Lactate is involved in the regulation of the expression of anti-microbial peptides in Chinese mitten crab (*Eriocheir sinensis*) under hypoxia stress through the HIF-1α-NF-κB signaling axis

Yuhan Ma, Yuhan Tu, Chang Liu, Yiyang Xu, Siyue Li, Junwei Guo, Yuting Xi, Jinming Yu, Haidong Zou, Jinming Yuan, Zihao Wu, Shu Huang* , Qilin Yi*

Dalian Ocean University College of Fisheries and Life Science, Dalian, Liaoning, 116023

Introduction

Hypoxia severely compromises the immune defense of aquatic animals. Although lactate, the end product of anaerobic glycolysis, has been demonstrated to exert immunomodulatory functions in higher vertebrates, its role in the hypoxic immune response of crustaceans remains unclear.

In this study, we conducted a hypoxia stress experiment with Chinese mitten crab (*Eriocheir sinensis*), an important aquatic economic crustacean in China. The results showed that both the mRNA expression of lactate dehydrogenase (LDH) and the level of histone lactylation (H3K18la) were significantly increased under hypoxia. Inhibition of LDH markedly downregulated the expression of Anti-lipopolysaccharide factors (ALFs), whereas activation of LDH upregulated their expression. Further mechanistic investigation revealed that lactate activates hypoxia-inducible factor-1α (HIF-1α), which in turn promotes the nuclear translocation of NF-κB, leading to enhanced ALFs production.

In summary, this study demonstrates that hypoxia stress enhances histone lactylation and LDH expression, and activates the HIF-1α–NF-κB signaling axis to positively regulate ALFs expression. These findings provide insights into the immune response mechanisms of crustaceans under hypoxia and support disease control strategies in aquaculture.

Results and discussion

1. The temporal mRNA expression pattern of *Es*LDH in haemocytes under hypoxia stress

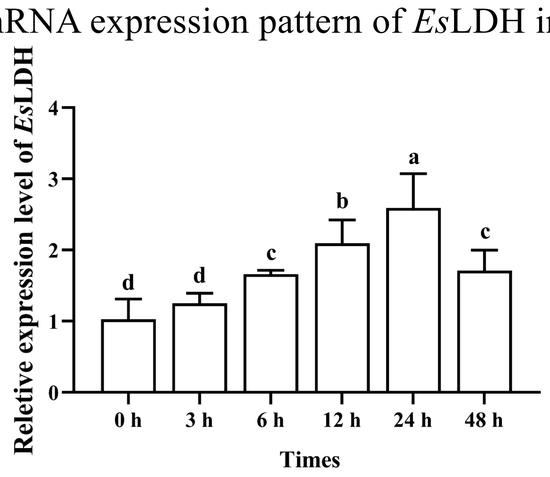

Fig. 1 The mRNA expression pattern of *Es*LDH in haemocytes under hypoxia stress after diffiterent times.

2. The histone lactylation (H3K18la) levels in haemocytes under hypoxia stress

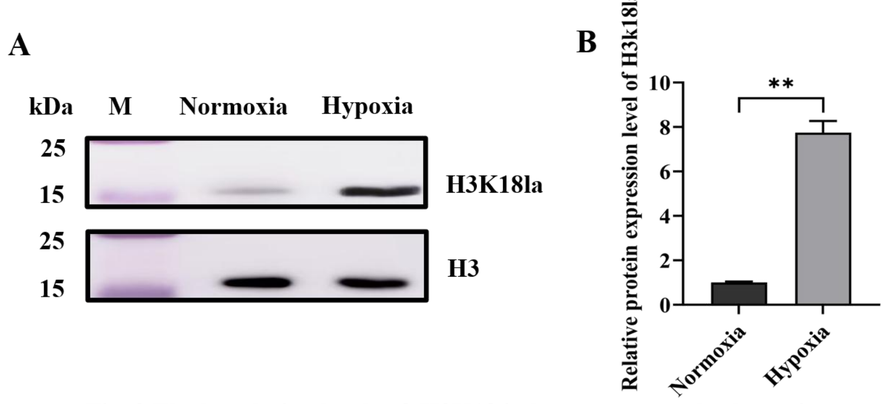

Fig. 2 The lactylation levels of H3K18 in haemocytes under hypoxia stress.

