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Article

Transcriptome Analysis of Multiple Tissues in the Shrimp *Penaeus vannamei* Reveals the Typical Physiological Response to Three Pathogens

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Abstract: Penaeid shrimp aquaculture is impacted by various diseases. However, most published studies on physiological responses to pathogens have focused on the changes in one or two tissues of shrimp infected by a single pathogen, or the effects of two pathogens infecting the shrimp in a single tissue. There has been limited systematic examination on the similarities and differences of immune responses in multiple tissues under various pathogen infection. Here, the transcriptomic changes of three immune tissues (gill, hepatopancreas and hemocytes) under the infection of white spot syndrome virus (WSSV), *Vibrio parahaemolyticus* acute hepatopancreatic necrosis disease (VP_{AHPND}), and decapod iridovirus 1 (DIV1) were examined to provide new insights regarding the immune responses of the most important cultured shrimp, *Penaeus vannamei*. The results showed tissue-specific differences in the immune responses of shrimp tissues. The significant differentially expressed genes (DEGs) in gill are mainly related to environmental information processing and cellular processes. The DEGs in hemocytes are mostly involved in cellular processes, while those in hepatopancreas are primarily associated with metabolism. In addition, cytoskeleton-related proteins, MAPK signaling pathway, complement and coagulation level pathway, and thermogenesis may play key roles in the shrimp–pathogen interactions across tissues. These findings shed light on the typical immune responses of *Penaeus vannamei* under the infection of pathogens and contribute to the sustainable development of penaeid shrimp farming.

Keywords: *Penaeus vannamei*; WSSV; VP_{AHPND}; DIV1; RNA profile

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1. Introduction

Penaeus vannamei, commonly known as the Pacific white shrimp, is a commercially important aquaculture species. It is currently the most cultured crustacean species worldwide [1], accounting for about 80% of the total production of cultured penaeid shrimp in Asia [2,3]. However, the frequent outbreaks of infectious diseases have caused large-scale mortality of cultured shrimp, seriously impacting this aquaculture industry as the main obstacle to its development [4–6]. The pathogens that cause large-scale mortality of shrimp are mainly viral or bacterial. White spot syndrome virus (WSSV), yellow head virus (YhV), and decapod iridescent virus 1 (DIV1) are the common viral pathogens, while *Vibrio parahaemolyticus* (VP) which causes acute hepatopancreatic necrosis disease (AHPND) is the common bacterial pathogen [7–9] (Table 1). Among these infectious diseases, white spot syndrome (WSS) and AHPND are most destructive, causing huge losses of up to 10 billion USD per year [3]. Recently, the newly emerged DIV1 has also caused substantial economic loss to *P. vannamei* aquaculture in China [10].

Table 1. Pathogens involved in this study.

Pathogen	Type of Infection	Major Target Tissues	Diagnosis
WSSV	Vira	Gills, hematopoietic tissues, lymph	White spots on the shell, with longer blood coagulation time, pale, swollen and erosive hepatopancreas [11–15].
DIV1	Viral	Hematopoietic tissues hemocytes, hepatopancreas, gills and muscles	Softening of the shell, the yellowish-whitening of hepatopancreas, and empty stomach and intestine [16–18].
VP_{AHPND}	Bacterial	Gut-associated tissues and organs, including the hepatopancreas, stomach, and intestine	Softening of the shrimp body, atrophy and whitening of hepatopancreas, reduced feeding with empty stomach and intestine [6,19].

Like other invertebrates, crustaceans do not have acquired immunity. They rely entirely on innate immunity to fight against pathogen infections. Their innate immune system includes physical defense, cellular immunity, and humoral immunity [20,21], which can be stimulated by certain feed additives [22,23]. Gills, hemocytes, and hepatopancreas are the three immune tissues that play crucial roles in penaeids' defense against pathogens [24–26]. Hemocytes play a crucial role in cellular and humoral immune responses [27,28]. Gills are vital tissues of aquatic organisms, responsible for controlling osmotic pressure, ion balance, and gas exchange [29]. Immune-related genes, such as phenol oxidase, are expressed in gill tissues during the infection of pathogenic bacteria [30]. The hepatopancreas plays a pivotal role in the immune defense of crustaceans. It is an essential organ for storage, metabolism, and detoxification, and produces immune proteins, such as hemocyanin and lectin [31].

