Hemocyte Transcriptome and Screening, Analysis of Genes Related to Hypoxia Tolerance of *Tegillarca granosa*

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Introduction

- *Tegillarca granosa*(blood clam) is an economical shellfish with strong hypoxia tolerance[1,2], but there is unclear on its molecular mechanism of hypoxic tolerance.
- Aquatic animals respond to low oxygen stress in different ways; Some fish, shrimp and crab species actively avoid low-oxygen waters, while others have evolved to adapt to low oxygen levels.
- As a kind of high-throughput sequencing, transcriptome sequencing has the characteristics of wide detection coverage and good accuracy, and has been widely used to explore the relationship between the external environment and individuals[2,3].

Objectives

- To analyze the differentially expressed genes (DEGs) and signal pathway changes of hemocyte transcripts and related experiment from *Tegillarca granosa* samples under hypoxia stress and normoxia conditions.
- To screen and analysis potential hypoxia tolerance genes.
- The changes of intracellular and extracellular calcium signals in hemocytes after *Tegillarca granosa* stress were observed by probes to verify the response of calcium signaling pathway.

Materials and Methods

- The healthy *Tegillarca granosa* was subjected to normoxia (9.5mg/L) and hypoxia (0.5mg/ L) for 6, 24, 72, and 120h, respectively, and then sampled from the control group and the experimental group.
- Transcriptome sequencing, DEGs analysis, GO biological process and KEGG pathway analysis were performed on the samples Data analysis.
- qPCR experiment was used to validate transcriptome data.
- Related hypoxia tolerance genes were screened according to the results of DEGs, GO and KEGG.
- Fluo-4 fluorescent probe was used to detect the changes of intracellular and extracellular calcium signals in *Tegillarca granosa* hemocytes after stress, so as to verify the calcium signaling pathway.

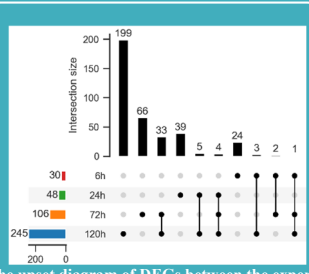

Fig.1 The upset diagram of DEGs between the experimental group and the control group after hypoxic treatment of blood clam hemocytes after 6, 24, 72 and 120h

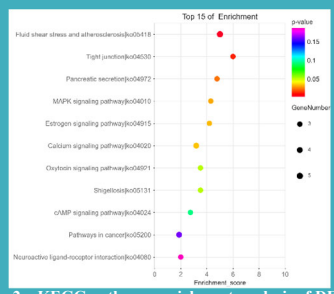

Fig. 2a KEGG pathway enrichment analysis of DEGs in blood clams' hemocytes of experimental group and control group after hypoxic treatment for 72h

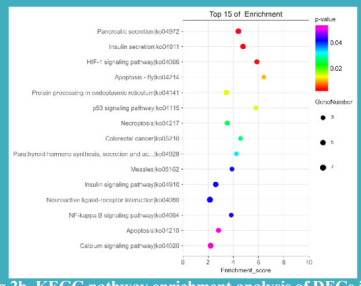

Fig.2b KEGG pathway enrichment analysis of DEGs in blood clams' hemocytes of experimental group and control group after hypoxic treatment for 120h

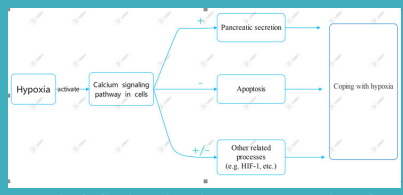

Fig. 3 Calcium signaling regulates a variety of biological processes[4]

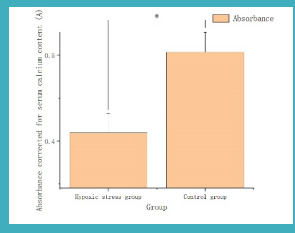

Fig.4a After hypoxia stress, extracellular calcium concentration of *Tegillarca granosa* hemocytes decreased significantly

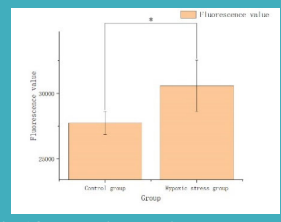

Fig.4b After hypoxia stress, intracellular calcium concentration of *Tegillarca granosa* hemocytes increased significantly

Results & Discussion

- As shown in Fig.1, the number of DEGs at four time points from 6h to 120h showed an increasing trend (30→48→106→245).
- As shown in Fig.2a&2b, pathway analysis was mainly enriched in Insulin and Pancreatic secretion signaling pathways, HIF-1 pathway, Calcium signaling pathway and Apoptosis pathway.
- As shown in Fig.3, Calcium signaling pathway is an important regulatory pathway that regulates pancreatic secretion, apoptosis, HIF-1 and other biological processes and pathways. The important genes related to these pathways are *JUN*, *PIM-3*, *TRIM45*, *MR-1B* and *CCKR*.
- As shown in Fig.4a&4b, After hypoxia stress, the extracellular calcium signal of *Tegillarca granosa* hemocytes decreased, while the intracellular calcium signal increased, suggesting that hypoxia activated the intracellular and extracellular flow of calcium ions in *Tegillarca granosa* hemocytes, indicating the activation of calcium signaling pathway.

Conclusions

- Our job speculated that the Pancreatic secretion signaling pathway, Calcium signaling pathway and Cell apoptosis played an important role in the tolerance to hypoxia of blood clam
- *JUN*, *PIM-3*, *TRIM45*, *MR-1B* and *CCKR* related to these pathways play a key role in the process of hypoxic tolerance.
- This work can provide reference for the exploration of the regulation mechanism and molecular breeding strains of hypoxic tolerance in shellfish.

References

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