3. The mRNA expression levels of *Es*ALFs and *Es*HIF-1α after inhibiting or activating LDH under hypoxia stress

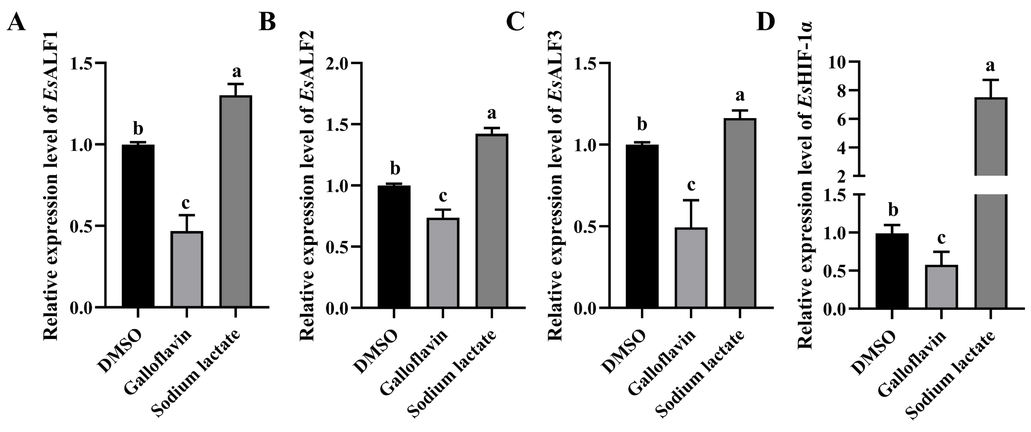

Fig. 3 The relative expression levels of *Es*ALFs and *Es*HIF-1α in haemocytes of Galloflavin (LDH inhibitor) and Sodium lactate (LDH activator) injected crabs post hypoxia treatment

4. The mRNA expression levels of *Es*ALFs and *Es*NF-κB after inhibiting HIF-1α crabs after Sodium lactate injection under hypoxia stress

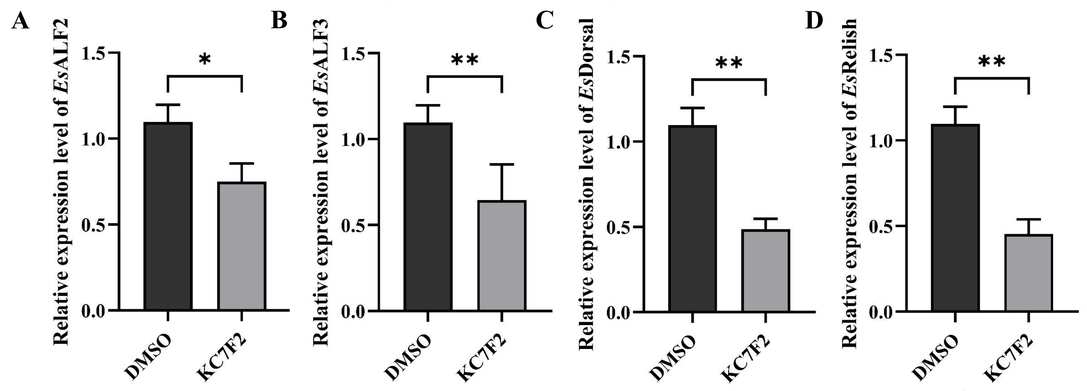

Fig. 4 The relative expression levels of *Es*ALFs and *Es*NF-κB in haemocytes of KC7F2 (HIF-1α inhibitor) injected crabs post hypoxia treatment crabs after Sodium lactate injection

5. The phosphorylation of NF-κB in HIF-1α inhibitor (KC7F2) injected crabs after Sodium lactate injection under hypoxia stress

