component of cellular membrane involved in energy metabolism. In fish, to maintain greater membrane fluidity at the lower temperatures, increasingly high cell membrane content of UFA was induced by the increasingly cold environment [40,41]. It has been reported that increasing the levels of plant oils (more UFA) in Nile tilapia (*Oreochromis niloticus*) feeds can enhance their cold tolerance [42]. In our present study, we deduced shrimp can activate UFA pathway to cope with cold stress.

In the energy metabolism, most DEPs related to oxidative phosphorylation were up-regulated, such as V-type proton ATPase subunit G, subunit of cytochrome *c* oxidase, cytochrome *b*-*c*1 complex, subunit of ATP synthase, and NADH dehydrogenase [ubiquinone] 1 (NDUA7, NDUB8, NDUFA12, NDUFB10). Oxidative phosphorylation is the process to form ATP by transferring the electrons from NADH or $FADH_2$ to O_2 by a series of electron carriers [43]. Therefore, ATP is the necessary energy for the shrimp to cope with cold stress.

4.2. DEPs related to organismal systems

The DEPs related to organismal systems in G1-VS-CK and G2-VS-CK groups were involved in immune system, digestive system, endocrine system, circulatory system, nervous system, development, excretory system, aging, sensory system, and environmental adaptation.

In the immune system, most DEPs related to hematopoietic cell lineage, Th1 and Th2 cell differentiation, platelet activation, toll-like receptor signaling pathway, NOD-like receptor signaling pathway, and cytosolic DNA-sensing pathway were down-regulated during cold stress stage. The down-regulated DEPs for hematopoietic cell lineage indicated that the reduction of hematopoietic function of shrimp gave rise to the total hemocyte count (THC) decrease, which was in accordance with our previous result of the THC decrease during cold stress using flow cytometer analysis [44]. The down-regulated DEPs related to Th1 and Th2 cell differentiation indicated that the shrimp immunity declined during the cold stress stage [45]. Another down-regulated protein at 13 °C for 24 h was beta-1,3-glucan-binding protein (XP_027214232.1), which was existed in various kinds of invertebrates including shrimp and played a vital role in innate immunity [46]. It also identified that the shrimp immunity was declined during cold stress stage. In shrimp farming, we should try to enhance the shrimp immunity when the climate is severely changed.

In the immune system, the DEPs related to IL-17 signaling pathway, RIG-I-like receptor signaling pathway, Chemokine signaling pathway, and leukocyte transendothelial migration were up-regulated only at 13 °C for 24 h. IL-17 signaling pathway played crucial roles in both acute and chronic inflammatory responses [47]. RIG-I-like receptor signaling pathway cooperated with other pathway or factors imparted innate immunity and modulated the adaptive immune response [48]. Chemokine signaling pathway was crucial in coordinating cell migration between health and disease and specifically regulating cell positioning in space and time [49]. Leukocyte transendothelial migration involved in the inflammatory response [50]. These up-regulated DEPs illustrated the shrimp can mobilize the body's immune response to against cold stress although the immunity was declined during cold stress stage.

In the digestive system, most DEPs were down-regulated, mainly focused on the pathway of pancreatic secretion, carbohydrate digestion and absorption, protein digestion and absorption, fat digestion and absorption. It was due to shrimp would reduce or stop eating during cold stress. In the circulatory system, we focused our interesting in cardiac muscle contraction pathway. The DEPs related to systole and diastole were all up-regulated, indicating shrimp enhanced the circulatory system to increase the body's energy supply during cold stress.

Among the DEPs, heat shock 70 kDa protein 14-A (HSPA14, XP_027238916.1) related to antigen processing and presentation in the immune system, was up-regulated at 13 °C for 24 h, which functioned in maintaining cellular homeostasis during environmental stress (temperature, oxidative, chemical, and pathogenic stresses) as a protein of HSP family [51–53]. Moreover, as molecular chaperones for other cellular proteins, HSPs had great cytoprotective effects.