While there have been many transcriptome studies on the immune tissues of *P. vannamei* infected by WSSV, VP_{AHPND}, and DIV1 for reviews on the common immune genes and signal pathways in [5,32–34], these studies mainly focused on a few key genes that are highly expressed in the tissues under pathogen infection. Genes that are relatively low in expression but differentially expressed are often ignored, but they may be related to important pathways common in multiple tissues or may shed light on the different roles played by various tissues in immune responses. Therefore, it is necessary to investigate in greater detail the differentially expressed genes and pathways through transcriptome analysis of different tissues under the influence of pathogens.

In this study, transcriptome profiles from nine studies on the differential gene expression in the gills, hemocytes, or hepatopancreas of *P. vannamei* infected by WSSV, VP_{AHPND}, or DIV1 were collected and re-analyzed. The differentially expressed genes in the three tissues of *P. vannamei* under different pathogen infections were compared. The overarching aim is to identify genes fundamental to the immune response of *P. vannamei*, and to illuminate the different roles of the immune tissues. The results would improve our understanding on the interactions between pathogens and shrimp, as well as the innate immune mechanisms of the shrimp, which would be conducive to disease prevention and treatment in penaeid shrimp farming.

2. Materials and Methods

2.1. Data Collection

All available RNAseq BioProject data relating to differential gene expression in the three target tissues of *Penaeus vannamei* in experimental studies on WSSV, VP_{AHPND}, or DIV1 infection were downloaded from NCBI SRA. Their accession numbers are PRJNA233549, PRJNA413606, PRJNA421143, PRJNA428228, PRJNA448614, PRJNA524934, PRJNA554075, PRJNA612147, and PRJNA716175 (Table 2) [4,32–38]. A total of 109 transcriptome expression profiles were obtained. Profiles from similar treatments were homogenized during analyses (see Table 2 for details).

Table 2. Transcriptome data analyzed in this study. N: Number of transcriptome expression profiles.

BioProject	Tissue	Pathogen	N	Remarks: Homogenization of Data (H) or Limitation (L)	DOI of Publications
PRJNA524934	Gills	WSSV	18	H: Data from 3 times points of WSSV challenge treatment were grouped as the "WSSV-challenged group", and data from the 3 time points of control treatment were grouped as "control group".	10.1038/s41598-019-49836-0 [33]
PRJNA716175	Gills	WSSV	48	H: Data from 6 times points of WSSV challenge treatment were grouped as the "WSSV-challenged group", and data from the 6 time points of control treatment were grouped as "control group".	10.3390/v13061140 [4]
PRJNA233549	Hemocyte	WSSV	8	H: No homogenization needed. L: WSSV isolate was not used in this study; instead, VP28, the major envelope proteins of WSSV was injected to shrimp to reveal immune response at the initial stage of infection.	10.1016/j.dci.2014.02.013 [34]
PRJNA448614	Hemocyte	VP_{AHPND}	3	H: Data from 2 times points of VP_{AHPND} challenge treatment were grouped as the "VP_{AHPND}-challenged group", and data from the 2 time points of control treatment were grouped as "control group". L: There were only 3 RNA-seq libraries in this study as it was difficult to yield adequate amount of RNA for library construction from hemocyte samples, but each library includes 10 individuals of VP_{AHPND}-challenged shrimp.	10.1016/j.fsi.2018.06.054 [32]
PRJNA612147	Hemocyte	DIV1	6	H: No homogenization needed.	10.3389/fimmu.2020.01904 [38]
PRJNA428228	Hepatopancreas	WSSV	8	H: Only hepatopancreas RNA-seq expression data was used.	10.3390/genes11070805 [37]
PRJNA554075	Hepatopancreas	WSSV	6	H: Only RNA-seq expression data in *P. vannamei* was used.	10.1016/j.dci.2019.103564 [35]
PRJNA413606	Hepatopancreas	VP_{AHPND}	6	H: Only hepatopancreas RNA-seq expression data was used.	10.1016/j.fsi.2018.10.005 [36]
PRJNA421143	Hepatopancreas	VP_{AHPND}	6	H: No homogenization needed.	Not available

2.2. Transcriptome Assembly and Differential Expression Gene Analysis

Trim Galore ver. 0.6.8 was used to filter low-quality reads and to remove adaptor sequences. After filtering, FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ (accessed on 19 June 2022) was used to check the quality of the reads. Using Hisat2 ver. 2.2.1 [39] with default parameter, clean reads were mapped to the genome of *P. vannamei* (GenBank accession number ASM397254v1) to obtain the corresponding transcripts. Then, featureCounts [40] were used for read summarization.

