Original Article

Evaluation of Beclin1 Effect on Apoptosis in Mouse Infected with Street Rabies Virus

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Abstract

Background: Apoptosis is a programmed cell death in which certain cellular components are packed into small membrane vacuoles by immune cells. Different strains of rabies virus (RABV) have their own biological features, but their effects on apoptosis have been little known. The aim of current research was to evaluate Beclin 1 effect on apoptosis in the mouse infected with the street rabies virus.

Materials and Methods: Exogenous Beclin1 overexpressed by the pIRES2-EGFP-Beclin1 vector in the cortex of NMRI mice. To evaluate the apoptosis, TUNEL assay was done on brain tissues of the rabid mice.

Results: TUNEL assay data showed that small apoptotic cells were seen in the four groups that received the vector alone or with the SRABV, but no significant changes were observed. There are no signs of apoptosis in mouse normal brain cells.

Conclusion: It was previously proven that overexpression of exogenous Beclin1 could induce autophagy but this study showed that overexpression of Beclin 1 does not cause apoptosis in rabies-infected cells.

Keywords: Apoptosis, Belin1 overexpression, Autophagy, Street rabies virus

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Introduction

Rabies virus (RABV) causes lethal encephalitis in mammals and poses a serious Public health threat in many parts of the world. Despite the availability of effective vaccines and possible protection by postexposure treatment, more than 60,000 humans die by rabies worldwide every year, with more than 15 million receiving postexposure prophylaxis. Rabies virus (RABV), has high neurotropism and belongs to the genus *Lyssavirus* in the family *Rhabdoviridae* (1). The genome of the RABV is a single negative-stranded RNA of approximately 12 kb and encodes five structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and large protein (L) (2). The only approved strategy for combating rabies virus (RABV) is vaccination by dead RABV in conjugation with immunoglobulins against RABV (3). Different strategies have been recruited for developing drugs or increasing the efficacy of the vaccination, such as vaccination by live genetically modified RABV to induce long-lasting immune protection (4) and employing monoclonal antibodies for controlling rabies (5).

The studies have shown that the pathogenicity of rabies

virus is inversely related to apoptosis and is affected by viral proteins N, M, and G (6). High levels of rabies virus glycoprotein have been shown to be a key factor in inducing apoptosis (7). Induction of apoptosis during rabies virus infection can be either caspase-dependent or caspase-independent (8). Apoptosis, as a type of cell death, is an active and energy-dependent level (9). Many viruses produce special products to control this biological process. In contrast, the immune system uses this pathway to fight many pathogens, including viruses.

Since mitochondria are the major crossroads for the integration of proapoptotic and anti-apoptotic signals, it has been reported that various viruses regulate cellular apoptosis at the mitochondrial level. Bax multi-domain protein proapoptosis plays a critical role in inducing mitochondrial apoptosis. It has been documented that the CVS strain induces Bax gene expression (10).

Beclin1 (Atg6/ /Vps30) is a well-known key regulator of apoptosis that belongs to the Class III phosphatidylinositol 3-kinase complex. It is necessary for the construction of a lipidic signal molecule (PI(3)P) on the phagophore membrane and autophagosome formation (11, 12). It implicated in numerous biological processes, including adaptation to stress, endocytosis, cytokinesis, immunity, tumorigenesis, aging, and cell death (13, 14). Acetylated Beclin1, inhibits autophagosome formation and result in a deficiency in macroautophagy in sporadic Alzheimer's disease (15). It was previously proven that overexpression of exogenous Beclin1 could induce autophagy. According to the contents, the aim of the study was to evaluate the Beclin1 effect on apoptosis in the mouse infected with the street rabies virus.

Methods

Ethical issues

Animals were maintained according to Pasteur institute of Iran guidelines for animals care and control. Animals were kept in the separate cages in a room with controlled temperature, light period (12 hours light/dark cycle), humidity (40-60%), and free access to food and water throughout the study. The Institutional Animal Ethics Committee in Pasteur Institute of Iran approved all experimental protocols of the present study (Certificate No: IR.PII.REC.1395.49).

Grouping

The male, six-weeks-old NMRI mice weighing 25-30g were purchased from the Pasteur institute of Iran and randomly divided into 3 groups. After the anesthetics of the NMRI mice with the combination of ketamine (100 mg/kg) and Xylene (10 mg/kg), the cannula was inserted into the cortex of the brain using a stereotaxic device (Figure 1). In each group, 30 LD_{50} of viruses were injected into each mouse intra-cranially. Group one (control group) received no treatment just saline. The second and third group received vector one hour post virus inoculation. 3µg of vectors (pIRES2-EGFP or pIRES2-EGFP-Beclin) were injected into the brains of mice using Poly plus transfection kit (poly plus, France) (16-18).