4.3. DEPs related to environmental information processing

The DEPs related to environmental information processing in G1-VS-CK and G2-VS-CK groups were involved in signal transduction (13 in G1-VS-CK and 29 in G2-VS-CK) and signaling molecules and interaction (5 in G1-VS-CK and 13 in G2-VS-CK). Most DEPs were involved in signal transduction, such as Notch signaling pathway, TGF-beta signaling pathway, AMPK signaling pathway, Sphingolipid signaling pathway and so on. The Notch signaling pathway was a conserved cell signaling pathway and played an important role in growth and development [54,55]. Recent studies indicated that the upregulation of the Notch signaling pathway may contribute to oxidative stress injuries (OSI) [56], therefore inhibiting the Notch signal might protect cells from OSI [57]. In this study, the down-regulated DEPs in Notch signaling pathway may mean that during cold stress stage shrimp activated the self-protection mechanism against OSI. As a down-regulated DEPs related to Notch signaling pathway, Astacins was a family of multidomain metalloendopeptidases which was either secreted or membrane-anchored [58]. However, the function of astacin to cold stress was unclear. We will focus on its function in further study.

4.4. DEPs related to human diseases

The DEPs related to human diseases in G1-VS-CK and G2-VS-CK groups were involved in immune diseases, infectious diseases: viral, infectious diseases: bacterial, infectious diseases: parasitic, cancers: overview, endocrine and metabolic diseases, neurodegenerative diseases, substance dependence, drug resistance: antineoplastic, cancers: specific types, cardiovascular diseases.

All DEPs related to systemic lupus erythematosus pathway in immune diseases were up-regulated (H2B, H3, H4), which were all belonging to histones. More studies have suggested that histones may participate in the reaction of cells to environmental stresses, such as heavy mental [59], osmotic stress [60], oxidative stress [61]. It also has been reported that high expression of core histone proteins (H2A, H2B, H3, and H4) was found in hemocytes of *L. vannamei* which had antimicrobial activity [62]. Antimicrobial histone proteins have been widely reported, such as H2B-like proteins in catfish skin [63], H4 in human sebocytes [64], Histone H5 [65], Histone H2A [66,67]. In our present study, the up-regulated histones may play important innate immunity function to avoid pathogen during cold stress.

Heat shock 70 kDa protein cognate 5 (HSC70-5, XP_027230517.1) related to tuberculosis in infectious diseases: bacterial, was up-regulated at 13 °C for 24 h, which was localized in the mitochondria and conferred thermal tolerance to regulate mitochondrial morphology and cellular homeostasis by preventing protein aggregation [68]. Additionally, HSC70-5 was confirmed to involve in WSSV toleration of *L. vannamei* [69]. In this study, the upregulated HSC70–5 may decrease the shrimp's cumulative mortality during cold-stress by reducing the aggregation of proteins. HSC70-5 and HSPA14 were the HSPs which were related to cold stress after GRP78 [4].

4.5. Correlation between transcription and translation level of DEPs

The transcription level for most of the genes was similar to the results of iTRAQ in the shrimp during cold stress stage, which indicated there was a good correlation between transcription and translation [70,71]. However, as an economic aquatic animal for *L. vannamei*, the limitation of antibodies limited the verification on translation level by Western blot analysis. In further research, we will focus on the proteins and pathways correlated with cold stress to study their function and molecular mechanism deeply.

5. Conclusion

In summary, this study is the first proteomic analysis of shrimp during cold stress by using the genome database of *L. vannamei*. Cold stress was involved in the disruption of homeostasis and triggered a series of molecular pathways which contained many key proteins regulating the organism homeostasis. Based on the proteomics data, the cold tolerance of *L. vannamei* might be related to the energy metabolism and body immunity. Moreover, *L. vannamei* could cope with cold stress by enhancing the production of ATP and UFA. Notably, arginine kinase, heat shock proteins, and histones may act as positive regulators in *L. vannamei* under cold stress. These results indicated that shrimp can maintain the organism homeostasis by a series of orderly regulatory process during cold stress. Furthermore, the results can provide guidance for shrimp farming.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31600322), Open Fund Project of Integrative Microbiology Research Centre, South China Agricultural University (IM20170307) and the China Scholarship Council Grant (201807630002). We acknowledged Shenzhen Wininnovate Bio for technological supports.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.06.037.