To identify differentially expressed genes in each BioProject, we used R/Bioconductor limma [41], EdgeR [42], and DESeq2 [43] for differential expression analysis, and log2Fold Change (log2FC) values and their respective *p*-values were calculated. Genes with absolute log2FC greater than 2 and the *p*-value less than or equal to 0.05 were considered differentially expressed candidate genes (DEGC). Genes identified as DEGC in at least two of the three analyses were considered significantly differentially expressed genes (DEGs) in each BioProject (See Supplementary Materials for details).

The amino acid sequences of the DEGs were searched against the non-redundant protein public database of eggNOG [44] for GO and KEGG pathway annotation using eggnog-mapper ver. 2.1.9 [45] with default parameters and an E-value threshold of 1e-5. Matching sequences from non-eukaryotes and chordates were considered contaminants and were eliminated. Clusterprofiler ver. 4.0 [46] was used to conduct GO and KEGG pathway enrichment analyses of the DEGs. GO terms and KEGG pathway descriptions with *p*-value ≤ 0.05 and ≤ 0.1, respectively, were considered significantly enriched.

The R package GOSemSim [47] was used to measure the functional similarity between GO terms or gene products, and R package ggplot2 was used to visualize the topological structure diagram of functional clustering. As a large number of processes were annotated in gills and hepatopancreas, to reveal key processes involved in the shrimp's immunity, we removed branches that shared low functional similarity with the others.

3. Results

3.1. Significant Differentially Expressed Genes (DEGs)

In gills, 2195 and 809 DEGC were detected by EdgeR and DESeq2, respectively, and 400 unigenes were DEGs (Table 3). In hemocytes, 388 and 377 DEGC were detected by EdgeR and limma, respectively, and 84 unigenes were DEGs (Table 3). In hepatopancreas, 767 and 1854 DEGC were detected by EdgeR and limma respectively, and 434 unigenes were DEGs (Table 3). We mapped DEGs to the KEGG level1 category (Figure 1). When compared with the other two tissues, DEGs in gills were enriched in "Environmental Information Processing" and "Cellular Processes", DEGs in hemocytes were mainly enriched in "Cellular Processes", while those in hepatopancreas were mainly enriched in "Metabolism".

Table 3. The number of differentially expressed genes (DEGs) found in gills, hemocytes, and hepatopancreas of *P. vannamei* under WSSV, VP_{AHPND}, or DIV1 infection compared to control group. Hyphen: the specific method could not identify any DEGC or was not suitable for the dataset.

BioProject	EdgeR	Limma	Deseq2	DEGs	Unigenes (DEGs) in Tissue	Tissue	Pathogen
PRJNA524934	1842	78	630	473	400	Gills	WSSV
PRJNA716175	353	2	179	156		Gills	WSSV
PRJNA233549	173	239	-	27	84	Hemocyte	WSSV
PRJNA448614	29	83	-	7		Hemocyte	VP_{AHPND}
PRJNA612147	186	55	13	50		Hemocyte	DIV1
PRJNA413606	53	54	1	19	434	Hepatopancreas	VP_{AHPND}
PRJNA421143	612	1289	240	609		Hepatopancreas	VP_{AHPND}
PRJNA428228	19	231	-	17		Hepatopancreas	WSSV
PRJNA554075	83	280	44	78		Hepatopancreas	WSSV

