Comparative analysis of mRNA, microRNA of transcriptome and proteomics on CIK cells responses to GCRV and *Aeromonas hydrophila*

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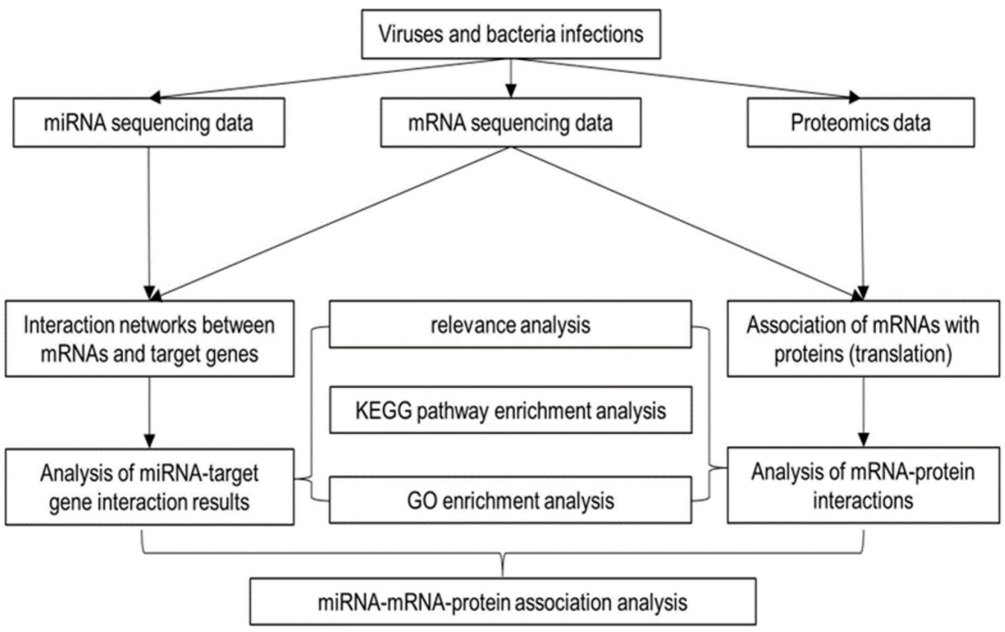
Introduction

Grass carp (*Ctenopharyngodon idellus*) is an important freshwater economy fish in China. However, due to the extremely complex and microbiologically rich environment that grass carp are exposed to during artificial intensive aquaculture , grass carp are highly susceptible to infection by microbial pathogens. In particular, outbreaks of haemorrhagic disease in grass carp caused by Grass Carp Reovirus (GCRV) and *Aeromonas hydrophila* can result in the death of a large number of grass carp, leading to significant economic losses and posing a serious threat to the sustainable development of China's freshwater aquaculture industry.

The study aimed to investigate the molecular mechanisms and immune responses at the miRNA, mRNA and protein levels in grass carp kidney cells (CIK) infected by Grass Carp Reovirus (GCRV) and *Aeromonas hydrophilus* (Bacteria) to gain insight into their pathogenesis.

Research ideas



Results

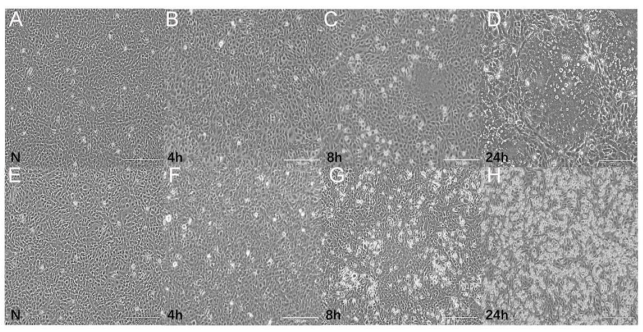


Figure 1. CIK cells infected with GCRV (A-D) and *Aeromonas hydrophila* (E-F) at different time points. N represents the control group, scale bar indicates 200 μm.