References

[1] D.S. Goldstein, I.J. Kopin, Evolution of concepts of stress, Stress 10 (2) (2007) 109–120 https://doi.org/10.1080/10253890701288935.
[2] R. Chovatiya, R. Medzhitov, Stress, inflammation, and defense of homeostasis, Mol. Cell 54 (2) (2014) 281–288 https://doi.org/10.1016/j.molcel.2014.03.030.
[3] K.J.A. Davies, Adaptive homeostasis, Mol. Asp. Med. 49 (2016) 1–7 https://doi.org/10.1016/j.mam.2016.04.007.
[4] L. Fan, A. Wang, Y. Miao, S. Liao, C. Ye, Q. Lin, Comparative proteomic identification of the hepatopancreas response to cold stress in white shrimp, Litopenaeus vannamei, Aquaculture 454 (2016) 27–34 https://doi.org/10.1016/j.aquaculture.2015.10.016.
[5] J. Liu, K. Wang, Y. Wang, W. Chen, Z. Jin, Z. Yao, D. Zhang, Strain-specific changes in the gut microbiota profiles of the white shrimp Litopenaeus vannamei in response to cold stress, Aquaculture 503 (2019) 357–366 https://doi.org/10.1016/j.aquaculture.2019.01.026.
[6] H.A. Abdelrahman, A. Abebe, C.E. Boyd, Influence of variation in water temperature on survival, growth and yield of Pacific white shrimp Litopenaeus vannamei in inland ponds for low-salinity culture, Aquacult. Res. 50 (2) (2019) 658–672 https://doi.org/10.1111/are.13943.
[7] T. Chen, T. Lin, H. Li, T. Lu, J. Li, W. Huang, H. Sun, X. Jiang, J. Zhang, A. Yan, C. Hu, P. Luo, C. Ren, Heat shock protein 40 (HSP40) in pacific white shrimp (Litopenaeus vannamei): molecular cloning, tissue distribution and ontogeny, response to temperature, acidity/alkalinity and salinity stresses, and potential role in ovarian development, Front. Physiol. 9 (1784) (2018), https://doi.org/10.3389/fphys.2018.01784.
[8] P. He, P. Wei, B. Zhang, Y. Zhao, Q. Li, X. Chen, D. Zeng, M. Peng, C. Yang, J. Peng, X. Chen, Identification of microRNAs involved in cold adaptation of Litopenaeus vannamei by high-throughput sequencing, Gene 677 (2018) 24–31 https://doi.org/10.1016/j.gene.2018.07.042.
[9] D. Xiong, Y. Duan, Y. Wang, J. Zhang, Elevated temperature disrupts the mucosal structure and induces an immune response in the intestine of whiteleg shrimp Litopenaeus vannamei (Boone, 1931) (Decapoda: dendrobranchiata: Penaeidae), J. Crustac Biol. 38 (5) (2018) 635–640 https://doi.org/10.1093/jcbiol/ruy055.
[10] X.-Y. Jia, D.-S. Zhong, D. Zhang, F. Wang, W.-L. Zhou, Energy metabolic enzyme responses of Litopenaeus vannamei to thermal stress: a comparative study in freshwater and seawater conditions, Aquacult. Int. 26 (4) (2018) 1067–1081 https://doi.org/10.1007/s10499-018-0268-9.
[11] J.-X. Peng, P.-P. He, P.-Y. Wei, B. Zhang, Y.-Z. Zhao, Q.-Y. Li, X.-L. Chen, M. Peng, D.-G. Zeng, C.-L. Yang, X. Chen, Proteomic responses under cold stress reveal unique cold tolerance mechanisms in the pacific white shrimp (Litopenaeus vannamei), Front. Physiol. 9 (2018) 1399 https://doi.org/10.3389/fphys.2018.01399.
[12] Q. Yin, J. Peng, L. Cui, D.-X. Xie, Z.-W. Wang, K. Li, X.-H. Chen, Molecular cloning of Litopenaeus vannamei TCP-1-eta gene and analysis on its relationship with cold tolerance, Hereditas 33 (2) (2011) 168–174 https://doi.org/10.3724/SP.J.1005.2011.00168.