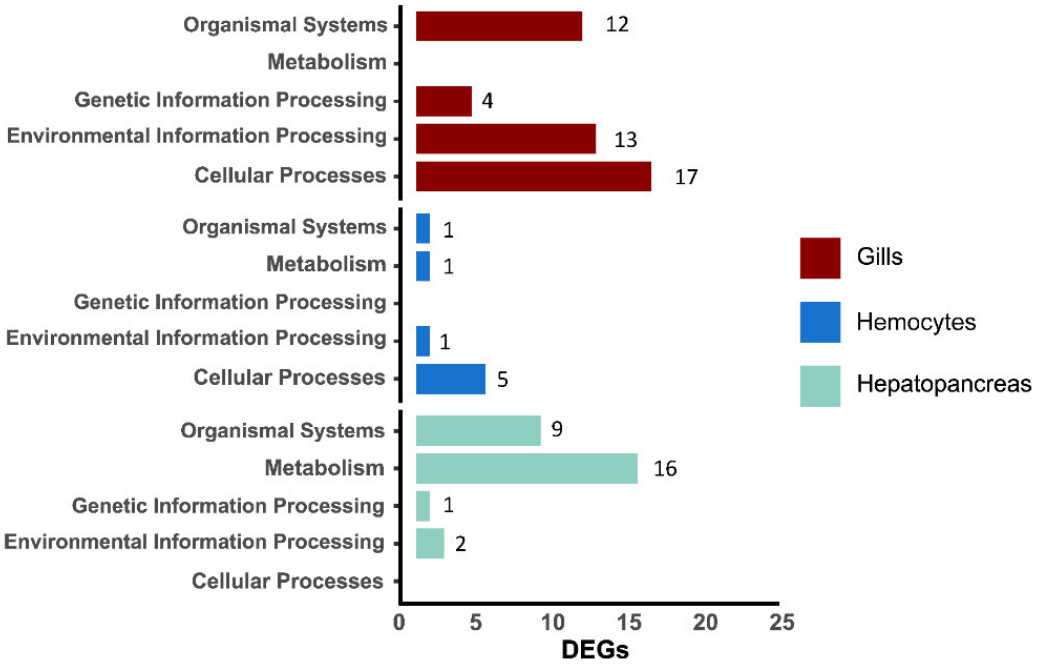

Figure 1. Histogram of the number of DEGs in the gills, hemolymph, and hepatopancreas of *P. vannamei* under three pathogens infection assigned to five KEGG level 1 categories, i.e., organismal systems, metabolism, genetic information processing, environmental information processing, and cellular processes.

3.2. GO Analysis of DEGs

GO analysis was conducted to obtain an overview of the molecular responses invoked during pathogen infection in *P. vannamei* (Figure 2A). While many of the enriched GO terms were tissue-specific and pathogen-specific, some were shared across tissues and pathogens. The GO term "Extracellular Region", containing 23 DEGs enriched in gills, hemocytes, and hepatopancreas under both WSSV and DIV1 infection, appeared to be the most affected molecular response (Figure 2B). Under the infection of WSSV and VP_{AHPND}, "Gated Channel Activity" (eight DEGs) and "Response to Growth Factor" (five DEGs) were affected in gills and hepatopancreas. "Epithelial Cell Differentiation" (total of seven DEGs) appeared to be markedly affected by all pathogens in hepatopancreas, while 10 DEGs of gills under WSSV and VP_{AHPND} infection were annotated to "R7 Cell Differentiation".

