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著者	QIN Weiyun, WANG Haifei, WU Zhengchang, WU Shenglong, BAO Wenbin
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Weiyun QIN, Haifei WANG, Zhengchang WU, Shenglong WU and Wenbin BAO

College of Animal Science and Technology, Yangzhou University

Intestinal barrier damage is one of the important factors in leading to diarrhea and intestinal inflammation in piglets. In order to screen candidate lncRNAs involved in PEDV infection through intestinal barrier, a total of 43 Duroc×Landrace×Yorkshire 8-day-old ternary crossbred pigs were used in this study, which included 28 diarrhea piglets and 15 normal piglets. Firstly, the pathogeny was identified by RT-PCR, and then the intestinal barrier function was evaluated by D-lactic acid- and DAO ELISA, HE and AB-PAS staining, scanning electron microscopy and transmission electron microscopy observation. Based on this, lncRNA-seq was performed using the confirmed phenotype of diarrhea piglets. 112 differentially expression lncRNAs were identified and we locked XR_002344446.1. lncRNA expression increased in intestinal mucosa after PEDV infection and mainly localized in the cytoplasm by FISH assays. Functionally, knockdown of lncRNA using siRNA in IPEC-J2 cells significantly increased their susceptibility to PEDV infection, Furthermore, we observed that lncRNA interference decreased the intestinal mucosal permeability and destroyed the connection of tight junction. Mechanistically, by using biotinylated-lncRNA probe to perform RNA pull-down assay in IPEC-J2 cells, we identified cytoskeletal proteins was abundantly pulled down by lncRNA in IPEC-J2 cells. In conclusion, we speculated lncRNA regulates tight junction through binding cytoskeletal proteins, the connection between lncRNA and cytoskeleton proteins was broken when PEDV enters the intestine, resulted in the disruption of the tight junctional distribution of ZO1 to the intracellular localization.