[13] M. Zhou, Z. Wu, R. Liang, N. Gu, Effects of dietary taurine, carnitine and cholesterol supplementation on growth performance and immunological status of Litopenaeus vannamei under cold exposure, Aquacult. Res. 48 (3) (2017) 1279–1290 https://doi.org/10.1111/are.12970.
[14] W.P. Blackstock, M.P. Weir, Proteomics: quantitative and physical mapping of cellular proteins, Trends Biotechnol. 17 (3) (1999) 121–127 https://doi.org/10.1016/S0167-7799(98)01245-1.
[15] G. Pottiez, J. Wiederin, H.S. Fox, P. Ciborowski, Comparison of 4-plex to 8-plex iTRAQ quantitative measurements of proteins in human plasma samples, J. Proteome Res. 11 (7) (2012) 3774–3781 https://doi.org/10.1021/pr300414z.
[16] C. Caubet, C. Lacroix, S. Decramer, J. Drube, J.H.H. Ehrich, H. Mischak, J.-L. Bascands, J.P. Schanstra, Advances in urinary proteome analysis and biomarker discovery in pediatric renal disease, Pediatr. Nephrol. 25 (1) (2009) 27 https://doi.org/10.1007/s00467-009-1251-5.
[17] Y. Shen, J. Li, R. Gu, X. Zhan, B. Xing, Proteomic analysis for phenanthrene-elicited wheat chloroplast deformation, Environ. Int. 123 (2019) 273–281 https://doi.org/10.1016/j.envint.2018.11.074.
[18] M. Li, X. Huo, Y. Pan, H. Cai, Y. Dai, X. Xu, Proteomic evaluation of human umbilical cord tissue exposed to polybrominated diphenyl ethers in an e-waste recycling area, Environ. Int. 111 (2018) 362–371 https://doi.org/10.1016/j.envint.2017.09.016.
[19] D. Ma, Q. Liu, M. Zhang, J. Feng, X. Li, Y. Zhou, X. Wang, iTRAQ-based quantitative proteomics analysis of the spleen reveals innate immunity and cell death pathways associated with heat stress in broilers (Gallus gallus), J. Proteomics. 196 (2019) 11–21 https://doi.org/10.1016/j.jprot.2019.01.012.
[20] W. Wang, Q. Lei, H. Liang, J. He, Towards a better understanding of allograft-induced stress response in the pearl oyster Pinctada fucata martensii: insights from iTRAQ-based comparative proteomic analysis, Fish Shellfish Immunol. 86 (2019) 186–195 https://doi.org/10.1016/j.fsi.2018.11.044.
[21] C. Chu, N. Yan, Y. Du, X. Liu, M. Chu, J. Shi, H. Zhang, Y. Liu, Z. Zhang, iTRAQ-based proteomic analysis reveals the accumulation of bioactive compounds in Chinese wild rice (Zizania latifolia) during germination, Food Chem. 289 (2019) 635–644 https://doi.org/10.1016/j.foodchem.2019.03.092.
[22] X. Zhang, J. Yuan, Y. Sun, S. Li, Y. Gao, Y. Yu, C. Liu, Q. Wang, X. Lv, X. Zhang, K.Y. Ma, X. Wang, W. Lin, L. Wang, X. Zhu, C. Zhang, J. Zhang, S. Jin, K. Yu, J. Kong, P. Xu, J. Chen, H. Zhang, P. Sorgeloos, A. Sagi, A. Alcivar-Warren, Z. Liu, L. Wang, J. Ruan, K.H. Chu, B. Liu, F. Li, J. Xiang, Penaeid shrimp genome provides insights into benthic adaptation and frequent molting, Nat. Commun. 10 (1) (2019) 356 https://doi.org/10.1038/s41467-018-08197-4.
[23] J.R. Wiśniewski, A. Zougman, N. Nagaraj, M. Mann, Universal sample preparation method for proteome analysis, Nat. Methods 6 (2009) 359 https://doi.org/10.1038/nmeth.1322.