TUNEL assay

For histological analysis, after the anesthetics of the mice with the combination of ketamine (100 mg/kg) and Xylene (10 mg/kg), transcardial perfusion was performed with 10% paraformaldehyde and brain samples were placed in 10% formalin for 72 hours. After Paraffin-embedded specimens' production, samples were cut into 4 µm sections. Moreover, a positive (mouse brain's cancer cell) and a negative control (mouse brain's normal cell) were probed with TUNEL assay. The procedure was performed according to the manufacturer's protocol (TUNEL Assay Kit-HRP-DAB (ab206386, Abcam). Counterstaining was done by methyl green for morphological evaluation and characterization of normal and apoptotic cells.

Results

Apoptosis in pIRES-EGFP-Beclin1 treated mice

TUNEL assay was carried out 72 hpi to appraise the apoptosis in five groups. Slides were prepared from paraffin-embedded specimens' in each group of mice and at least 5 fields of each slide were tested for investigation of apoptotic signs. All images are taken at 40 magnification. Mice that had received only the empty vector (Fig. 2A) and the Beclin 1 containing vector (Fig.2 B) showed very little apoptotic bodies. In the groups that received SRABV (Fig.2 C) or SRAVB and pIRES2-EGFP-Beclin1 vector (Fig.2 D), a slight apoptosis was seen but no significant differences were observed comparing with the positive control (Fig.2 F).

Figure 1. Street rabies virus detection with UV microscopy. A) Left: Normal BSR cells Right: BSR cells' infected with SRABV. B) SRABV *in vivo* inoculation. The sample prepared from the brain tissue of rabid mice after 24hour and stained with anti-rabies nucleocapsid FITC-labeled antibody. Left: Cannulation using a stereotaxic device in the cortical part of the mouse brain. Right: Mice one day after Cannulation.

There are no signs of apoptosis in normal brain cells and negative control (Fig.2 E & G).

Discussion

Rabies is one of the zoonotic diseases, which, after clinical symptoms appear, are not treated. Today, rabies is still leads to death in the world. For researchers, discovered a treatment for human rabies remains a problem. Autophagy involves the various pathways that cells utilize to render cytoplasmic components to lysosomes for demolition (19). Rabies phosphoprotein binds to the BECN1 gene and RABV replication stimulates by induction of BECN1signaling pathway, which is related to incomplete autophagy. That process would be a potential target for antiviral drugs against RABV (20). By reducing the complications of the rabies disease, some scientists suggest further study on the mechanism of action and side effects of the virus in cells. It can be said that not much information is available about the SRABV. SRABV behaviors are very different from challenge RABV or vaccinal strains' of RABV.

The pathogenicity of RABV strains depend on the glycoprotein. The glycoprotein gene expression have been correlated with apoptosis reversely. Glycoprotein gene expression inhibits the proapoptotic genes (21) then infected cells are not destroyed. It enables the virus to remain in host cell's neurons .Prabhavathy (22) compared the ability of apoptosis induction in the Indian SRABV and challenge RABV in murine neuroblastoma cell line. He found that SRABV glycoprotein gene expression was low and proapoptotic genes' expression were high. Some scientists suggested that the pathogenesis of a fixed strain of the rabies virus (CVS-11) in mice begins with induction of apoptosis with the RABV replication (23). Taji et al. (24) demonstrated that the Beclin1 overexpression in MDCK cells diminishes telomerase activity and increased apoptosis. The results of our experiments were in line with the results of other researchers. We performed a TUNEL assay to observe the effect of pIRES2-EGFP-Beclin1 and pIRES2-EGFP used

