Iranian Journal of Medical Microbiology | ISSN:2345-4342

Change in the Basic Structure of the Rabies Virus Glycoprotein by Reverse Genetics

Narges Miandehi [1], Seyed Kazem Bidoki [1], Mehdi Ajorloo [2], Alireza Gholami [*3]

1. *Department of Biology, Faculty of Basic Sciences, Payam-e Noor University, East Tehran Branch*
2. *Department of Clinical Laboratory Sciences, School of Allied Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran*
3. *Human Rabies Vaccine Department, Research Production Complex, Pasteur Institute of Iran, Tehran, Iran*

10.30699/ijmm.14.4.348

ABSTRACT

Background: Rabies is a deadly zoonotic disease that is caused by the rabies virus. The virus can infect and disrupt the central nervous system of a rabid patient. The rabies virus is a neurotropic single stranded RNA virus. Glycoprotein (G) is the most important protein that binds to the cellular receptors and also induces an immune response against the virus in the host. Using reverse genetics technology, the glycoprotein gene could be modified and a virus with higher immunogenicity or lower pathogenicity.

Materials & Methods: In this study, we designed a mutation in the sequence of glycoprotein gene using a software, on the main antigenic site II of the Pasteur virus strain at the position of 42-34 amino acids. Agene fragment in the cloning vector containing the rabies virus genome was replaced by the synthesized construct containing the altered gene by two restricted enzymes, and then cloned. The T7-BHK cell under the T7 phage promoter control was transfected to express the glycoprotein gene, along with the construct and vectors expressing the N, P, and L genes of the rabies virus as well as the full genome. After expressing and confirming viral genes, it was cultured and amplified in BSR cell.

Results: after cloning and expression of the recombinant virus in the target cell, the vector containing the mutated gene led to the rescue of the recombinant virus. The recombinant virus cultured and propagated in the BSR cells, then the genome was extracted and finally confirmed by sequencing.

Conclusion: The rescued recombinant virus can be used for research studies or in the vaccines manufacturing, provide that the antigenicity is maintained or increased.

Keywords: Rabies, Reverse genetics, Vaccine, Glycoprotein, Recombinant virus

Received: 2020/04/05; **Accepted**: 2020/06/02; **Published Online:** 2020/07/20

Corresponding Information: **Alireza Gholami,** Human Rabies Vaccine Department, Research Production Complex, Pasteur Institute of Iran, Tehran, Iran.
Email: a.gholami@pasteur.ac.ir

Miandehi N, bidoki K, Ajorloo M, Gholami A. Change in the Basic Structure of the Rabies Virus Glycoprotein by Reverse Genetics. Iran J Med Microbiol. 2020; 14 (4) :348-360

Download citation: BibTeX | RIS | EndNote | Medlars | ProCite | Reference Manager | RefWorks
Send citation to: Mendeley Zotero RefWorks

Introduction

Rabies is a deadly zoonotic disease caused by rabies virus, spread around the world and can infect all mammals, including humans. The virus infects the central nervous system and leads to disorders in central nervous system **(1)**, with horrible and acute neurological symptoms in the clinical stage. The rabies virus belongs to the rhabdovirus family and lyssavirus genus. Their genetic material is a single-stranded RNA with a negative polarity and a length of about 12kb. The genome of virus encodes five proteins (N, P, M, G, and L) **(2)**. The G protein (glycoprotein) binds to some specific cell's receptors and also provokes immune response against the virus in the host. Antigenic sites on G protein are essential roles in this regard. The antigenic site I carries linear and three-dimensional epitopes located between226-231 amino acid residues.

Another major antigenic site II is located between the amino acids 34-42 (II_b) and 198-200 (II_a). The main antigenic site III is a continuous epitope located between the amino acids 330-338.Epitope IV consists of only one amino acid at position 251. The G1 (also called the antigenic site a) and G5 sub-antigenic sites are also in positions 342-343 and 261-264, respectively **(3)** (Figure 1).