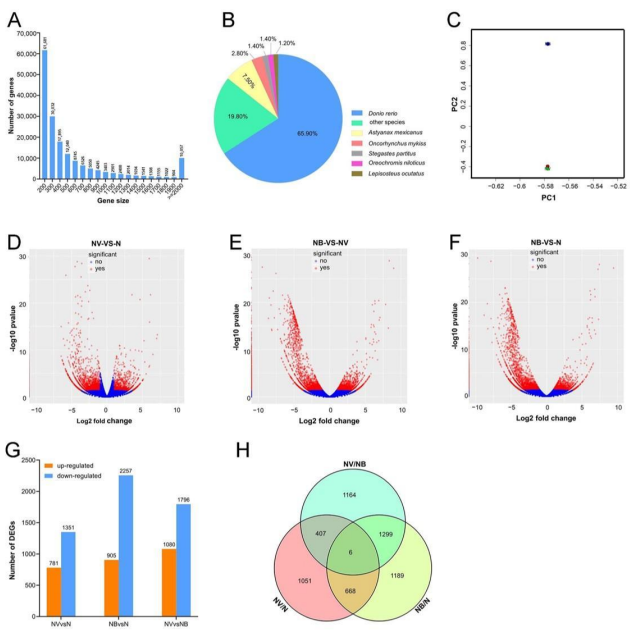


Figure 2. Transcriptome sequencing analysis. (A) Length distribution of unigenes. (B) Blastx analysis of unigenes from the grass carp transcriptome. Different colors represent different species, the size of the area indicates the proportion of each species. (C) Principal Component Analysis. PC1 represents the difference in infected samples, while PC2 represents the difference between the control group and the experimental group. The blue dot represents the bacterial group (NB), the green dot represents the viral group (NV), and the red dot represents the control group (N). (D-F) The DEGs from three treatment samples were visualized by volcano plots. The absolute values of log2 ratio ≥ 2 and P ≤ 0.05(-log 10 ≥ 1.31) were performed as the threshold to assigned DEGs. (G) The number of up-regulated and down-regulated DEGs in each group. (H) The Venn diagram illustrates the overlapping situation of DEGs within each group.

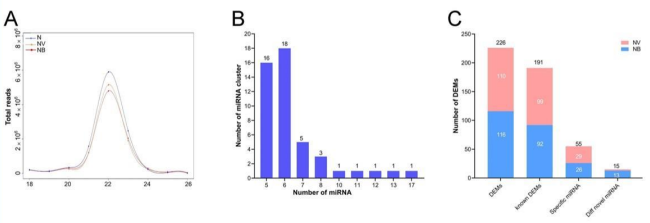


Figure 3. Analysis of small RNA sequencing. (A) Length distribution of miRNA. The red line represents the N group, the yellow line represents the NV group, and the blue line represents the NB group. (B) Statistics of miRNA clusters. The x-coordinate are number of miRNA clusters, and the y-coordinate are number of miRNAs. (C) Statistics of DEMs and specific miRNAs.

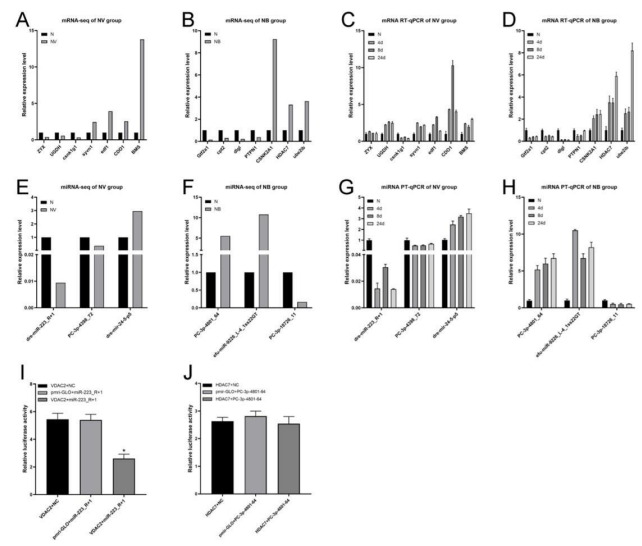


Figure 4. Validation of mRNA-seq/small RNA-seq by RT-qPCR. (A-D) DEGs validation of NV and NB groups. A and B represent transcriptome sequencing data of DEGs of NV and NB groups, C and D represent RT-qPCR data of DEGs of NV and NB groups. (E-H) DEMs validation of NV and NB group. E and F represent transcriptome sequencing data of DEMs of NV and NB groups, G and H represent RT-qPCR data of DEMs of NV and NB groups. (I, J) miR-223_R+1 and PC-3p-4801-64 target validation. CIK cells were transfected with miR-223_R+1 and PC-3p-4801-64, along with VDAC2 and HDAC7-3'UTR for 24h, respectively, the luciferase activity was determined. All data represent the mean ± S.D. of three replicates.