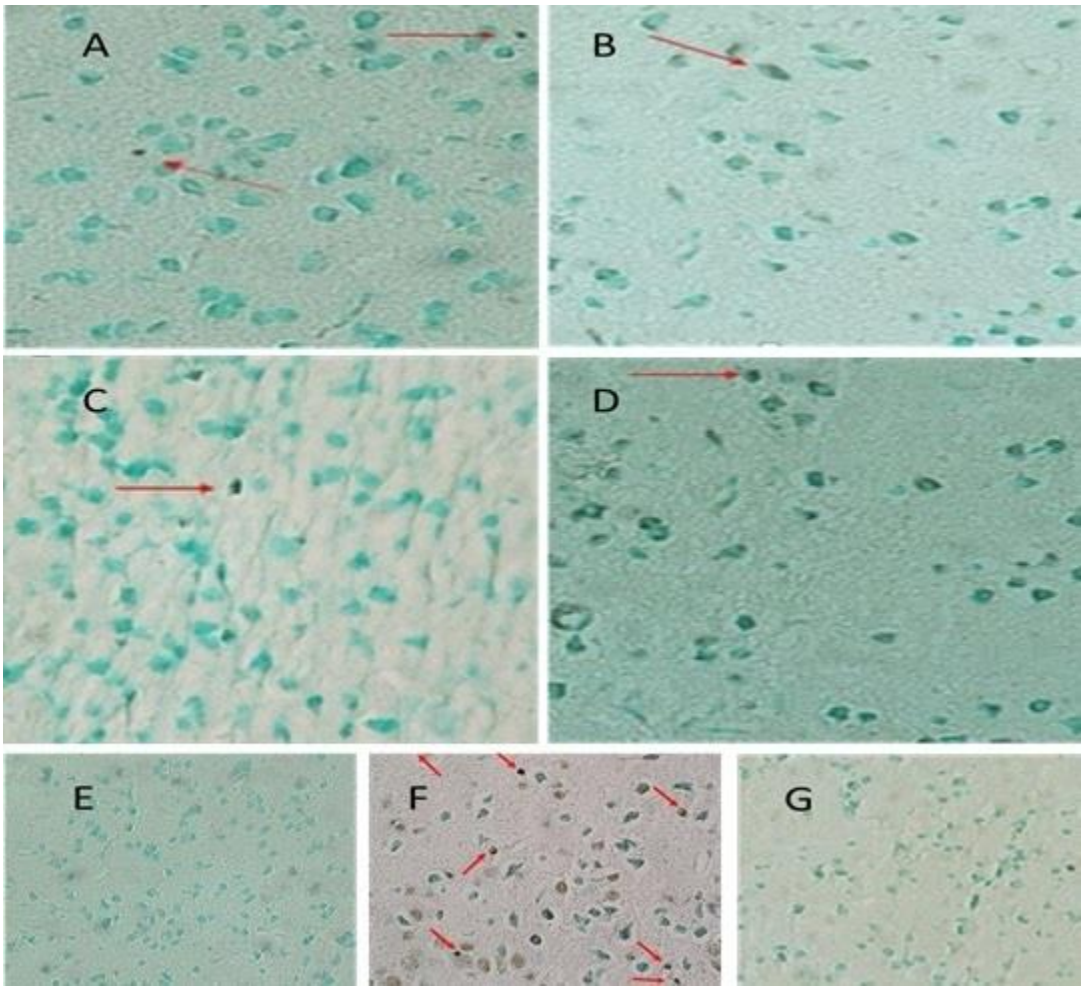

Figure 2. TUNEL Assay results in experimental groups. A) Mouse brain tissue transfected with empty vector, B) mouse brain tissue transfected with *Beclin1* vector, C) RABV inoculated mouse brain, D) RABV inoculated mouse brain, and treated with *Beclin1* vector, E) negative control, F) positive control, G) Normal brain tissue. Five fields per each section were used for determination of the number of TUNEL-positive cells.

vectors on the apoptosis in brain cells of mice. Our findings did not show any significant increase in the amounts of apoptosis in all groups. That is, in the brain cells of mice, none of the vectors or SRABV induced apoptosis. Data reveal important points about the behavior of SRBV in apoptosis in a mouse model; remember that in addition to the little information that exists about this type of SRBV, there is a handful of *in vivo* studies.

Conclusion

We found exogenous Beclin1 overexpression have no significant effect on apoptosis in rabies infected mice model. This may be due to overexpression of RABV glycoprotein, which inhibits the expression of apoptosis related genes.

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References

1. Yamaoka S, Ito N, Ohka S, Kaneda S, Nakamura H, Agari T, et al. Involvement of the rabies virus phosphoprotein gene in neuroinvasiveness. Journal of virology. 2013;87(22):12327-38.
2. Finke S, Mueller-Waldeck R, Conzelmann K-K. Rabies virus matrix protein regulates the balance of virus transcription and replication. Journal of General Virology. 2003;84(6):1613-21.
3. Zhu S, Guo C. Rabies control and treatment: from prophylaxis to strategies with curative potential. Viruses. 2016;8(11):279.
4. Shuai L, Feng N, Wang X, Ge J, Wen Z, Chen W, et al. Genetically modified rabies virus ERA strain is safe and induces long-lasting