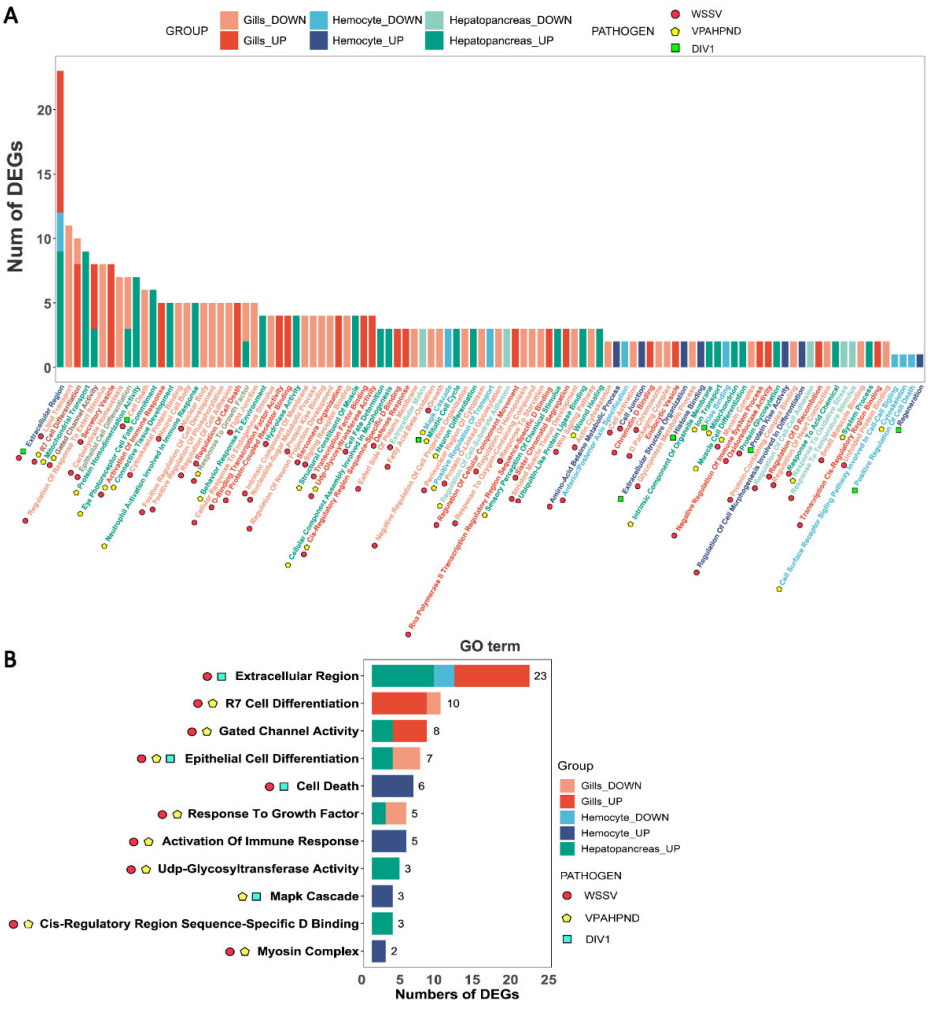

Figure 2. (**A**) Distribution of GO terms in all DEGs annotated in the gills, hemocytes, and hepatopancreas of *P. vannamei* under WSSV, VPAHPND, or DIV1 infection. (**B**) GO terms of DEGs in the gills, hemolymph, and hepatopancreas that are shared between at least two of the three pathogen infections. The colors of the bars represent the tissue types and expressional changes (down- or up-regulated). The icons next to the KEGG level 3 categories indicate the types of infection.

To reveal the broad categories of responses under different pathogen infection and to compare the response across tissues, we calculated the functional similarity between GO terms or gene products for constructing the functional clustering diagram of DEGs using the GOSemSim algorithm (Figure 3). With respect to WSSV's influence on the three tissues, the main GO terms of DEGs in the gills could be divided into seven broad categories, namely cell division, differentiation, regeneration, signal transduction, cell connection,

and response to external stimuli (Figure 3A). In contrast, the GO terms of DEGs in the hepatopancreas could be divided into only three categories, namely extracellular tissue, cell connection, and cell differentiation (Figure 3B). In the hemocytes, GO terms were mainly related to cell connection and adhesion (Figure 3C). Overall, the commonly enriched DEGs of the three tissues were related to cell differentiation and cell connection. DEGs in the gills were enriched in functions related to external stimulation (Figure 3A).