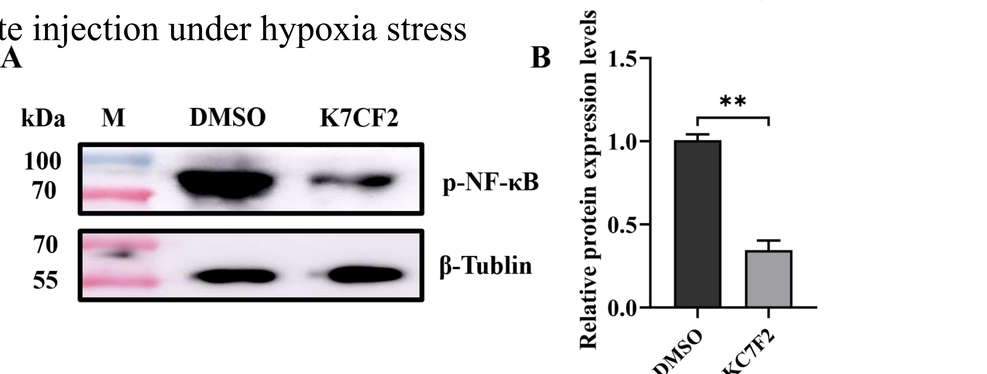

Fig. 5 The phosphorylation of NF-κB in haemocytes of HIF-1α inhibitor injected crabs after Sodium lactate injection post hypoxia treatment

6. The nuclear translocation of NF-κB in haemocytes of in HIF-1α inhibitor (KC7F2) injected crabs after Sodium lactate injection under hypoxia stress

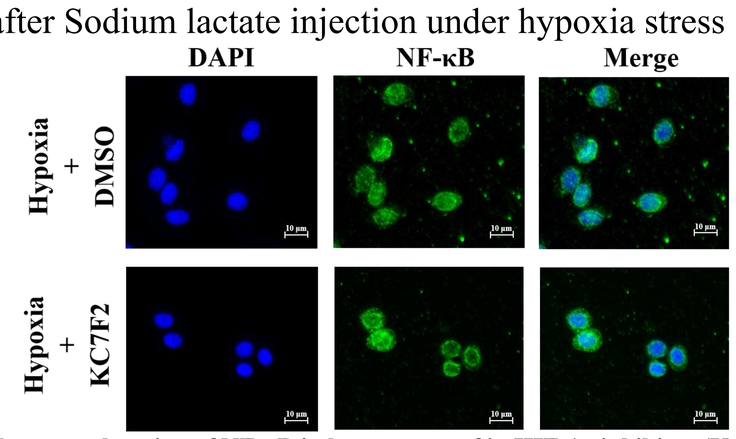

Fig. 6 The nuclear translocation of NF-κB in haemocytes of in HIF-1α inhibitor (KC7F2) injected crabs after Sodium lactate injection under hypoxia stress

Conclusion

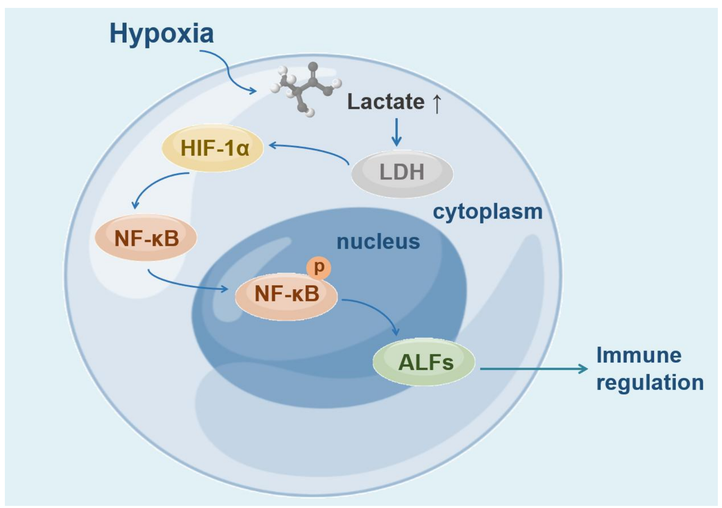

Fig. 7 Overview of the relationship among LDH, HIF-1α, NF-κB and ALFs in *E. sinensis*

- Under hypoxic stress, both LDH mRNA expression and histone lactylation levels were significantly elevated.
- Activation of LDH enhanced ALFs and HIF-1α expression, while inhibition of LDH downregulated the expression of ALFs and HIF-1α.
- Inhibition of HIF-1α downregulated the mRNA expression of ALFs and NF-κB.
- Inhibition of HIF-1α inhibited the phosphorylation of NF-κB and the nuclear translocation of NF-κB.

Hypoxia activated the HIF-1α–NF-κB axis through enhancing histone lactylation and LDH expression to promote the production of antimicrobial peptide.