The Inhibition of EV71 Virus-Induced Apoptosis by ZVAD Through ROS and mTOR Pathway

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Abstract

Background: Emerging evidence that Enterovirus 71 (EV71) infection closely related to infection with the generation of apoptosis. Excess apoptosis exacerbates inflammatory reactions and can even result in the death of the cell. The ZVAD is a broad caspase inhibitor, can prevent apoptosis and regulate the survival in many different cell types.

Methods: The aims of this project were to elucidate the mechanism of the ZVAD in anti-EV71 virus.

Results: Our data showed that reduced viral replication was observed after treated with ZVAD in EV71 infected Vero and SK-N-SH cells. Meanwhile, Vero and SK-N-SH cells infected with EV71virus were both exhibit higher cells viability in the presence of ZVAD. Additionally, ZVAD can decrease the apoptosis, ROS and inflammation induced by EV71 in the infected Vero cells. However, ZVAD may play an antiviral role in SK-N-SH cells through the mTOR signaling pathway

Conclusions: In addition to inhibiting caspase, ZVAD also inhibits apoptosis by regulating ROS and inflammation cytokines to achieve antiviral effect.

Full Text

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Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

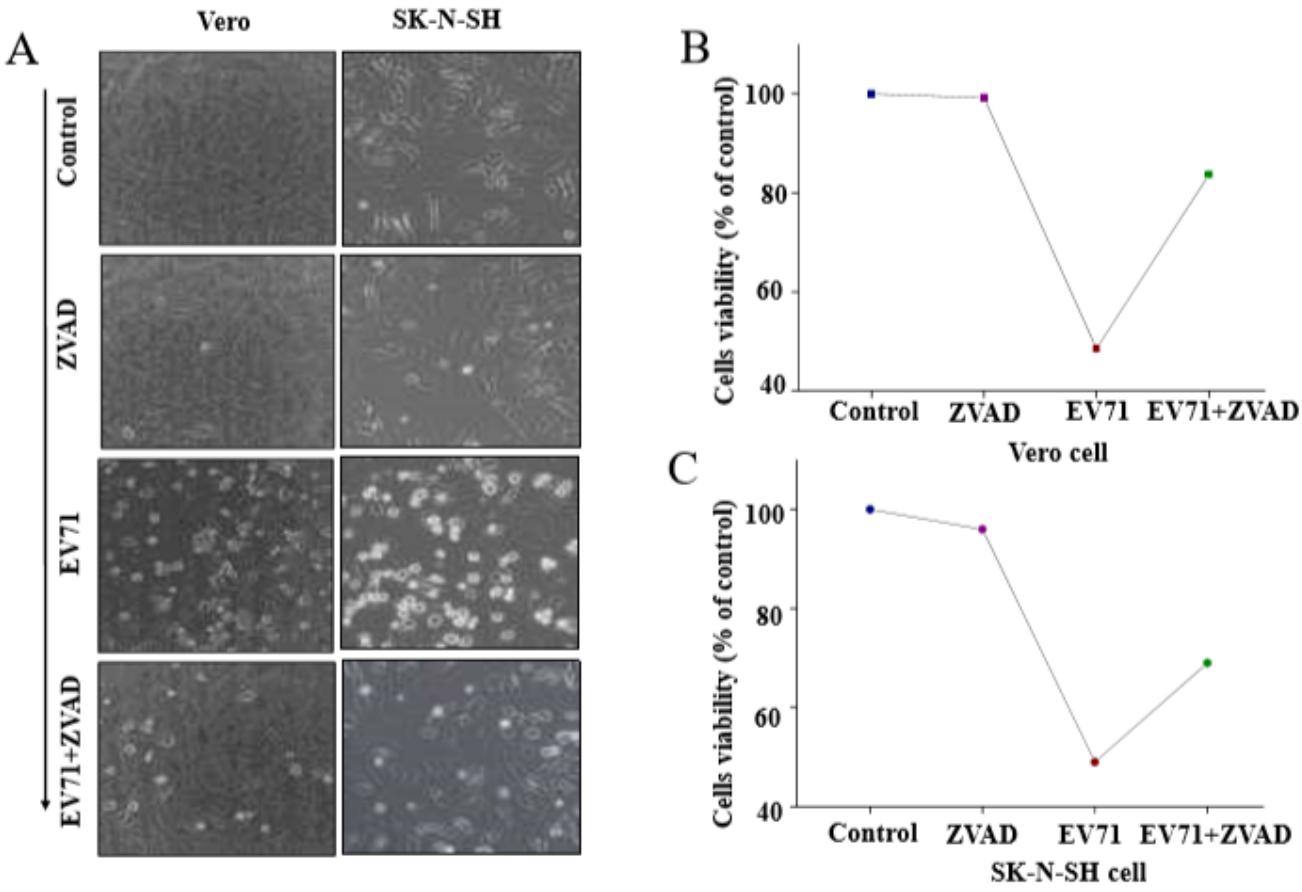

Figure 1

Cells viability with ZVAD. (A) ZVAD reduced the EV71-induced CPE in Vero and SKN-SH cells respectively. Cells were examined using a microscopy (×20). (B) The CCK8 assay of ZVAD treated EV71 infected Vero cell. (C) The CCK8 assay of ZVAD treated EV71 infected SKN-SH cell. The concentration of ZVAD were 25 μmol/L. Control, Vero /SK-N-SH cells without any treatment; ZVAD, Vero/SK-N-SH cells treated with ZVAD; EV71, Vero/SK-N-SH cells infected with EV71; EV71+ZVAD, EV71 infected Vero/SK-N-SH cells treated with ZVAD.

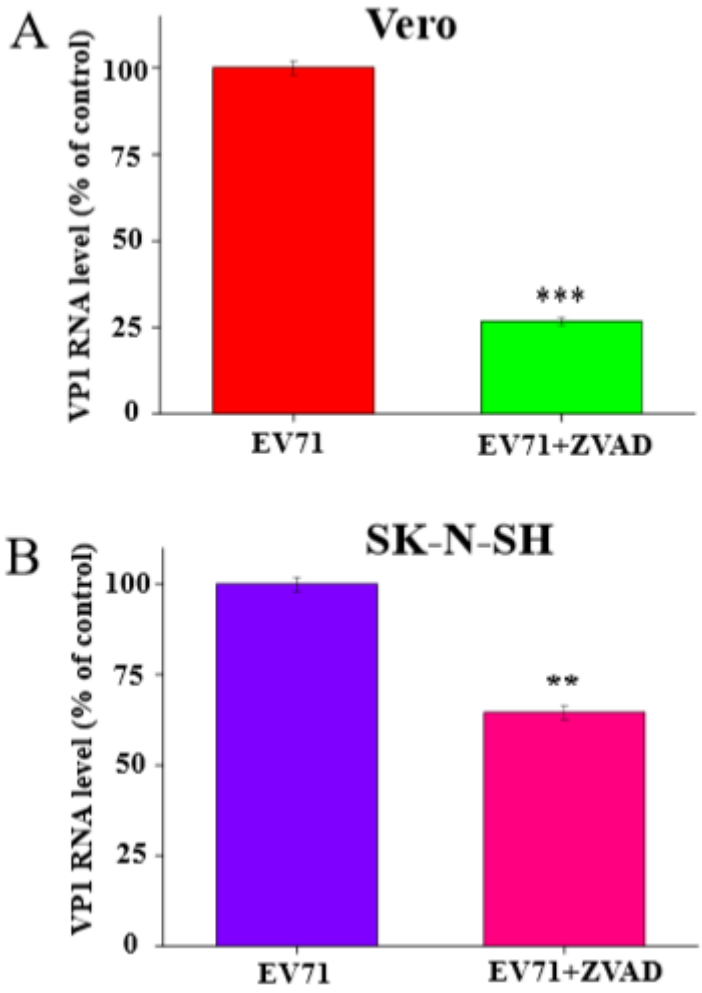

Figure 2

The antiviral effect of ZVAD against EV71 in Vero and SK-N-SH cells. EV71-VP1 RNA were analysis by QRT-PCR. By comparison with the control group (without infection), the EV71VP1 RNA were both decreases in EV71 infected Vero cells (A) and SK-N-SH cells (B). ***P < 0.001, **P < 0.01, *P < 0.05, compared with the EV71 infected model. EV71, enterovirus 71, QRT-PCR, quantitative real-time polymerase chain reaction.

