Iranian Journal of Virology 2012;6(2): 8-11
©2012, Iranian Society of Virology

Original Article

Cloning and Expression of Rabies Virus Glycoprotein Gene into Eukaryotic System

Borhani K[1], Bamdad T[1*], Ajorloo M[1,2], Mozhgani SHR[2,3], Miandehi N[4], Moradi-Joshaghan A[4], Gholami AR[2,4]

1. Department of Virology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
2. Human Rabies Vaccine Laboratory, Production and Research Complex Pasteur, Institute of Iran, Tehran, Iran.
3. Department of Virology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.
4. Viral Vaccines Production Department, Production and Research Complex Pasteur, Institute of Iran, Tehran, Iran.

Abstract

Background and Aims: The aim of this study was cloning and expression of rabies virus glycoprotein by a eukaryotic expression plasmid pcDNA3.1(+) in BSR cell line. This construct might be used for a potential DNA vaccine.

Materials and Methods: Glycoprotein gene was synthesized and cloned into pBluescript vector and then sub cloned into eukaryotic expression vector (pcDNA3.1(+)). After verification of the cloning, the recombinant plasmid was transfected into BSR cell line (a clone of BHK-21 cell), and its expression was detected by RT-PCR.

Results: The authenticity of the recombinant plasmid pcDNA3.1(+)-Gp has been confirmed by a quick check method and restriction endonuclease digestion analysis, and after transfection into eukaryotic cells, the presence of mRNA transcripts was verified by reverse transcriptase-polymerase chain reaction (RT-PCR).

Conclusion: This study demonstrated that the construction of eukaryotic expression plasmid for rabies virus glycoprotein is possible. Nevertheless, more work is necessary to develop this kind of vaccine for final use.

Keywords: Rabies virus, glycoprotein; DNA vaccine; Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Introduction

Rabies virus is the etiological agent of a major zoonotic disease that triggers fatal encephalitis in all of mammals including humans (1). This virus is a member of Lyssavirus genus in Rhabdovridae family (2). The nucleocapsid of rabies virus contained three proteins including nucleoprotein, phosphoprotein and RNA dependent RNA polymerase, which is surrounded by a lipid bilayer that is associated with matrix protein and glycoprotein (3). Glycoprotein (Gp) of rabies virus is a type one membrane glycoprotein. This protein is a trimer which extends from the viral membrane. This glycoprotein contains 505 amino acids and is responsible for binding to cellular receptor and is involved in cellular entry of the virus (4, 5). This protein is also involved in the transsynaptic movement of the virus (6-8). In addition, antibodies that are induced against glycoprotein protect against rabies virus infection (9).

***Corresponding author:** Taravat Bamdad, Ph.D. Department of Virology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran.. Email: Bamdad_T@modares.ac.ir

[Downloaded from journal.isv.org.ir on 2026-11-18]

[DOI: 10.21859/isv.6.2.8]

In DNA vaccines, a naked DNA is being transferred in to target cell and induces expression of the desired protein. If this happens successfully in an animal target, the expressed protein could be presented along with MHC antigens in order to raise an immune response. Moreever in the DNA vaccine production process the produced protein is native, in its tertiary structure which has advantages over the subunit vaccines that are based on chemically treated virion (10-12). In this study we demonstrate cloning and expression of rabies virus glycoprotein gene in BSR cell line. This construct which is able to produce rabies virus glycoprotein, could potentially be used as a DNA vaccine.

Methods

Rabies virus glycoprotein gene cloning

The Gp gene of rabies virus sequence (1575 bp) has been synthesized and cloned into pBluescript vector. This circular plasmid and circular pcDNA3.1(+) (Invitrogen), was digested by NheI and EcoRI restriction enzymes (fermentas), and identified by 1% agarose gel electrophoresis. The liner pcDNA3.1(+) and the Gp fragment were excised and recovered using gel extraction kit (fermentas). The target fragment was ligated into linearized pcDNA3.1(+) plasmid using T4 DNA ligase (fermentas) based on the standard protocoles. The ligation product was transformed into competent E.coli TOP10F' strain. A number of colonies carrying antibiotic resistance gene were selected and screened by a quick check method using phenol for denaturing of bacterial proteins, then centrifuge in 12000 rpm. The upper phase contained recombinant plasmid. After conformation, individual colonies were isolated and the plasmid was purified using plasmid extraction kit (fermentas). The purified plasmid was further checked by digestion using NheI and EcoRI restriction enzymes (fermentas).