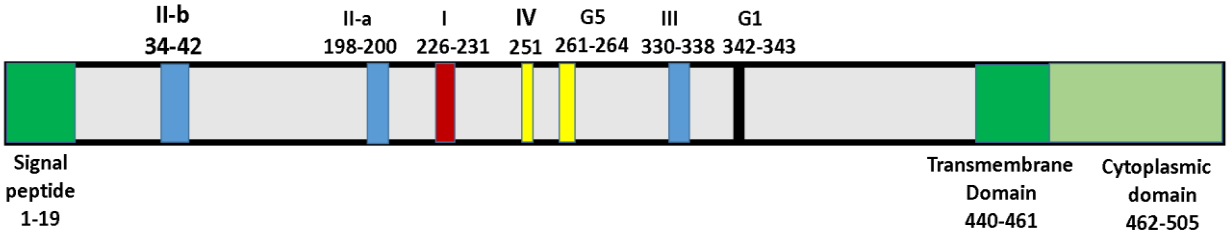

Figure 1. Schematic view of the glycoprotein structure of rabies virus. The main antigenic sites and the location of the amino acids associated with them.

The virus enters the cell via the endocytic pathway, and its replication and propagation cycle is entirely cytoplasmic **(5)**. Once the virus genome is released into the cytoplasm, its genome is used as mRNA to express viral proteins genes, as well as to synthesisantigenome (positive polarity) in order to amplify the virus genome. The rabies virus polymerase complex is consisted of phosphoprotein and the polymerase protein (P and L respectively), which is responsible for transcription and replication of the virus genome **(6)**. According to the above, in the construction of the recombinant rabies virus with reverse genetic technology, only N, P, and L proteins are necessary to form the virus-producing devices. Reverse genetics is a type of technology that manipulates and makes genetic changes in an organism in order to study the altered phenotype in the organism **(7)**.In order to rescue a recombinant and active RNA virus, the cDNA clone from the virus should be prepared and converted into an RNA molecule inside the host cell **(9)**.Genetic modification of the rabies virus toward improving its immunogenicity is one of the valuable applications of this technology. Accordingly, with the aim of establishing a suitable basis for this method in the country and using it in future research, genetic modification in the glycoprotein of rabies virus was done and its release of the active virus particle from the cell was shown.

Materials and Methods

Design and Manufacture of Mutated Glycoprotein

The genetic sequence of the rabies virus glycoprotein strain of Pasteur virus (PV) was obtained from the NCBI database. To generate the desired genetic mutation, the Mega4 software was used to change the genetic code from glycine (GGG) to glutamic acid (GAG) at the main antigenic site II_b, amino acid number 40.

Insertion of Mutated Glycoprotein into the Expression Vector

The mutated glycoprotein-carrying plasmid of the rabies virus was cut by the two restricted enzymes Nco I and SnaB I (Thermo Fisher) and the glycoprotein gene was removed and replaced in the expressing construct of the rabies virus (PV strain) genome instead of the original gene. After cloning in the Top 10 bacteria, PCR was performed and send to gene sequencing.

Animal Cell Culture

After transfection of the T7-BHK cell with expression vectors of N,P, L and modified G genes by lipofectamin 2000 (Invitrogen), the recombinant virus was rescued and inoculated to the BSR (Baby Syrian Related) cells.

Confirmation of Mutant Virus Sequence After Recovery

In order to compare the sequence of the mutated virus with other samples studied in this study, the cells of which were collected and RNA extraction was performed. cDNA was made from extracted RNA. The glycoprotein gene was then multiplied using PCR and specific primers and the PCR product was sent for sequencing.

Results

Confirmation of the Mutated Glycoprotein Gene Sequence in the Expression Vector

Mutated glycoprotein gene was transferred into the construct expressing the genome of the rabies virus by two restriction enzymes. The new cloned gene was generated by suitable PCR primers (Figure 2).

Transfected T7-BHK Cell by Expression Plasmids Containing Virus Genes

The expression genes are depended on the T7-RNA polymerase promoter, produced in this cells. To ensure the correct function of the cells in polymerase expression, the cells were transfected with 4T7A construct and their presence of eGFP protein in microscopic evaluation was well confirmed (Figure 3).Transfection of the above cells with full genomes and expression constructs of virus (N, P, and L) confirmed the expression of viral proteins in T7-BHK cells after specific staining (Figure 4).