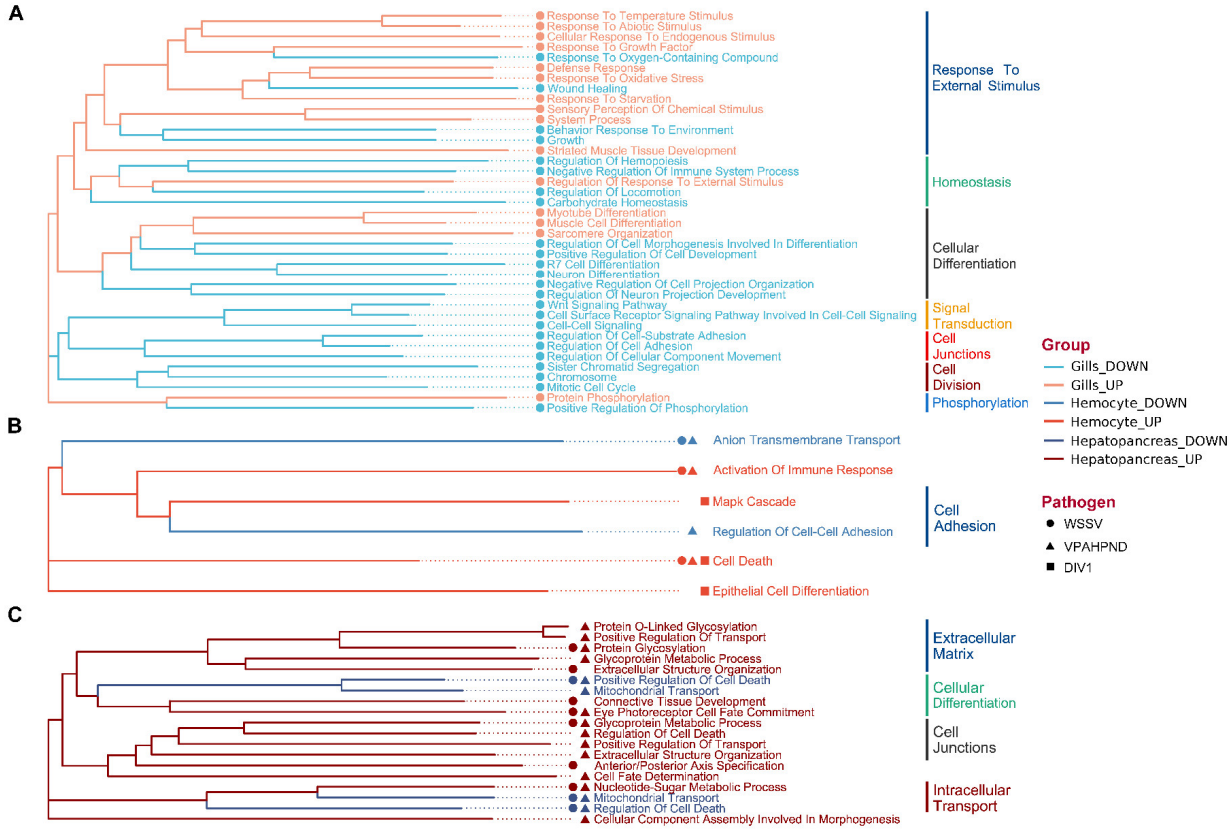

Figure 3. GO clustering diagram based on DEGs functional similarity in (**A**) gills, (**B**) hemocytes, and (**C**) hepatopancreas, constructed using the R package GOSemSim (branches that shared low functional similarity with the others were removed to reveal critical processes involved in the shrimp's immunity). DEGs functional categories are grouped according to functional themes. The colors of the branches indicate tissue types and expressional changes (down- or up-regulated), while icons at the tip indicate the types of infection.

3.3. Pathway Enrichment Analysis of DEGs

We analyzed the KEGG pathway of DEGs in the three tissues under different pathogen infections. Regardless of the pathogen infected, the MAPK pathway was affected in all three tissues, though it was up-regulated in hemocytes infected by WSSV and DIV1 but down-regulated in gills and hepatopancreas under WSSV infection (Figure 4). Under WSSV infection, several immune pathways, including phagosome and actin, which are related to the cytoskeleton or cell connection, were enriched in both gills and hemocytes, while the thermogenic pathway was enriched in gills and hepatopancreas (Figure 4A,C). Regarding the tissue-specific response under WSSV infection, it is noteworthy that up-regulated DEGs in gills were annotated to pathways related to immunity (e.g., Rap1 signaling pathway) and cell junction, those in hemocytes were annotated to pathways related to tight junctions, phagosome, and platelet activation, while those in hepatopancreas were mostly associated with glycosaminoglycan biosynthesis.

Figure 4. Results of KEGG enrichment analysis of DEGs in (**A**) gill, (**B**) hemocytes, and (**C**) hepatopancreas in WSSV, VPAHPND, or DIV1 infected *P. vannamei*, showing the pathway identifiers, the numbers of up-regulated DEGs (GEH, in red) and down-regulated DEGs (DEL, in green), and the corresponding KEGG level 3, 2 and 1 functional categories. The icons next to the KEGG level 3 categories indicate the types of infection, with grey diamond indicating the KEGG level 3 categories enriched in two of the tissues (gills and hemocytes or hepatopancreas), while the black diamond indicates categories enriched in all three tissues. DEGs were first assigned to a specific KEGG pathway level 2, then assigned to either one of the five KEGG level 1 category (i.e., organismal systems, metabolism, genetic information processing, environmental information processing, and cellular processes).