Cell transfection

The pcDNA3.1(+)-Gp gene was transfected into BSR cell line (a clone of BHK-21 cell). This cell line was cultured and maintained in Dulbecco's Modified Eagles Medium (DMEM, Invitrogen) with 7% fetal bovine serum (FBS, PAA). Two day before transfection, cells from the confluent culture were trypsinazed (0.25% trypsin-EDTA, Invitrogen) and transferred to 6 well plates (Greiner) containing $2.5x10^5$ cells per well. The cells were incubated for 48 hours at 37˚C in incubator with 5% CO2. When 75-90% confluency was observed each well of cells was transfected with 4 µg of plasmid encoding Gp gene. The transfection process was done using lipofectamin 2000 reagent (Invitrogen) according to the manufacturer instruction.

Confirmation of expression

For confirmation of the presence of Gp gene mRNA, after 24 hours of transfection, total RNA was extracted from harvested cells with high pure RNA isolation kit (Roche), and treated with RNase-free DNaseI enzyme (fermentas). Complementary DNA (cDNA) was synthesis by using of random hexamer primer (fermentas), and Reverse Transcriptase enzyme (fermentas). This cDNA was used as template for PCR using 5'-ACCATGGTTCCTCAGGCTC-3' as forward and 5'- TCTCACAGTCCGGTCTCACC-3' as reverse primers. The amplified fragment was visualized by 1% agarose gel electrophoresis. pcDNA3.1(+) transfected cells were subjected to RNA extraction as negative control.

Results

To study the insertion of Gp gene in our target plasmid, a number of colonies caring antibiotic resistance gene were selected and screened by a quick check method as a described in Materials and Methods. Figure 1 shows the pcDNA3.1(+) band shift during 1% agarose gel electrophoresis that could be due to insertion of Gp gene. The Presence of Gp gene (1575bp) in the recombinant plasmid pcDNA3.1(+)-Gp was further checked by restriction enzymes digestion and the results are shown in figure 2. After transfection of recombinant pcDNA3.1(+)-Gp plasmid in BSR cell line, the presences of transcript mRNA was verified by Reverse Transcriptase polymerase chain

[Downloaded from journal.isv.org.ir on 2026-11-18]

[DOI: 10.21859/isv.6.2.8]

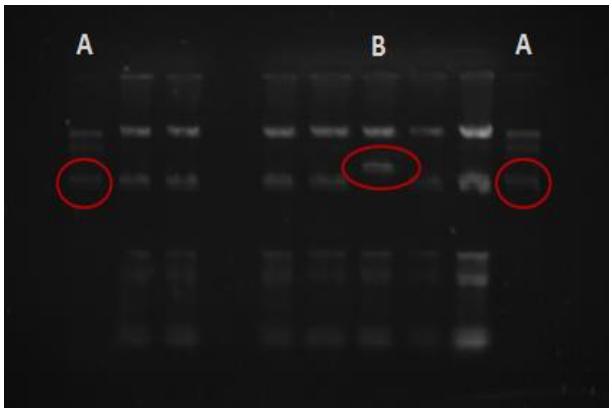


Fig. 1. Quick check analysis of pcDNA3.1(+) plasmids after ligation with Gp. A) pcDNA3.1(+) without recombinant Gp fragments. B) pcDNA3.1(+) Recombination with Gp gene which shows a larger size.

