Journal of Microbiology, Immunology and Infection (2017) xx, 1–9

Available online at www.sciencedirect.com

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Original Article

Anti-apoptotic activity of Japanese encephalitis virus NS5 protein in human medulloblastoma cells treated with interferon-β

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Received 9 August 2016; received in revised form 3 January 2017; accepted 25 January 2017
Available online ■ ■ ■

KEYWORDS
Japanese encephalitis virus;
NS5;
Interferon;
Anti-apoptosis;
Hsp70

Abstract *Background:* Japanese encephalitis virus (JEV) non-structural protein 5 (NS5) exhibits type I interferon (IFN) antagonists, contributing to immune escape, and even inducing viral anti-apoptosis. This study investigated the anti-apoptotic mechanism of JEV NS5 protein on type I IFN-induced apoptosis of human medulloblastoma cells.
Methods: Vector control and NS5-expressing cells were treated with IFN-β, and then harvested for analyzing apoptotic pathways with flow cytometry, Western blotting, subcellular localization, etc.
Results: Annexin V-FITC/PI staining indicated that IFN-β triggered apoptosis of human medulloblastoma cells, but JEV NS5 protein significantly inhibited IFN-β-induced apoptosis. Phage display technology and co-immunoprecipitation assay identified the anti-apoptotic protein Hsp70 as a NS5-interacting protein. In addition, Western blotting demonstrated that NS5 protein up-regulated the Hsp70 expression, and reduced IFN-β-induced phosphorylation of ERK2, p38 MAPK and STAT1. Hsp70 down-regulation by quercetin significantly recovered IFN-β-

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http://dx.doi.org/10.1016/j.jmii.2017.01.005

induced apoptosis of NS5-expressing cells, correlating with the increase in the phosphorylation of ERK2, p38 MAPK, and STAT1. Inhibiting the ATPase activity of Hsp70 by VER-155008 resulted in the elevated IFN-β-induced apoptosis in vector control and NS5-expressing cells.
Conclusions: The results indicated Hsp70 up-regulation by JEV NS5 not only involved in type I IFN antagonism, but also responded to the anti-apoptotic action of JEV NS5 protein through the blocking IFN-β-induced p38 MAPK/STAT1-mediated apoptosis.

Introduction

Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus in the Flaviviridae family. Like other mosquito-borne flaviviruses, such as dengue (DEN), yellow fever (YF), St. Louis encephalitis, and West Nile (WNV), JEV is a life-threatening pathogen causing acute flaccid paralysis, meningitis and encephalitis.[1] JEV particle widely appears within the nervous system, including thalamus, basal ganglia, brainstem, cerebellum, cerebral cortex and spinal cord.[2] Especially, JEV infects the basal ganglia and thalamus in 71% patients as well as the brainstem in 43% patients, which are responsible for the movement disorders, acute respiratory failure, and even death.[2] Japanese encephalitis (JE) with a high fatality rate of 30% occurs in East and Southeast Asia, and Northern Australia.[1] Remarkably, estimated 30,000 to 50,000 JE cases with 10,000−15,000 deaths are reported annually in Asian countries.[3]

Flavivirus contains a plus-sense, single strand RNA genome with one open reading frame encoding a large polyprotein. Viral polyprotein is cleaved by viral and cellular proteases, and then divided into three structural proteins (capsid (C), membrane (prM/M), and envelope (E)) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5).[4] Interestingly, several NS proteins like NS2A, NS4A, NS4B and NS5 show type I interferon (IFN) antagonistic activity through blocking JAK-STAT signaling.[5–8] In IFN-stimulated JAK-STAT signaling pathways, JEV NS5 suppresses nuclear translocation and tyrosine phosphorylation of SATA1; WNV NS5 protein prevents phosphorylated STAT1 accumulation; DEN NS5 inhibits phosphorylation of STAT2. Interaction of PDZ protein scribble (hScrib) with tick-borne encephalitis virus (TBEV) NS5 is associated with type I IFN antagonism.[7,9,10] A recent report indicates type I interferon antagonism by JEV NS5 as the inhibition of Ca^{2+}/calreticulin/calcineurin in STAT1-mediated signaling.[11]

JEV-induced neuronal apoptosis and inflammation are responsible for JE pathogenesis. However, JEV could be isolated from the cerebrospinal fluid in JE cases more 3 weeks after occurring encephalitis symptoms.[12] JEV persistence is also detected in peripheral mononuclear cells in infected children several months after acute infection.[13] Type IFN antagonism has been considered as the essential role in the establishment of viral persistence.[14] Since JEV NS5 protein showed type I IFN antagonistic ability, thus this study intends to the anti-apoptotic role of JEV NS5 in IFNβ-induced apoptosis of TE671 human medulloblastoma cells. Heat shock protein 70 (Hsp70) was identified as a JEV NS5-interacting protein using phage display technology; the binding interaction between Hsp70 and NS5 was confirmed by immunoprecipitation assay. In particular, Hsp70 was up-regulated in NS5-expressing cells compared to mock cells, in which involved in anti-apoptotic mechanism of JEV NS5 on IFN-induced apoptosis. Quercetin, reducing the Hsp70 expression, significantly augmented IFNβ-induced apoptosis in human medulloblastoma cells via the activation of p38 MAPK and STAT1.

Materials and methods

Cells

Human medulloblastoma TE671 cells were grown in the minimum essential medium (MEM) with 2 mM L-glutamine, 1 mM sodium pyruvate and 10% fetal bovine serum. Stably-transfected TE671 cell lines containing the pCR3.1 vector or JEV NS5 recombinant plasmid were generated in our previous report,[11] and cultured in MEM plus 2 mM L-glutamine, 1 mM sodium pyruvate, 10% fetal bovine serum (FBS), and 800 μg/ml G418.

Apoptosis assay with flow cytometry

Stably-transfected cell lines were treated with 1000 U/ml IFN-β, photographed under microscope 24 and 48 post treatment, and then harvested for apoptosis analysis with the Annexin V-fluorescein isothiocyanate (FITC) apoptosis Detection Kit (BioVision, Milpitas, CA, USA). Stained cells (at least 10,000 cells per sample) were quantitated by flow cytometry with an excitation wavelength of 488 nm and the emission wavelengths at 620 nm for propidium iodide (PI) and 530 nm for FITC, respectively.[15]

Western blotting analysis of protein expression and phosphorylation

For testing expression and phosphorylation levels of indicated proteins, stably-transfected cell lines were treated with 0, 250, 500, or 1000 U/ml IFN-β (Merck-Serono, Darmstadt, Germany) for 30 min, 60 min, or 24 h, and then harvested for Western blotting with anti-caspase 3, anti-phospho-STAT1 (Tyr701), STAT1, anti-phospho-ERK1/2, anti-phospho-p38 MAPK (Thr180/Tyr182), anti-Hsp70, and anti-β actin Abs (Cell Signaling, Danvers, MA, USA). The

Please cite this article in press as: Weng J-R, et al., Anti-apoptotic activity of Japanese encephalitis virus NS5 protein in human medulloblastoma cells treated with interferon-β, Journal of Microbiology, Immunology and Infection (2017), http://dx.doi.org/10.1016/j.jmii.2017.01.005