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**The study of the pathogenesis of the virus of the snake was studied based on the transcription group and the proteomics**

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**Abstract:**

**Background/Objective:** Equid herpesvirus 8 (EHV-8), one of the most important pathogens infecting donkeys and horses, mainly causes abortion, perinatal fetal death, pneumonia and encephalomyelitis in female donkeys. Currently, relatively few studies on EHV-8 have been conducted at home and abroad, and the specific pathogenic mechanism is still unclear, which has caused great trouble in the prevention and control of this disease.

**Methods:** In this study, we determined the pathogenicity of the EHV-8 strain and analyzed its pathogenic mechanism based on transcriptomics and proteomics.

**Results:**1. Pathogenicity study of EHV-8

To understand the pathogenicity of EHV-8, the EHV-8 LCDC01 strain isolated in our laboratory was inoculated with rabbit kidney cells (RK-13) and breast hamster kidney cells (BHK-21), and its pathogenicity in mice was investigated. The results showed that the LCDC01 isolate could form typical cytopathic lesions such as cell rounding, detachment, grapevine-like shape and apoptosis on RK-13 and BHK-21 cells. The mice showed no clinical signs except weight loss after the attack; histopathological sections showed inflammatory damage in the lungs, livers and brains, with the lungs being particularly damaged; the lung index of the mice reached its highest level (2.48%) at the 5th day after the infection; the mice were positive for EHV-8 nucleic acid in the lungs by PCR, and the mice were positive for EHV-8 nucleic acid in the lungs, livers, brains, and intestines by fluorescent quantitative PCR. ELISA results showed that the inflammatory factors Interleukin-β (IL-β), Interleukin-6 (IL-6), Interleukin-8 (IL-8) and Interferon-α (IFN-α) were detected in mouse lungs. (IFN-α) secretion was significantly increased (*P*<0.05).

2. Transcriptomic analysis of lung injury in EHV-8-attacked mice

RNA-seq analysis of mouse lung injury samples identified 3841 differentially expressed genes (DEGs), of which 1922 genes were up-regulated and 1919 genes were down-regulated. The results of GO enrichment analysis showed that the differentially expressed genes were mainly related to the activities of immune cells, including the biological processes of T-cell activation, neutrophil chemotaxis regulation, T-cell migration regulation, etc. The results of KEGG enrichment analysis showed that the differentially expressed genes were mainly related to the activities of immune cells, including the biological processes of T-cell activation, neutrophil chemotaxis regulation and T-cell migration regulation. Enrichment analysis showed that the differentially expressed genes were mainly enriched in the TNF signaling pathway, B cell receptor signaling pathway, NF-κB signaling pathway, cell adhesion asthma and other important biological pathways. Eight differential genes (*Mapk13*, *Hspb1*, *Tnfsf14*, *Hsp90aa1*, *Csf2*, *Cxcl12*, *Tnfaip3*, and *Syk*) were randomly screened according to the differential ploidy and validated by fluorescence quantitative PCR, of which seven genes, *Mapk13*, *Hspb1*, *Csf2*, *Hsp90aa1*, *Tnfsf14*, *Tnfaip3* and *Cxcl12* were consistent with the results of transcriptional analysis, proving the reliability of the RNA-Sep results.

3. Transcriptomic and proteomic analysis of EHV-8 infected RK-13 cells

The LCDC01 isolate was infected with RK-13 cells for 24 h and 48 h, respectively, and all the RNA and proteins in the cells were extracted for RNA-seq transcriptomics and TMT proteomics analyses, respectively. The results of the RNA-seq analyses showed that a total of 824 shared differentially expressed genes were identified in the 24 h-infected groups (R2) and the 48 h-infected groups (R3), of which a total of 1176 were up-regulated, 638 were co-regulated and 638 were up-regulated. 1176 genes were up-regulated and 638 genes were down-regulated.GO enrichment analysis showed that the shared differentially expressed factors were mainly involved in inflammatory response, cell migration, response to interleukin-1, etc.KEGG enrichment analysis showed that the shared differentially expressed factors were mainly associated with the TNF signaling pathway, axon regeneration, DNA replication and other pathways.

TMT analysis showed that a total of 1814 differentially expressed proteins were identified in the 24 h infection group (R2) and 48 h infection group (R3), and GO enrichment analysis showed that the co-expressed proteins in the two infection groups were mainly involved in the processes of DNA replication, inflammatory response, cell migration, etc. KEGG enrichment analysis showed that the co-expressed proteins were mainly enriched in pathways such as the TNF signaling pathway, DNA replication, and NF-κB pathway. KEGG enrichment analysis showed that the co-expressed proteins were mainly enriched in the TNF signaling pathway, DNA replication, NF-κB and other pathways.

4. Combining transcriptomics and proteomics approaches to explore the key pathways of EHV-8 infection

The TNF signaling pathway was found to play an important role in EHV-8 infection by transcriptomics and proteomic association analysis. Four key regulators (*TNF*-α, NFκB2, *Map3k8*, and *CXCL10*) on this pathway were validated, and all of them were significantly up-regulated and expressed during EHV-8 infection (*P*<0.05), which was consistent with the trend of association analysis.

**Conclusion:** In this study, the pathogenicity of EHV-8 was preliminarily determined by infecting susceptible cells and mice, and RNA-seq analysis was performed on injured lung samples from mice. To further investigate the pathogenic mechanism of the EHV-8 virus, transcriptomics combined with proteomics was utilized to investigate the expression of differential factors in RK-13 cells of EHV-8 infection, which provided data support for further investigation of the pathogenic mechanism of EHV-8.

**Keywords:** EHV-8; Transcriptomics; Proteomics; Correlation analysis