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Microbiomic and transcriptomic insight into the pathogenesis of meningitis-like disease in cultured *Pelophylax nigromaculatus*

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Abstract

In recent years, a deadly infectious disease named frog meningitis (or cataract and torticollis) raged the frog farms in China. Diseased frogs manifested torticollis, cataract, edema, and finally died. Yet, the molecular pathogenesis of this disease has not been studied. In this study, the etiologic factor of frog meningitis broken in a black-spotted frog (*Pelophylax nigromaculatus*) farm was investigated by comparative microbiomics (brain, eyeball, and intestine). And the pathogenesis of two major types of symptomatic frogs (individuals with both torticollis and cataract and individuals with only torticollis) were investigated by histopathological section and comparative transcriptomics of brain, liver, and muscle. Our results indicated that *Elizabethkingia* bacteria had increased proportions in the brain and eyeball microbiomes (but not intestine microbiomes) of both types of symptomatic frogs, and *E. miricola* was dominant in the eyeball and brain microbiomes of most torticollis-cataract frogs. In the torticollis-only individuals, vascular congestion and reduced hepatic fat can be detected in their leptomeninges vessels and liver, respectively. While in the torticollis-cataract ones, we observed additional leukocyte extravasation in their leptomeninges vessel, and neurologic damage in their brain and spiral cord. Transcriptional analyses suggested that frogs could be well divided into asymptomatic, torticollis-only, and torticollis-cataract groups by the variation of their brain transcriptomes. The upregulated brain genes in symptomatic frogs were mainly enriched in immune-related pathways. And the Toll-like receptor and NOD-like receptor signaling pathways, inflammation, and adaptive humoral immunity were activated in response to *E. miricola* infection. Torticollis-only and torticollis-cataract individuals differed in the level of immune gene expression remarkably, and the emerge of cataract symptom indicated the onset of strong innate immune and inflammatory responses. Overall, our results suggested that *E. miricola* was likely the etiologic factor of meningitis emerged in this farm. In regards to pathological stages, the infection progressed to leptomeninges invasion in torticollis-only individuals, while it had caused meningitis in torticollis-cataract ones. Correspondingly, the torticollis symptom was likely due to leptomeninges infection, and the cataract was likely a concomitant symptom of meningitis due to disruption of vascular permeability during excessive inflammation. Our results provided a first molecular insight into the pathogenesis of frog meningitis.

Keywords:

Cataract, Torticollis, *Elizabethkingia miricola*, Amphibian,Immune response

Introduction

Since 1993, the frequency of outbreak of epidemic meningitis-like disease (also called “frog torticollis” or “frog cataract") in frog farms have increased in China .Stricken species include *Lithobates catesbianus*, *L. grylio*, *Hopulobatrachus chinensis*, *Quasipaa spinosa*, *Q. boulengeri*, and *Pelophylax nigromaculatus*. This disease brings high lethality to frogs within several days (Lei et al., 2018; Li et al., 2016; Liu et al., 2018; Xie et al., 2009). Typical symptoms in diseased frogs included torticollis (a systematic symptom resembling hemiplegia), cataract (or proptosis and hyperemia), abdominal edema, lethargy, and anepithymia (Hu et al., 2017; Lei et al., 2019). Frog meningitis was caused by bacterial infection (Hu et al., 2017), and at least 10 bacterial species have been identified to be the etiology of this disease so far (Table 1). Some outbreak events were even caused by mixed bacterial infection (Yang, 2010).

Previous pathological investigations on frog meningitis focused on the identification of infected organs and tissue damage. Histopathologic examination showed severe meningitis with denatured and incrassated meninges in frogs with typical symptoms (Hu et al., 2017). Moreover, cell degeneration, inflammation, and lymphocyte infiltration can be detected in the liver, spleen, kidney, heart, brain, and muscle of diseased frog. Multiple organ failure and necrosis were the direct causation of individual death (Lei et al., 2018; Lei et al., 2019; Liu et al., 2018). However, the pathological stages of frog meningitis and host immune response after pathogen infection are still rarely concerned, especially at a molecular level. Indeed, gene expression variations, especially the transcriptional activation of immune related genes, can be useful indicators to distinguish the different pathological stages of this disease. And the combination of histopathological and transcriptional approaches could clarify the pathogenesis of this disease.

We observed two types of diseased *P. nigromaculatus* individuals in infected ponds. Some individuals exhibited combined symptoms of torticollis, cataract, inactivity, and different degree of edema (called torticollis-cataract/T + C group in this study). Other individuals manifested torticollis, but neither cataract nor edema (called torticollis-only/T group in this study) Torticollis-only and torticollis-cataract individuals always co-existed in ponds with high infection rate and severe mortality.

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