protective immune response in dogs after oral vaccination. Antiviral research. 2015;121:9-15.
5. Sparrow E, Torvaldsen S, Newall AT, Wood JG, Sheikh M, Kieny MP, et al. Recent advances in the development of monoclonal antibodies for rabies post exposure prophylaxis: A review of the current status of the clinical development pipeline. Vaccine. 2019;37:A132-A9.
6. Morimoto K, Hooper DC, Spitsin S, Koprowski H, Dietzschold B. Pathogenicity of different rabies virus variants inversely correlates with apoptosis and rabies virus glycoprotein expression in infected primary neuron cultures. Journal of virology. 1999;73(1):510-8.
7. Faber M, Pulmanausahakul R, Hodawadekar SS, Spitsin S, McGettigan JP, Schnell MJ, et al. Overexpression of the rabies virus glycoprotein results in enhancement of apoptosis and antiviral immune response. Journal of virology. 2002;76(7):3374-81.
8. Sarmento L, Tseggai T, Dhingra V, Fu ZF. Rabies virus-induced apoptosis involves caspase-dependent and caspase-independent pathways. Virus research. 2006;121(2):144-51.
9. Elmore S. Apoptosis: a review of programmed cell death. Toxicologic pathology. 2007;35(4):495-516.
10. Ubol S, Sukwattanapan C, Utaisincharoen P. Rabies virus replication induces Bax-related, caspase dependent apoptosis in mouse neuroblastoma cells. Virus research. 1998;56(2):207-15.
11. Shibata M, Lu T, Furuya T, Degterev A, Mizushima N, Yoshimori T, et al. Regulation of intracellular accumulation of mutant Huntingtin by Beclin 1. Journal of Biological Chemistry. 2006;281(20):14474-85.
12. Hill SM, Wrobel L, Rubinsztein DC. Post-translational modifications of Beclin 1 provide multiple strategies for autophagy regulation. Cell Death & Differentiation. 2019;26(4):617-29.
13. Houtman J, Freitag K, Gimber N, Schmoranzer J, Heppner FL, Jendrach M. Beclin1- driven autophagy modulates the inflammatory response of microglia via NLRP 3. The EMBO journal. 2019;38(4):e99430.
14. Kaur S, Changotra H. The beclin 1 interactome: Modification and roles in the pathology of autophagy-related disorders. Biochimie. 2020.
15. Esteves AR, Filipe F, Magalhães JD, Silva DF, Cardoso SM. The role of Beclin-1 acetylation on autophagic flux in Alzheimer's disease. Molecular neurobiology. 2019;56(8):5654-70.
16. Goula D, Remy J, Erbacher P, Wasowicz M, Levi G, Abdallah B, et al. Size, diffusibility and transfection performance of linear PEI/DNA complexes in the mouse central nervous system. Gene therapy. 1998;5(5):712-7.
17. Demeneix BA, Ghorbel M, Goula D. Optimizing polyethylenimine-based gene transfer into mammalian brain for analysis of promoter regulation and protein function. Gene targeting protocols: Springer; 2000. p. 21-35.
18. Remy J-S, Abdallah B, Zanta MA, Boussif O, Behr J-P, Demeneix B. Gene transfer with lipospermines and polyethylenimines. Advanced Drug Delivery Reviews. 1998;30(1-3):85-95.
19. Rubinsztein DC, Mariño G, Kroemer G. Autophagy and aging. Cell. 2011;146(5):682-95.
20. Liu J, Liao M, Yan Y, Yang H, Wang H, Zhou J. Rabies virus phosphoprotein P5 binding to BECN1 regulates self-replication by BECN1-mediated autophagy signaling pathway. Cell Communication and Signaling. 2020;18(1):1-12.
21. Alcami A, Koszinowski UH. Viral mechanisms of immune evasion. Trends in microbiology. 2000;8(9):410-8.
22. Prabhavathy H, editor Comparison of Apoptosis in Street Rabies Virus Isolates and Challenge Virus Standard by Flowcytometry using Tunel Staining Method: Scientific Research Forum.
23. Theerasurakarn S, Ubol S. Apoptosis induction in brain during the fixed strain of rabies virus infection correlates with onset and severity of illness. Journal of neurovirology. 1998;4(4):407-14.
24. Taji F, Abdoli A, Baesi K, Sheikholeslami F, Kouchesfahani HM. Overexpression of Beclin1 gene leads to reduction of telomerase activity in MDCK cells and enhances apoptosis. 2020.