[24] B. Wen, C. Du, G. Li, F. Ghali, A.R. Jones, L. Käll, S. Xu, R. Zhou, Z. Ren, Q. Feng, X. Xu, J. Wang, IPeak: an open source tool to combine results from multiple MS/MS search engines, Proteomics 15 (17) (2015) 2916–2920 https://doi.org/10.1002/pmic.201400208.
[25] B. Wen, R. Zhou, Q. Feng, Q. Wang, J. Wang, S. Liu, IQuant: an automated pipeline for quantitative proteomics based upon isobaric tags, Proteomics 14 (20) (2014) 2280–2285 https://doi.org/10.1002/pmic.201300361.
[26] M. Hattori, M. Itoh, M. Araki, M. Hirakawa, S. Kawashima, S. Okuda, S. Goto, T. Katayama, T. Tokimatsu, Y. Yamanishi, M. Kanehisa, KEGG for linking genomes to life and the environment, Nucleic Acids Res. 36 (suppl_1) (2007) D480–D484 https://doi.org/10.1093/nar/gkm882.
[27] J. Muller, L.J. Jensen, M. Kuhn, P. Julien, T. Doerks, P. Bork, C. von Mering, eggNOG: automated construction and annotation of orthologous groups of genes, Nucleic Acids Res. 36 (suppl_1) (2007) D250–D254 https://doi.org/10.1093/nar/gkm796.
[28] J. Huerta-Cepas, K. Forslund, S. Sunagawa, P. Bork, D. Szklarczyk, D. Heller, C. von Mering, H. Cook, L.J. Jensen, M.C. Walter, T. Rattei, D.R. Mende, M. Kuhn, eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences, Nucleic Acids Res. 44 (D1) (2015) D286–D293 https://doi.org/10.1093/nar/gkv1248.
[29] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method, Methods 25 (4) (2001) 402–408 https://doi.org/10.1006/meth.2001.1262.
[30] K. Uda, N. Fujimoto, Y. Akiyama, K. Mizuta, K. Tanaka, W.R. Ellington, T. Suzuki, Evolution of the arginine kinase gene family, Comp. Biochem. Physiol. Genom. Proteonom. 1 (2) (2006) 209–218 https://doi.org/10.1016/j.cbd.2005.10.007.
[31] C.-L. Yao, C.-G. Wu, J.-H. Xiang, B. Dong, Molecular cloning and response to laminarin stimulation of arginine kinase in haemolymph in Chinese shrimp, Fenneropenaeus chinensis, Fish Shellfish Immunol. 19 (4) (2005) 317–329 https://doi.org/10.1016/j.fsi.2005.01.006.
[32] S.-J. Yin, L. Zhang, L. Zhang, J. Wan, W. Song, X. Jiang, Y.-D. Park, Y.-X. Si, Metabolic responses and arginine kinase expression of juvenile cuttlefish (Sepia pharaonis) under salinity stress, Int. J. Biol. Macromol. 113 (2018) 881–888 https://doi.org/10.1016/j.ijbiomac.2018.03.036.
[33] M.S. Shekhar, J. Kiruthika, A.G. Ponniah, Identification and expression analysis of differentially expressed genes from shrimp (Penaeus monodon) in response to low salinity stress, Fish Shellfish Immunol. 35 (6) (2013) 1957–1968 https://doi.org/10.1016/j.fsi.2013.09.038.
[34] H. Abe, S. Hirai, S. Okada, Metabolic responses and arginine kinase expression under hypoxic stress of the kuruma prawn Marsupenaeus japonicus, Comp. Biochem. Physiol. Mol. Integr. Physiol. 146 (1) (2007) 40–46 https://doi.org/10.1016/j.cbpa.2006.08.027.
[35] H. Jiang, F. Li, Y. Xie, B. Huang, J. Zhang, J. Zhang, C. Zhang, S. Li, J. Xiang, Comparative proteomic profiles of the hepatopancreas in Fenneropenaeus chinensis