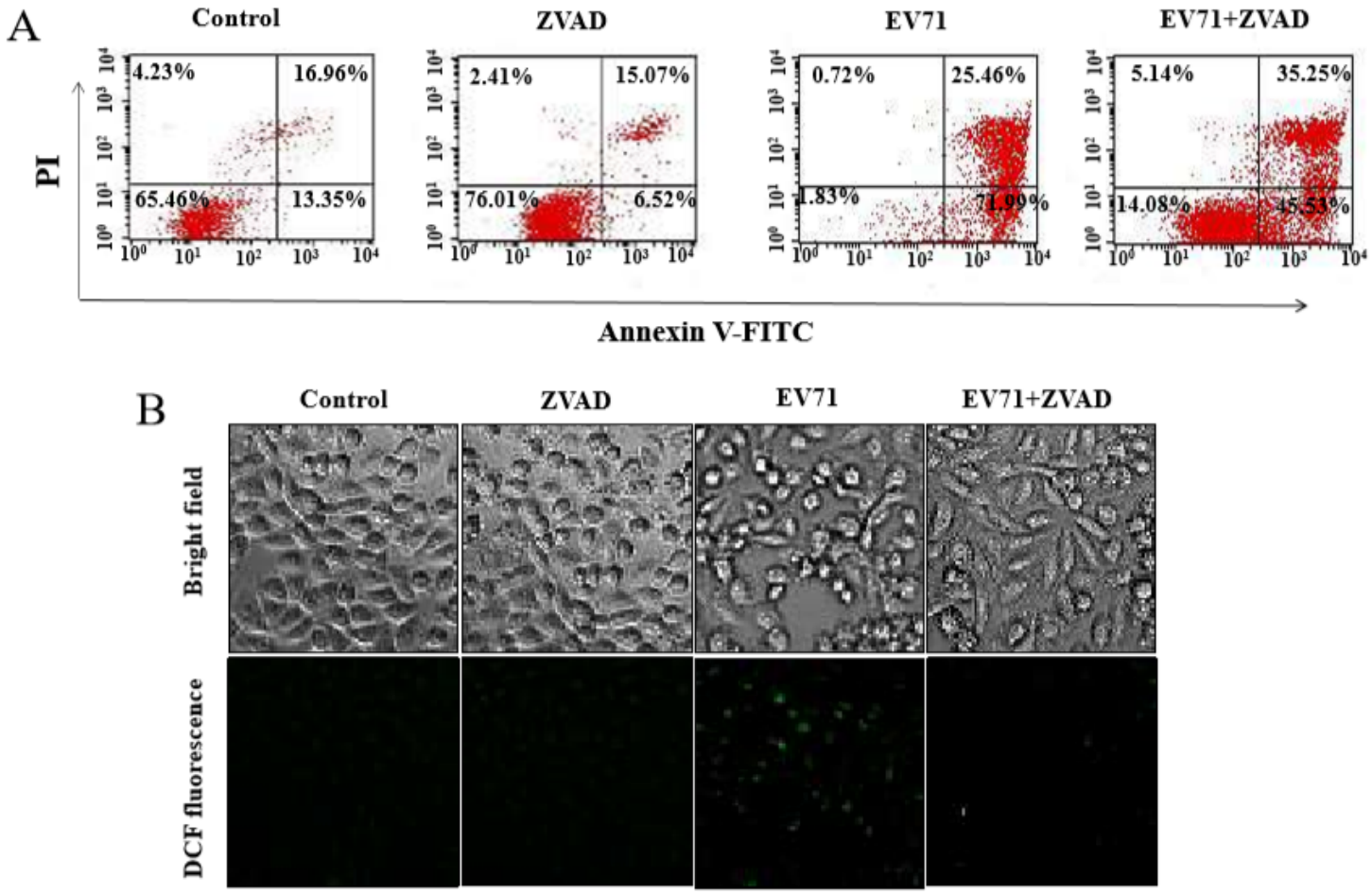

Figure 3

ZVAD suppressant the apoptosis induced by EV71 in Vero cells. (A) Annexin V-FITC and PI flow cytometry was performed to detect the the number of apoptosis in EV71-infected Vero cells. The upper right quadrant of every plot represents late dead cells. (B) DCF fluorescence intensity assay was conducted to investigate the production of ROS restrain by ZVAD in EV71infected Vero cells, compared to non-infected normal cells (Control). Control, Vero cells without any treatment; ZVAD, Vero cells treated with ZVAD; EV71, Vero cells infected with EV71; EV71+ZVAD, EV71 infected Vero cells treated with ZVAD. EV71, enterovirus 71; ROS, reactive oxygen species; DCF, Dichlorofluorescein;