The KEGG pathways of DEGs from hemocytes infected by WSSV and VP_{AHPND} were almost identical. Lipid metabolism, tight junction, cytoskeleton, phagosome, and platelet activation pathways were enriched in both cases, while MAPK signaling pathway and lysosome pathway were enriched in hemocytes in DIV1-infected shrimp (Figure 4B). In contrast, VP_{AHPND} and WSSV elicited different responses in hepatopancreas, and only the glycosaminoglycan biosynthesis related pathway was invoked in both cases.

4. Discussion

With the global demand for seafood, the aquaculture production of penaeid shrimp has been increasing exponentially. However, shrimp farming is plagued by diseases, which seriously restricts the further development of this industry [48]. There is still a lack of multi-tissue studies on penaeid shrimp infected by various pathogens. In the present study, we investigated the gene expression profiles in multiple tissues of *P. vannamei* infected with three pathogens of WSSV, VP_{AHPND}, and DIV1 to explore the shrimp's immune responses to pathogen attack at the molecular level.

4.1. Tissue-Specific Responses

Penaeid shrimp can only protect themselves from pathogens through the innate immune system [49], in which the hepatopancreas, hemocytes, and gills play crucial roles [50–52]. In this study, we found tissue-specific immune responses. DEGs in the gills are mainly related to environmental information processing and cellular processes,

while DEGs in hemocytes are mainly associated with cellular processes. In contrast, DEGs in the hepatopancreas are primarily related to metabolism. Apparently, the structural and functional differences between the three tissues lead to the tissue-specific responses under pathogen's attack. The gills are in direct contact with the external environment and are mainly responsible for gas exchange, osmoregulation, and ion balance [25,29]. Thus, the gills may play a role in protection, blocking, and signal transmission against pathogen infection. They receive external stimuli and transmit signals to various tissues of the body. Hemocytes play a role in maintaining homeostasis, immune regulation, and wound healing, while the hepatopancreas is an essential tissue for storage, metabolism, and detoxification [24,26]. Hence, upon pathogen infection, hemocytes and hepatopancreas may play important roles in killing and removing the pathogens to achieve recovery.

Hemolymph contains many immune cells, such as hemocytes which are involved in the phagocytosis of apoptotic cells, as well as immune active substances, such as phenol oxidase, complement factor, lectin, and antimicrobial peptides. They represent one of the critical barriers to pathogen invasion in invertebrates [53]. Some studies have shown that VP_{AHPND} and WSSV stimulate different immune responses in penaeid shrimp lymphoid tissues: VP_{AHPND} causes more extensive immune responses in lymphoid tissues, while WSSV affects metabolic processes [14,54]. However, the DEGs upon infection of both pathogens have been annotated to similar pathways, such as the pro activation system, lysosomes, extracellular matrix, cytoskeleton proteins, and chitin-binding proteins [14]. In our analyses, the pathways annotated by DEGs of hemocytes under WSSV and VP_{AHPND} infection were similar, mainly related to fatty acid metabolism, tight junction, actin cytoskeleton, and phagosome. Moreover, compared with WSSV or DIV1, more upregulated pathways were caused by VP_{AHPND} stimulation, which may indicate that the hemolymph plays a more important role in the anti-bacterial immune responses.

In addition, we found that the complement and coagulation cascade pathways in gill and hepatopancreas tissues infected with WSSV were down-regulated. Previous study indicated that WSSV-infected *P. vannamei* lost blood coagulation ability after 48 h of infection [55], and the coagulation system seems to be a target of viral and bacterial attacks in crustaceans [56]. A prolonged exposure viral attack may reduce hemocytes abundance, as indicated by a study on *P. indicus* [57], further causing the loss of blood coagulation ability [55].

Hence, the present study reveals markedly difference responses of gills, hepatopancreas, and hemocytes under pathogen infection. This may be attributable to their different structural attributes, roles in immunity, and their unique interaction with the pathogens.