response to hypoxic stress, Proteomics 9 (12) (2009) 3353–3367 https://doi.org/10.1002/pmic.200800518.
[36] C. Song, Z. Cui, Y. Liu, Q. Li, S. Wang, Cloning and expression of arginine kinase from a swimming crab, Portunus trituberculatus, Mol. Biol. Rep. 39 (4) (2012) 4879–4888 https://doi.org/10.1007/s11033-011-1283-3.
[37] X. Shi, L. Wang, Z. Zhou, C. Yang, Y. Gao, L. Wang, L. Song, The arginine kinase in Zhikong scallop Chlamys farreri is involved in immunomodulation, Dev. Comp. Immunol. 37 (2) (2012) 270–278 https://doi.org/10.1016/j.dci.2012.03.008.
[38] X. Wang, S. Wang, C. Li, K. Chen, J.G. Qin, L. Chen, E. Li, Molecular pathway and gene responses of the pacific white shrimp *Litopenaeus vannamei* to acute low salinity stress, J. Shellfish Res. 34 (3) (2015) 1037–1048 https://doi.org/10.2983/035.034.0330.
[39] Z.-Q. Du, Y.-H. Jin, Comparative transcriptome and potential antiviral signaling pathways analysis of the gills in the red swamp crayfish, Procambarus clarkii infected with White Spot Syndrome Virus (WSSV), Genet. Mol. Biol. 40 (2017) 168–180 https://doi.org/10.1590/1678-4685-GMB-2016-0133.
[40] A.J. Hulbert, Life, death and membrane bilayers, J. Exp. Biol. 206 (14) (2003) 2303–2311 https://doi.org/10.1242/jeb.00399.
[41] R.S. Raynard, A.R. Cossins, Homeoviscous adaptation and thermal compensation of sodium pump of trout erythrocytes, Am. J. Physiol. Regul. Integr. Comp. Physiol. 260 (5) (1991) R916–R924 https://doi.org/10.1152/ajpregu.1991.260.5.R916.
[42] H.M. Abdel-Ghany, A.-F.M. El-Sayed, A.A. Ezzat, M.A. Essa, A.M. Helal, Dietary lipid sources affect cold tolerance of Nile tilapia (Oreochromis niloticus), J. Therm. Biol. 79 (2019) 50–55 https://doi.org/10.1016/j.jtherbio.2018.11.009.
[43] H. Lodish, A. Berk, S.L. Zipursky, P. Matsudaira, D. Baltimore, J. Darnell, Electron Transport and Oxidative Phosphorylation, Molecular Cell Biology, fourth ed., WH Freeman, 2000.
[44] L. Fan, A. Wang, Y. Wu, Comparative proteomic identification of the hemocyte response to cold stress in white shrimp, Litopenaeus vannamei, J. Proteomics. 80 (2013) 196–206 https://doi.org/10.1016/j.jprot.2012.12.017.
[45] T. Chitnis, A.D. Salama, M.J. Grusby, M.H. Sayegh, S.J. Khoury, Defining Th1 and Th2 immune responses in a reciprocal cytokine environment in vivo, J. Immunol. 172 (7) (2004) 4260–4265 https://doi.org/10.4049/jimmunol.172.7.4260.
[46] B. Phupet, T. Pitakpornpreecha, N. Baowubon, P. Runsaeng, P. Utarabhand, Lipopolysaccharide- and β-1,3-glucan-binding protein from Litopenaeus vannamei: purification, cloning and contribution in shrimp defense immunity via phenoloxidase activation, Dev. Comp. Immunol. 81 (2018) 167–179 https://doi.org/10.1016/j.dci.2017.11.016.
[47] C. Gu, L. Wu, X. Li, IL-17 family: cytokines, receptors and signaling, Cytokine 64 (2) (2013) 477–485 https://doi.org/10.1016/j.cyto.2013.07.022.
