ScRNA-seq reveals intrinsic regulatory genes of shrimp sand diving behavior in *Penaeus japonicus*

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Background

- ***Penaeus japonicus*** is the third largest artificially cultivated marine shrimp in the world, whose selling price is 4 to 5 times that of the same-sized *Litopenaeus vannamei* in China. Thus it has important economic value.
- The unit area aquaculture yield of *Penaeus japonicus* is not much, mainly because their **sand diving habits** limit the increase of **aquaculture density**.
- In order to **increase** the breeding density of *Penaeus japonicus*, we tried to establish a variety of breeding modes, which significantly increased the breeding yield per unit area.

Experiment model

The hemolymph of different groups of *Penaeus japonicus* were extracted and sequenced by ScRNA-seq.
We conducted tissue localization of cell population marker genes through mRNA-FISH validation.
We conducted RNAi and mRNA overexpression verification experiments to Investigate the function of genes.

Screening of highly expressed genes in each cell cluster

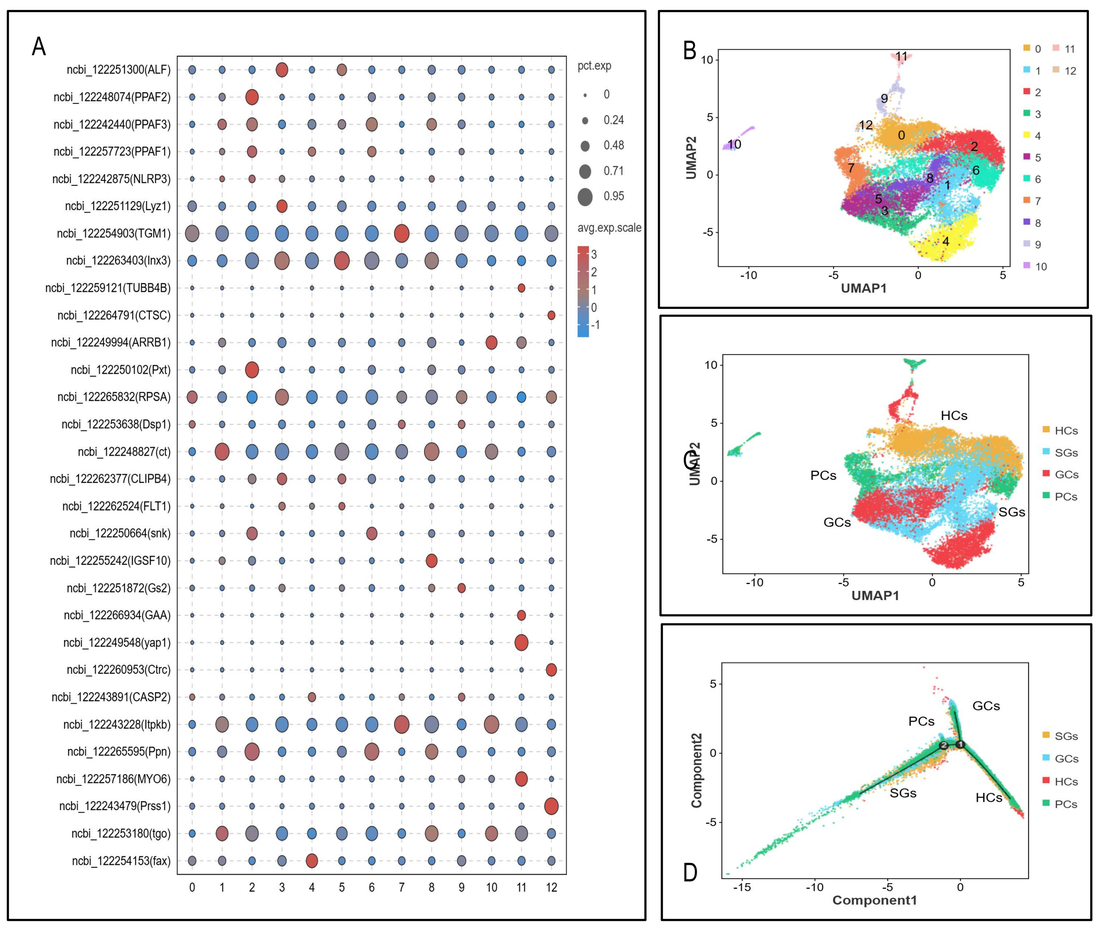