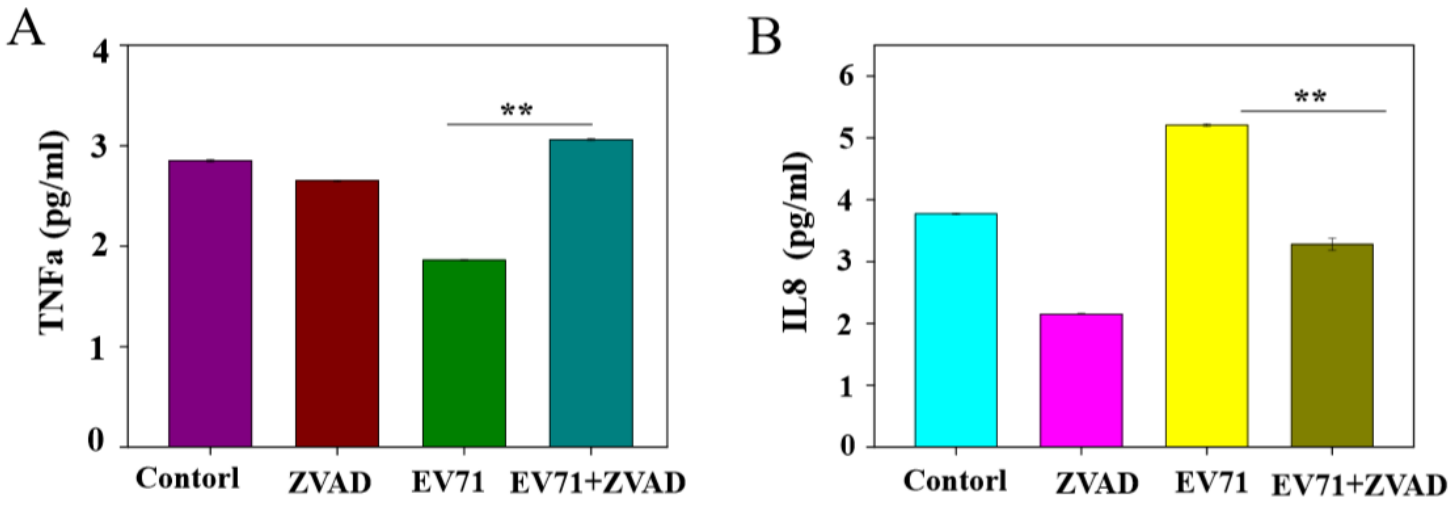

Figure 4

Flow cytometry assay was done to study the expression level of the inflammatory cytokines. (A) ZVAD increased the expression of TNFa in EV71 infected Vero cells. (B) ZVAD decreased the expression of IL8 in EV71 infected Vero cells. ***P <0 .001, **P < .01, *P < .05, compared with the EV71 infected model. Control, Vero cells without any treatment; ZVAD, Vero cells treated with ZVAD; EV71, Vero cells infected with EV71; EV71+ZVAD, EV71 infected Vero cells treated with ZVAD.

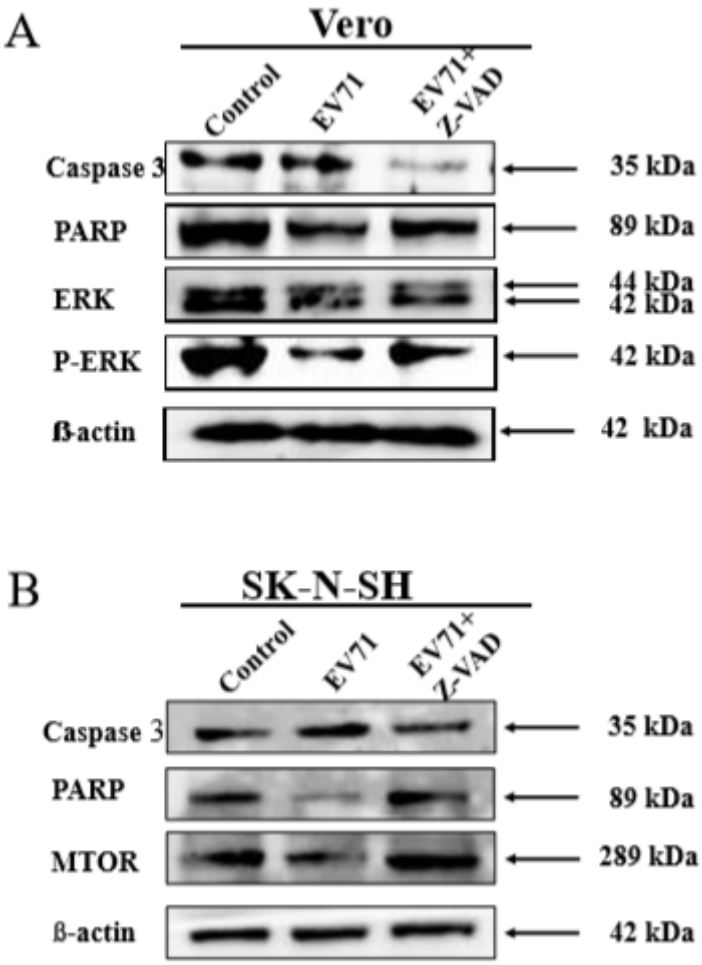

Figure 5

ZVAD increased the phosphorylation of ERK and the expression of mTOR in Vero cells (A) and in SK-N-SH cells (B). Vero and SK-N-SH cells were plated into 10 cm dish respectively. Cells were uninfected or infected with 100TCID50 EV71 for 2 h. The cells were then treated with ZVAD for 48 h. Then cells were harvested and proteins were examined by western blot. Control, Vero /SK-N-SH cells without any treatment; EV71, Vero/SK-N-SH cells infected with EV71; EV71+ZVAD, EV71 infected Vero/SK-N-SH cells treated with ZVAD.

Supplementary Files

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- Table1.pdf