[48] Y.-M. Loo, M. Gale Jr., Immune signaling by RIG-I-like receptors, Immunity 34 (5) (2011) 680–692 https://doi.org/10.1016/j.immuni.2011.05.003.
[49] D.F. Legler, M. Thelen, New insights in chemokine signaling, F1000Research 7 (2018) 95-95 https://doi.org/10.12688/f1000research.13130.1.
[50] W.A. Muller, Mechanisms of leukocyte transendothelial migration, Ann. Rev. Pathol. 6 (2011) 323–344 https://doi.org/10.1146/annurev-pathol-011110-130224.
[51] D. Lanneau, M. Brunet, E. Frisan, E. Solary, M. Fontenay, C. Garrido, Heat shock proteins: essential proteins for apoptosis regulation, J. Cell Mol. Med. 12 (3) (2008) 743–761 https://doi.org/10.1111/j.1582-4934.2008.00273.x.
[52] C. Garrido, M. Brunet, C. Didelot, Y. Zermati, E. Schmitt, G. Kroemer, Heat shock proteins 27 and 70: anti-apoptotic proteins with tumorigenic properties, Cell Cycle 5 (22) (2006) 2592–2601 https://doi.org/10.4161/cc.5.22.3448.
[53] J.M. Matz, K.P. LaVoi, R.J. Moen, M.J. Blake, Cold-induced heat shock protein expression in rat aorta and Brown adipose tissue, Physiol. Behav. 60 (5) (1996) 1369–1374 https://doi.org/10.1016/S0031-9384(96)00220-X.
[54] S. Artavanis-Tsakonas, M.D. Rand, R.J. Lake, Notch signaling: cell fate control and signal integration in development, Science 284 (5415) (1999) 770–776 https://doi.org/10.1126/science.284.5415.770.
[55] Y. Yang, W. Duan, Z. Jin, S. Bi, J. Yan, Y. Jin, J. Lu, J. Yang, D. Yi, New role of Notch-mediated signaling pathway in myocardial ischemic preconditioning, Med. Hypotheses 76 (3) (2011) 427–428 https://doi.org/10.1016/j.mehy.2010.11.011.
[56] A.V. Boopathy, K.D. Pendergrass, P.L. Che, Y.-S. Yoon, M.E. Davis, Oxidative stress-induced Notch1 signaling promotes cardiogenic gene expression in mesenchymal stem cells, Stem Cell Res. Ther. 4 (2) (2013) 43-43 https://doi.org/10.1186/scrt190.
[57] Y. Yang, W. Duan, Z. Liang, W. Yi, J. Yan, N. Wang, Y. Li, W. Chen, S. Yu, Z. Jin, D. Yi, Curcumin attenuates endothelial cell oxidative stress injury through Notch signaling inhibition, Cell. Signal. 25 (3) (2013) 615–629 https://doi.org/10.1016/j.cellsig.2012.11.025.
[58] F.X. Gomis-Rüth, S. Trillo-Muyo, W. Stöcker, Functional and Structural Insights into Astacin Metallopeptidases, (2012), p. 1027 https://doi.org/10.1515/hsz-2012-0149.
[59] J. Kang, C. Lin, J. Chen, Q. Liu, Copper induces histone hypoacetylation through directly inhibiting histone acetyltransferase activity, Chem. Biol. Interact. 148 (3) (2004) 115–123 https://doi.org/10.1016/j.cbi.2004.05.003.
[60] B.A. Burkhart, S.B. Kennett, T.K. Archer, Osmotic stress-dependent repression is mediated by histone H3 phosphorylation and chromatin structure, J. Biol. Chem. 282 (7) (2007) 4400–4407 https://doi.org/10.1074/jbc.M609041200.