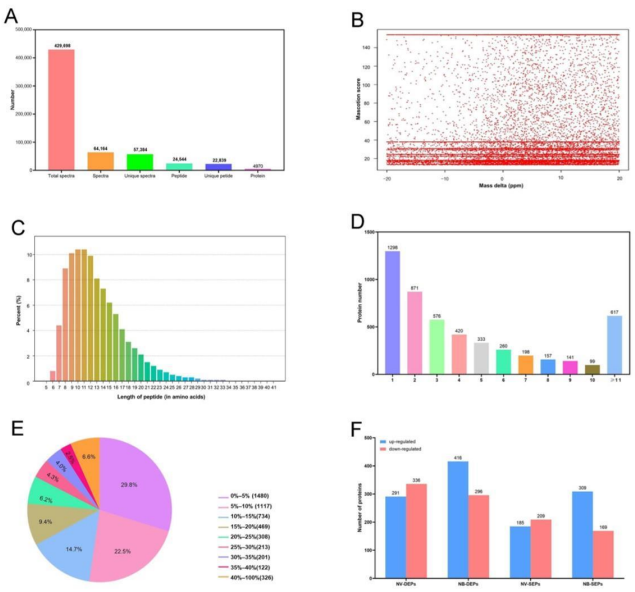


Figure 5. Quality control validation of mass spectrometry (MS) data. (A) A number of total proteins. (B) Mass error distribution of all identified peptides. The distribution of mass error is near 20. (C) Peptide length distribution. The length of most peptides is distributed between 7 and 16, which is consistent with the properties of the tryptic peptides. (D) Distribution of peptide numbers Most proteins included 1-4 peptides, which is credible. (E) Protein coverage distribution. Protein coverage of approximately 50% peptides was more than 10%. (F) The number of up-regulated and down-regulated DEPs in each group.

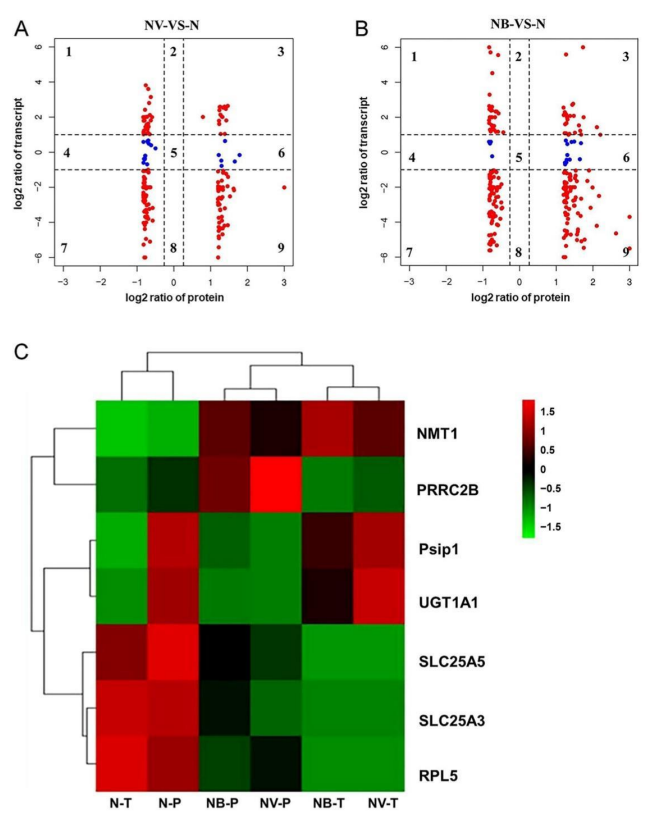


Figure 6. Overview interactions of miRNA-mRNA-protein. (A-B) The relationship between mRNA and protein expression levels. (A) NV group compared with N group. (B) NB group compared with N group. In the figure, it is divided into 9 quadrants. The ordinate represents the fold change of the gene expression profile, and the abscess represents the fold change of the protein expression profile. When | fold change | (FC) ≥ 2 was assigned as DEGs (log2=1 or -1) and fold change | (FC) ≥1.2 was assigned as DEPs (log2=0.5849, or -0.5849). (C) Hierarchical clustering of DEGs and DEPs common in N, NV and NB. DEGs expression level of the transcriptome in different groups (N-T, NV-T, NB-T). DEPs expression level of the proteome in different groups (N-P, NV-P, NB-P).

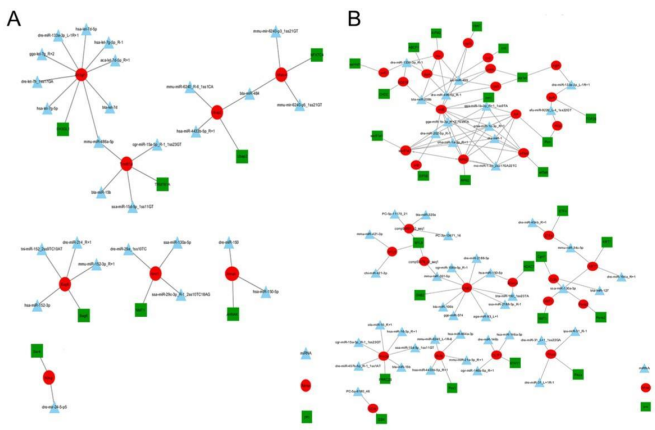


Figure 7. miRNA-mRNA-protein negative correlation network in NV group(A) and NB group(B). Triangles represented DEMs, circles represented DEGs, and squares represented DEPs.

Conclusion

The present study revealed that the pathogenesis of haemorrhage in grass carp caused by GCRV infection was different from that caused by *Aeromonas hydrophila* infection, in that most of the DEGs in the viral group were mainly involved in cellular processes, while most of the DEGs in the bacterial group were associated with metabolic pathways according to KEGG enrichment analysis. Both the innate and adaptive immune systems are highly responsive to viral and bacterial infections of CIK cells.