Figure 1 **A** :The bubble distribution of marker genes screened by different cell clusters, in which PPAF, ALF, Pxt and other genes were significantly expressed in their cell clusters, and TGM1 and Ihx3 were expressed in each cell cluster, which provided a basis for subsequent classification. **B**: The visualization of dimensionality reduction of different cell clusters; **C**: The visualization dimensionality reduction of cell subsets according to the function of marker genes, including granular cells(HCs), hyalurinous cells(HCs), granulocytes(GCs)and precursor cells(PCs), with the largest number of GCs cells and the least number of PCs cells. **D**: The differentiation trajectory of four types of cells, the total subset has two branch points, and the cells of each subset are expressed between the branch points, the location of HCs is the starting site of the differentiation trajectory, the location of SGs is the end of the differentiation trajectory, and PCs are evenly distributed in the differentiation trajectory.

localization of cell population marker genes

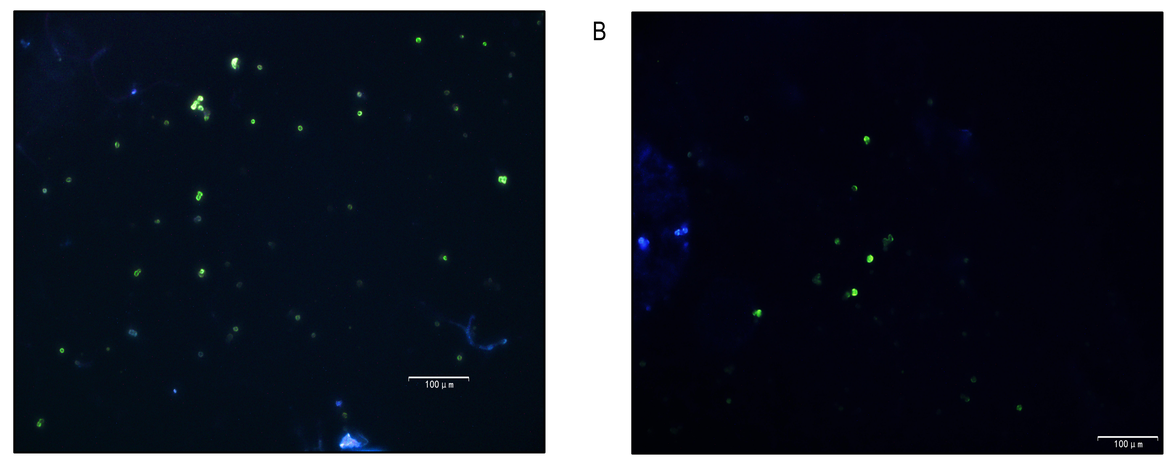

Figure 2 Results of mRNA-FISH microscopy on hemolymph smears of *Penaeus japonicus* .Using PPAF2-pSPT18 probe, green fluorescent cells were identified as GCs.