[61] Y. Niu, T.L. DesMarais, Z. Tong, Y. Yao, M. Costa, Oxidative stress alters global histone modification and DNA methylation, Free Radic. Biol. Med. 82 (2015) 22–28 https://doi.org/10.1016/j.freeradbiomed.2015.01.028.
[62] S.A. Patat, R.B. Carnegie, C. Kingsbury, P.S. Gross, R. Chapman, K.L. SCHEY, Antimicrobial activity of histones from hemocytes of the Pacific white shrimp, Eur. J. Biochem. 271 (23-24) (2004) 4825–4833 https://doi.org/10.1111/j.1432-1033.2004.04448.x.
[63] D. Robinette, S. Wada, T. Arroll, M.G. Levy, W.L. Miller, E.J. Noga, Antimicrobial activity in the skin of the channel catfish Ictalurus punctatus: characterization of broad-spectrum histone-like antimicrobial proteins, Cell. Mol. Life Sci. CMLS 54 (5) (1998) 467–475 https://doi.org/10.1007/s000180050175.
[64] D.-Y. Lee, C.-M. Huang, T. Nakatsuji, D. Thiboutot, S.-A. Kang, M. Monestier, R.L. Gallo, Histone H4 is a major component of the antimicrobial action of human sebocytes, J. Investig. Dermatol. 129 (10) (2009) 2489–2496 https://doi.org/10.1038/jid.2009.106.
[65] J. Jodoin, M.T. Hincke, Histone H5 is a potent antimicrobial agent and a template for novel antimicrobial peptides, Sci. Rep. 8 (1) (2018) 2411 https://doi.org/10.1038/s41598-018-20912-1.
[66] A.L. Fischer, M. Bustillo, D.E. Elmore, A modular approach to the histone H2A family of antimicrobial peptides, FASEB J. 27 (1_supplement) (2013) 1021.4-1021.4 https://www.fasebj.org/doi/abs/10.1096/fasebj.27.1_supplement.1021.4.
[67] B. Chen, D.-Q. Fan, K.-X. Zhu, Z.-G. Shan, F.-Y. Chen, L. Hou, L. Cai, K.-J. Wang, Mechanism study on a new antimicrobial peptide Sphistin derived from the N-terminus of crab histone H2A identified in haemolymphs of Scylla paramamosain, Fish Shellfish Immunol. 47 (2) (2015) 833–846 https://doi.org/10.1016/j.fsi.2015.10.010.
[68] S. Banerjee, B. Chinthapalli, A proteomic screen with Drosophila Opa1-like identifies Hsc70-5/Mortalin as a regulator of mitochondrial morphology and cellular homeostasis, Int. J. Biochem. Cell Biol. 54 (2014) 36–48 https://doi.org/10.1016/j.biocel.2014.05.041.
[69] K. Yuan, F.-H. Yuan, H.-H. He, H.-T. Bi, S.-P. Weng, J.-G. He, Y.-H. Chen, Heat shock 70 kDa protein cognate 5 involved in WSSV toleration of Litopenaeus vannamei, Dev. Comp. Immunol. 72 (2017) 9–20 https://doi.org/10.1016/j.dci.2017.02.003.
[70] R. Mi, Y. Sun, J. Li, S. Ma, Z. Wen, X. Li, N. Meng, Y. Li, X. Du, S. Li, Immune-related proteins detected through iTRAQ-based proteomics analysis of intestines from Apostichopus japonicus in response to tussah immunoreactive substances, Fish Shellfish Immunol. 74 (2018) 436–443 https://doi.org/10.1016/j.fsi.2018.01.002.
[71] H. Zhang, C. Song, J. Xie, X. Ge, B. Liu, Y. Zhang, C. Sun, Q. Zhou, Z. Yang, Comparative proteomic analysis of hepatic mechanisms of Megalobrama amblycephala infected by Aeromonas hydrophila, Fish Shellfish Immunol. 82 (2018) 339–349 https://doi.org/10.1016/j.fsi.2018.08.006.