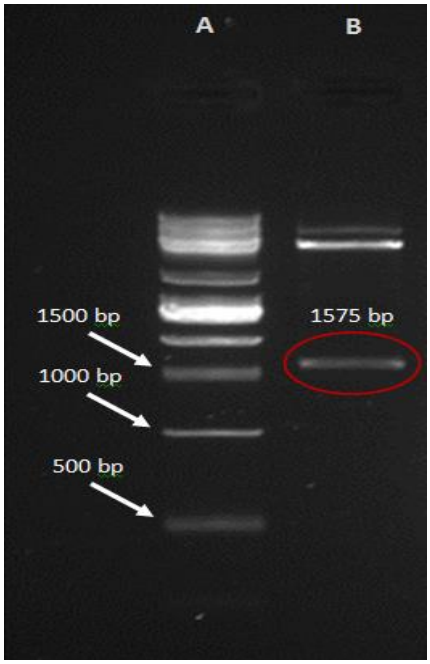


Fig. 2. Restriction enzyme analysis of recombinant plasmid. A) DNA Ladder 1kb (Vivantis). B) Double digestion of recombinant plasmid pcDNA3.1(+)-Gp by NheI and EcoRI restriction enzymes resulted in a 1575 bp Fragment.

reaction (RT-PCR). The result was shown in figure 3.

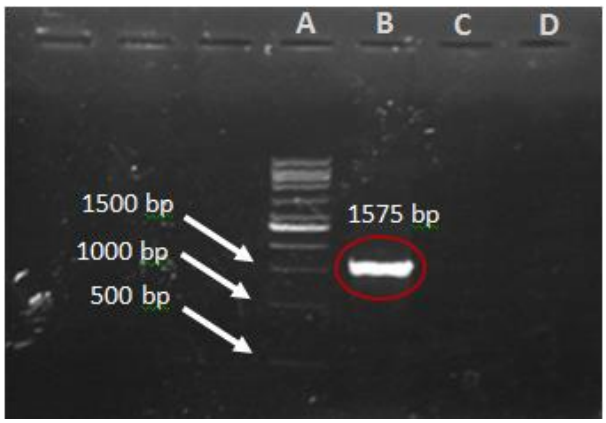


Fig. 3. Expression analysis of Gp in transfected cells by RT-PCR. A) DNA Ladder 1kb (Vivantis). B) RT-PCR result of RNA that was treated with DNaseI. C) PCR of pcDNA3.1(+)-Gp that was treated with DNaseI has no result. D) RT-PCR of RNA from cell control (was transfected by pcDNA3.1(+)) and treated with DNaseI, there was no positive result.

Discussion

One of the most important ways to control rabies is vaccination of domestic and wild animals. The importance of Rabies vaccination is well recognized (13). DNA vaccination technology has grown very fast since 1990s. In this technology the plasmid DNA codes an antigenic protein that could induces an immune response to the desired protein (14, 15). Like other types of vaccines, DNA vaccines engage both MHC-I and MHC-II defense mechanism and are able to induce CD8+ and CD4+ T cells, unlike recombinant proteins that generally induce only humoral response (16). This successful new method has been named as the "the third generation of vaccines" (17). Bahloul et al. have compared two rabies post-exposure prophylaxis. They demonstrated that a single administration of rabies DNA vaccine in BALB/c mice was as effective as five injections of cell culture-derived vaccine (18). The other study that was done by Bahloul et al. showed for the first time that rabies DNA vaccination could be more efficient under experimental or field conditions than the available cell culture-derived vaccine (19).

[Downloaded from journal.isv.org.ir on 2026-11-18]

[DOI: 10.21859/isv.6.2.8]

In this study rabies virus glycoprotein was cloned and expressed under the pcDNA3.1(+)-Gp recombinant plasmid. Successful construction and expression vector might potentially be used as a DNA vaccine. To evaluate protein expression in eukaryotic cells, Western blot analysis using specific antibodies must be done. In the following studies we will monitor rabies virus glycoprotein expression and will evaluate immunogenicity of this potential DNA vaccine in laboratory animals. Further studies would also be done to compare this product with recombinant rabies virus glycoprotein (a parallel study of our team) expressed in eukaryotic cells and commercial rabies vaccine in animal model.

