The Caspase-8/Caspase-3/GSDME-mediated pyroptosis contributes to inflammation and antibacterial immunity in oysters

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ABSTRACT

Pyroptosis is a form of lytic programmed cell death mediated by the cleavage of gasdermins, which functions as an innate immune mechanism to facilitate host defense against invasive bacteria. In the present study, a gasdermin E (GSDME) was identified from the Pacific oyster *Crassostrea gigas* (defined as *Cg*GSDME) with a conserved N-terminal pyroptosis-triggering domain and a C-terminal repressor domain. There were four Caspase-3 cleavage sites in *Cg*GSDME sequence, which generated four N-terminal fragments (*Cg*GSDME-Ns). The obvious cleavage of *Cg*GSDME protein into fragments (*Cg*GSDME-N and *Cg*GSDME-C) in haemocytes and the swollen haemocytes with the presence of many vesicles were observed after *V. splendidus* stimulation. The binding of *Cg*GSDME/*Cg*Caspase-3 was evident in haemocytes pulled down by either *Cg*Caspase-3 or *Cg*GSDME immunoprecipitation after *V. splendidus* stimulation. When the activation of *Cg*Caspase-8 and *Cg*Caspase-3 was inhibited, the amount of *Cg*GSDME-N protein in haemocytes was reduced after *V. splendidus* stimulation. *Cg*Caspase-8, *Cg*Caspase-3 and *Cg*GSDME could induce the expressions and secretions of cytokines, as well as histological damage in gills. The recombinant *Cg*GSDME-N (r*Cg*GSDME-N) was able to bind multiple bacteria and assemble on the bacterial surface to generate pores. It displayed directly bactericidal activity and inhibited the growth of *V. splendidus* and *Staphylococcus aureus*. These results indicated that the cleavage of *Cg*GSDME by *Cg*Caspase-3 and *Cg*Caspase-8 not only mediated haemocyte pyroptosis and induced inflammation, and for the first time also showed direct bacteriostatic/bactericidal activity in the immune response of bivalve molluscs.

RESULTS

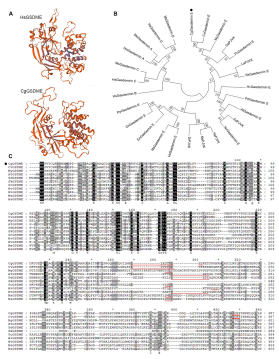

Fig.1 The molecular features of GSDM proteins from the Pacific oyster and other species.

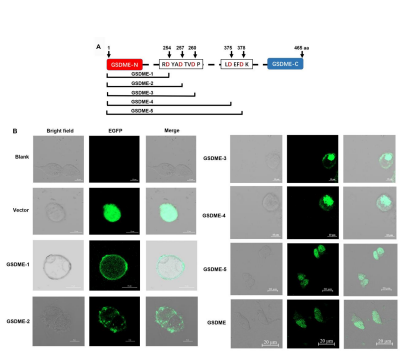

Fig.2 The subcellular location of *Cg*GSDME N-terminal domains in HEK293 cells.

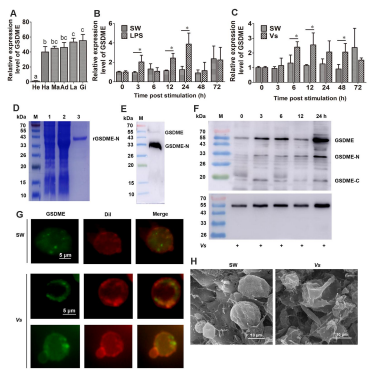

Fig.3 The spatiotemporal expressions of *Cg*GSDME, the haemocyte pyroptosis and *Cg*GSDME cleavage.

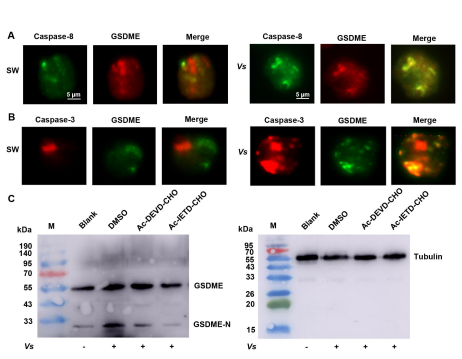

Fig. 4 The effects of *Cg*Caspase-8 and *Cg*Caspase-3 for *Cg*GSDME activation.

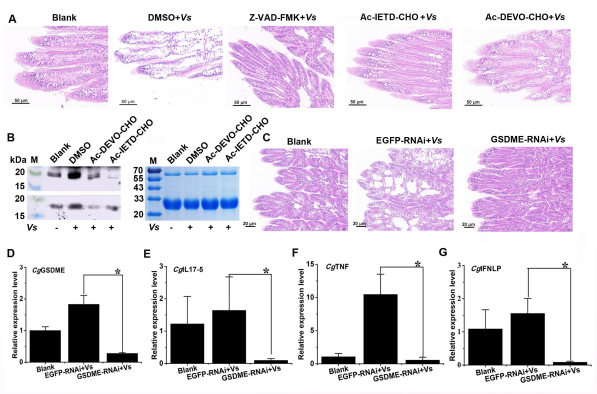

Fig.5 The effect of pyroptosis pathway on the inflammatory response and cytokine expressions.

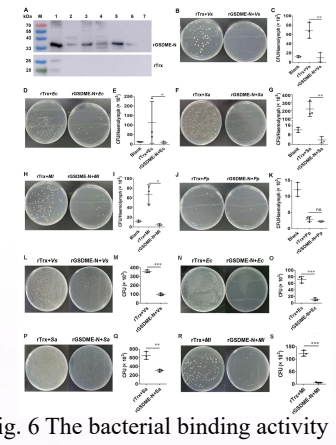

Fig. 6 The bacterial binding activity and bacteriostatic/bacteriostatic activity of r*Cg*GSDME-N.

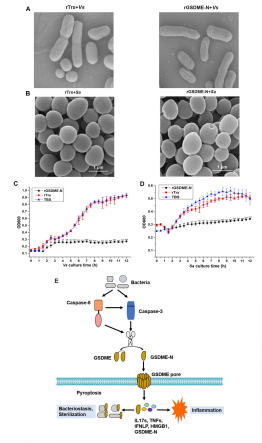

Fig. 7 The bacteriostatic activity of r*Cg*GSDME-N.

CONCLUSION

The pyroptosis signaling pathway was characterized in invertebrates for the first time. The GSDME homologue (*Cg*GSDME) identified in the Pacific oyster could be cleaved by *Cg*Caspase-8 and *Cg*Caspase-3 to induce pyroptosis of haemocytes. The generated N-terminal fragment of *Cg*GSDME displayed directly and broad bactericidal/bacteriostatic activity and induced the inflammatory responses .These results were helpful for understanding the evolution of GSDME-mediated pyroptosis pathway and its roles in immune responses of molluscs.