Comparative results of the expression of differential genes between groups

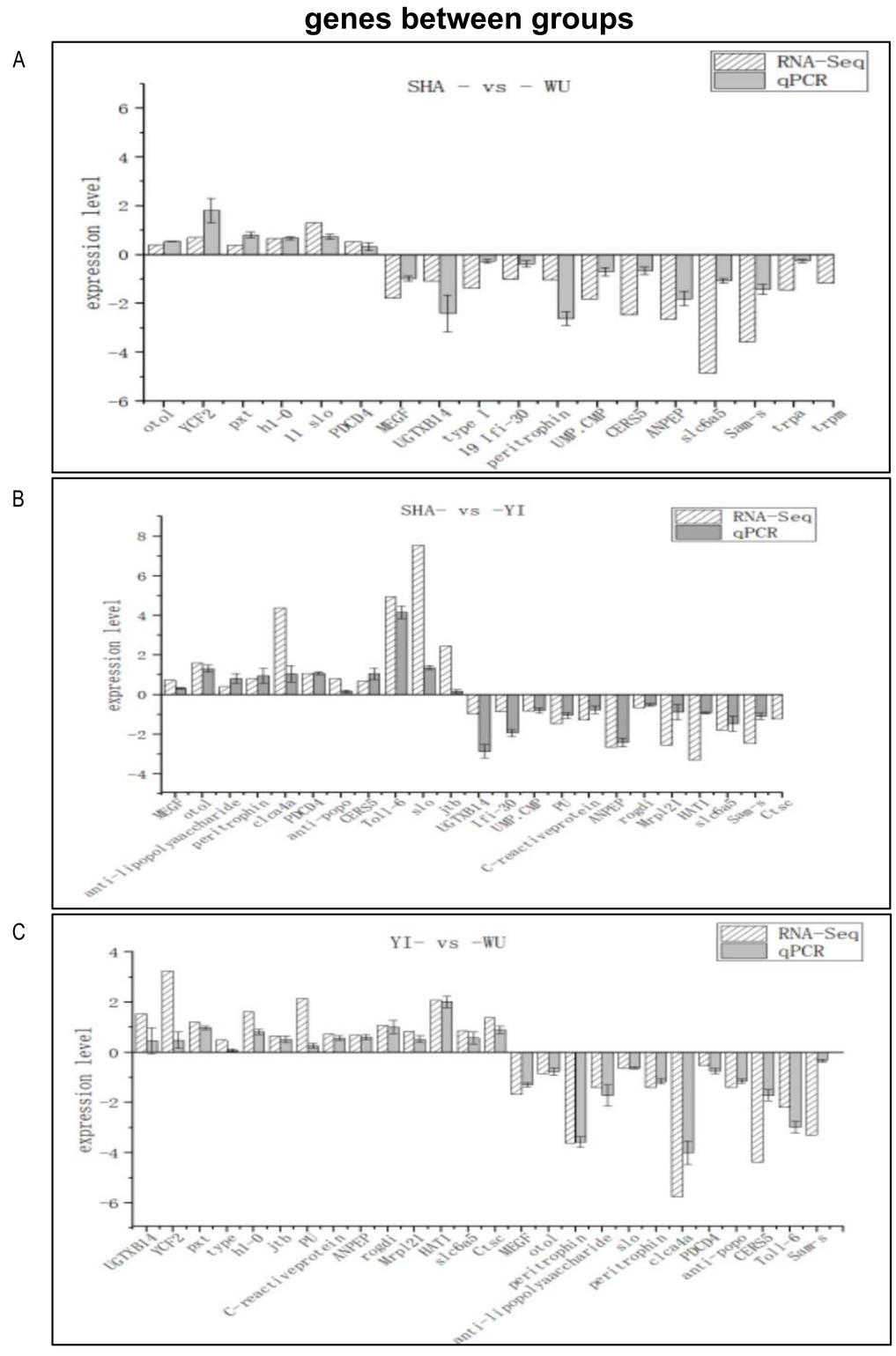

Figure 3 Organize the sequencing results and analyze the differences in the expression levels of differentially expressed genes among the three groups, screen for significantly upregulated and downregulated genes, and use qPCR to measure the expression levels in the hemolymph of different experimental groups. The expression trend is consistent with the ScRNA seq results.

Expression of trpa after knockdown

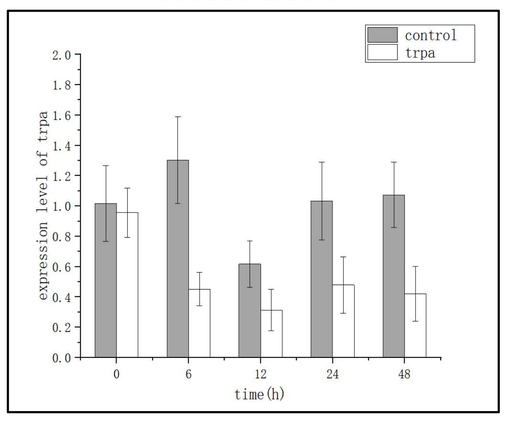

Figure 4 Trpa-1 was significantly expressed in XO-SG complex, and 28.5% of the shrimp in the interference group kept swimming at night after 12h of interference.

Expression of trpm after knockdown

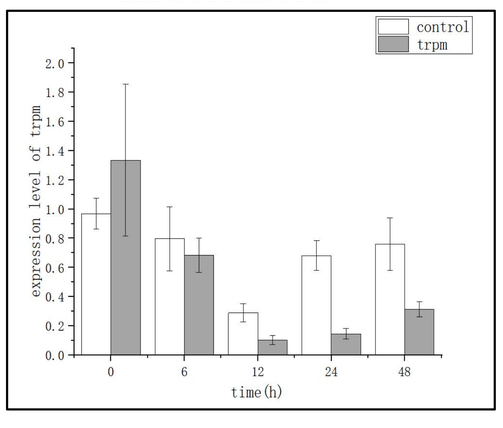

Figure 5 trpm was significantly expressed in the heart tissue, and the shrimp with trpm gene expression knocked down remained in the sand diving state at all sampling sites.

Conclusions

We found two genes may play an important role in regulating the sand diving behavior of *Penaeus japonicus*. The results of this study will be helpful for breeding new varieties of *Penaeus japonicus* without creeping sand behavior.