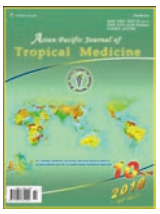

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Pig IFITMs are restriction factors for Japanese encephalitis virus

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ABSTRACT **Objective:** Japanese encephalitis virus (JEV) is responsible for one of the most serious epidemics of encephalitis in the world. JEV uses pigs as its main hosts and spreads among vertebrates and humans mediated by *Culex* mosquitoes. The prevention and control of JEV spread in pigs is one of the most effective measures to protect global public health. Interferon-inducible transmembrane proteins (IFITMs) are small membrane-spanning proteins that were identified as innate antiviral factors against multiple pathogenic viruses, especially enveloped viral pathogens. This study aims to verify whether pig interferon-inducible transmembrane proteins (pIFITMs) inhibit JEV and investigate the related molecular mechanisms of anti-JEV. **Methods:** Transient expression and RNA interference technology were used to overexpress and silence *IFITMs* gene. Three different cell lines, PK15, HEK293 and Huh7, were transfected with recombinant pIFITMs-expressing plasmids. The lentiviral vectors harboring RNAi sequences targeting pIFITMs were introduced into PK15 cells. Quantitative real-time PCR was used to determine the antiviral activities of pIFITMs through measuring and analyzing the virus copy number of JEV (SA14-14-2 strain) in the supernant of pIFITMs overexpression or silencing cells 48 hours post transfection. The expression of the related proteins was examined by western blot. The fusion vectors inserted with pig IFITM1-EGFP were constructed and introduced into three different cell lines respectively. Then Laser Co-focus light microscopy was used to observe the subcellular localization. The key active amino acids of pIFITM1 were analyzed by investigating the anti-JEV effect of the cysteine mutants produced with PCR site directed mutagenesistechnology. **Results:** In three different cell lines, PK15, HEK293 and Huh7, all of three pig IFITM proteins, pIFITM1, pIFITM2, and pIFITM3 could inhibit the replication of JEV whether through transient gene over-expression methods or RNA interference silencing. And that, among three pig IFITMs, pIFITM1 showed the strongest anti-JEV effect. The anti-JEV activity of pIFITM1 manifested at the early entry stage. In PK15, BHK21, and HEK293 cells, before virus infection, pIFITM1 was located in the plasma membrane area, and after infection, transferred to the membranous structures outside the nucleus. The S-palmitoylated cysteines at position 50, 51 and 84 of pIFITM1 had significant effect on virus replication. **Conclusions:** Pig interferon-inducible transmembrane proteins are restriction factors for JEV infection and have potentials in the prevention of virus spread. Our results provide some new sights into understanding the antiviral activity of pig IFITMs.

Keywords: Japanese encephalitis virus; pIFITMs; Anti-JEV; Subcellular localization

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