4.2. Immune Responses Shared among Tissues

MAPK signaling pathway plays a vital role in the responses of cells to extracellular stimuli. In vertebrates, the MAPK signaling pathway plays a crucial role in the innate immune system, especially in the fight against pathogenic infections [53,58]. The differential expression of proteins related to MAPK signaling pathway, Ras signaling pathway, phagosome, and Hippo signaling pathway was also found in mud crabs infected with WSSV and VP_{AHPND} [59]. However, some studies have also reported that virus may activate hosts' MAPK pathway in cytoskeleton, which might help viral replication in cells, and thus promote pathogen infection [60,61]. Hence, a lowered expression of genes involved in MAPK signaling pathways could indicate that the virus is inefficient in activating the said pathway, or that the host actively down-regulate the pathway to hamper viral replication. Our study shows that the MAPK signaling pathway was down-regulated in the gills and hepatopancreas of WSSV challenged, but up-regulated in the hemocytes of DIV1 and VP_{AHPND} challenged shrimps, which may reflect complex interactions between the host and pathogens, warranting further investigation.

The present study shows that WSSV elicited thermogenic responses in the hepatopancreas and gills. Chen et al. found that in the early stage of WSSV infection, slightly warming the penaeid shrimp pond could effectively increase shrimp's resistance to WSSV and re-

duced the cumulative mortality [21]. Lin et al. found that the fever response can positively regulate the immune response through heat shock protein [62]. Yuan et al. found that the activation of heat shock protein can reduce the cumulative mortality of penaeid shrimp infected with WSSV [21,62,63]. Thus, under the attack of WSSV, thermogenic changes may be part of the shrimp's immune responses which may entail the activation of heat shock protein. The exact mechanism in this respect needs to be further studied.

Genes related to cytoskeleton or cell junction, including actin and focal adhesion, are generally upregulated upon pathogen infection, and it is believed that the cytoskeleton plays an essential role in the innate immunity of invertebrates through cell adhesion and phagocytosis [64–66]. However, pathogens might have evolved to regulate the host's cytoskeleton structure to gain easy passage to tissues, as evidenced in a study of gill, gut, and cuticular epithelium of *P. vannamei* under WSSV infection [4]. This study showed that cytoskeleton-related proteins such as actin and focal adhesion were significantly upregulated in gills, hemocytes, and hepatopancreas of shrimp infected by the three pathogens, but the underlying cause and the consequence are yet to be determined in future studies.

5. Conclusions

To improve the understanding of the immune responses of *P. vannamei* upon pathogen infection, we compared the transcriptome data from 109 transcriptome expression profiles of *P. vannamei* infected by three common pathogens. Though shrimps exhibit various responses under the attack of different pathogens, we also identify common molecular responses potentially related to immunity, including the upregulation of cytoskeletal transcripts related to viral trafficking or cell phagocytosis during infection, MAPK signaling pathway, complement and coagulation level communication, and thermogenesis. The differences in the functions of the three tissues may lead to their tissue-specific differences under pathogen infection. Compared with WSSV and DIV1, VP_{AHPND} caused more immune responses in the hemolymph, which may indicate that the hemolymph plays an essential role in the anti-bacterial immune responses. In addition, there may be a relationship between thermogenic response and the activation of heat shock proteins. The MAPK signaling pathway may be a double-edged sword in host–pathogen interactions. In conclusion, our findings provide new insights into the interactions between *P. vannamei* and pathogens. The results improve the understanding of the immune response mechanisms of penaeid shrimp, provide a theoretical basis for the prevention and control of diseases in cultured penaeid shrimp, and are important for promoting their health for the sustainable development of shrimp farming.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse11020389/s1, Figure S1: Transcriptomic changes of gills infected by WSSV based on two studies (BioProject accession number: (A) PRJNA524934; (B) PRJNA716175), showing results from principal component analysis (PCA), gene abundance heat map, Wien diagrams showing number of differentially expressed candidate genes; Figure S2: Transcriptomic changes of haemolymph infected by WSSV, VP_{AHPND} and DIV1 based on two studies (BioProject accession number: (A) PRJNA233549; (B) PRJNA448614; (C) PRJNA612147), showing results from principal component analysis (PCA), gene abundance heat map, Wien diagrams showing number of differentially expressed candidate genes; Figure S3: Transcriptomic changes of hepatopancreas infected by WSSV and VPAHPND based on two studies (BioProject accession number: (A) PRJNA413606; (B) PRJNA421143; (C) PRJNA428228; (D) PRJNA554075), showing results from principal component analysis (PCA), gene abundance heat map, Wien diagrams showing number of differentially expressed candidate genes.

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