Acknowledgment

This study funded and supported by Human Rabies Vaccine Project, Production and Research Complex, Pasteur Institute of Iran.

References

1. Schnell MJ, McGettigan JP, Wirblich C, Papaneri A. The cell biology of rabies virus: using stealth to reach the brain. Nature. 2010;8:51-61.
2. Conzelmann KK. Reverse genetics of mononegavirales. Curr. Top. Microbiol. Immunol. 2004;283:1-41.
3. Tordo N, Meslin FX, Kaplan MM, Koprowski H. Characteristics and molecular biology of the rabies virus, in: Laboratory Techniques in Rabies, World Health Organization, Geneva, 1996, pp. 28-51.
4. Anilionis A, Wunner WH, Curtis PJ. Structure of the glycoprotein gene in rabies virus. Nature. 1981;294:275-8.
5. Coll JM. The glycoprotein G of rhabdoviruses. Arch. Virol. 1995;140:851-987.
6. Coulon P, Rollin PE, Flamand A. Molecular basis of rabies virus virulence. II. Identification of a site on the CVS glycoprotein associated with virulence. J. Gen. Virol. 1983;64:693-6.
7. Etessami R, Conzelmann KK, Fadai-Ghotbi B, Natelson B, Tsiang H, Ceccaldi PE. Spread and pathogenic characteristics of a Gdeficient rabies virus recombinant: an in vitro and in vivo study. J. Gen. Virol. 2000;81:2147-53.
8. Kucera P, Dolivo M, Coulon P, Flamand A. Pathways of the early propagation of virulent and avirulent rabies strains from the eye to the brain. J. Virol. 1985;55:158-62.
9. Perrin P, Thibodeau L, Sureau P. Rabies immunosome (subunit vaccine) structure and immunogenicity. Pre- and post-exposure protection studies. Vaccine. 1985;3(3):325-32.
10. Hassan UA, Abai AM, Harper DR, Wren BW, Morrow JW. Nucleic acid immunization: concepts and techniques associated with third generation vaccines. J. Immunol. Meth. 1999;229:1-22.
11. Pasquini S, Xiang Z, Wang Y, He Z, Deng H, Blaszczyk-Thurin M. Cytokines and costimulatory molecules as genetic adjuvants. Immunology and cell biology. 1997;74:397-401.
12. Smith HA, Klinman DM. The regulation of DNA vaccines. Curr. Opin. Biotech. 2001;12:299-303.
13. Ertl HCJ. Novel Vaccines to Human Rabies. PLoS Negl Trop Dis. 2009;3(9):e515.1-9
14. Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A, Felgner PL. Direct gene transfer into mouse muscle in vivo. Science. 1990;247:1465-8.
15. Tang DC, DeVit M, Johnston SA. Genetic immunization is a simple method for eliciting an immune response. Nature. 1992;356:152-4.
16. Wang B, Godillot AP, Madaio MP, Weiner DB, Williams WV. Vaccination against pathogenic cells by DNA inoculation. Curr. Top. Microbiol. Immunol. 1998;226:21-35.
17. Babiuk LA, Lewis J, Suradhat S, Baca-Estrada M, Foldvari M, Babiuk S. Polynucleotide vaccines: potential for inducing immunity in animals. Journal of biotechnology. 1999;73:131-40.
18. Bahloul C, Ben Hadj Ahmed S, B'chir BI, Kharmachi H, Hayouni E, Dellagi K. Post-exposure therapy in mice against experimental rabies: a single injection of DNA vaccine is as effective as five injections of cell culturederived vaccine. Vaccine. 2003;22:177-84.
19. Bahloul C, Taieb D, Diouani MF, Ben Hadj Ahmed S, Chtourou Y, B'chir BI, Kharmachi H, Dellagi K. Field trials of a very potent rabies DNA vaccine which induced long lasting virus-neutralizing antibodies and protection in dogs in experimental conditions. Vaccine. 2006;24:1063-72.

[Downloaded from journal.isv.org.ir on 2026-11-18]

[DOI: 10.21859/isv.6.2.8]