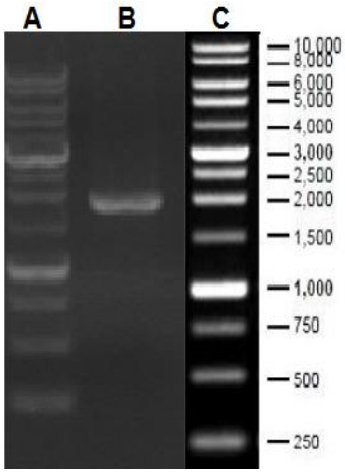

Figure 2. The PCR result on the mutated glycoprotein gene after cloning it into the expression vector. 1 kb marker (A), mutated glycoprotein gene (B), weight marker pattern used (C).

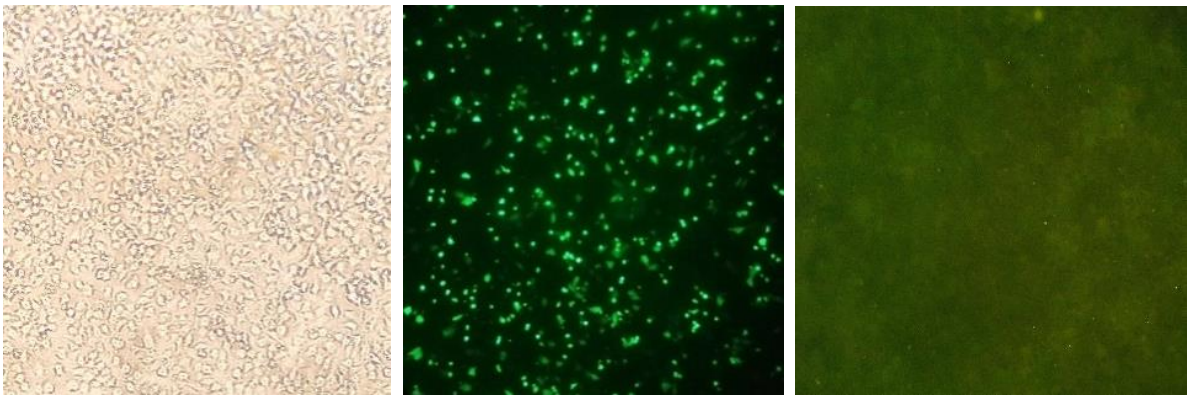

Figure 3. T7-BHK cells transfection results. T7-BHK cells (right), T7-BHK cells transfected with 4T7A structure to confirm T7 polymerase function (middle), the negative control cells with immunofluorescence staining (left).

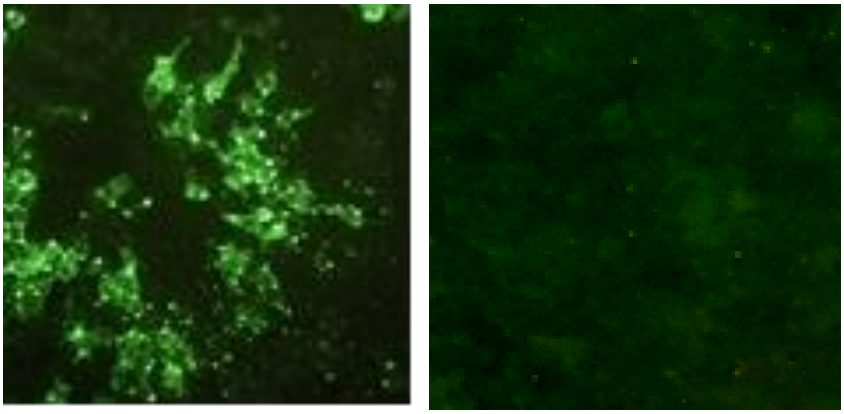

Figure 4. T7BHK cell transfected with expression plasmids. The cell containing the virus genome, with the expression vectors containing the L, P, and N genes of immunofluorescence staining with specific antibodies, shows the production of viral proteins (left). Negative control cells transfected with the same plasmids except L (right).

Infectivity of Recovered Viruses in the BSR Cells

Microscopic observation of BSR cells after specific staining confirmed the ability of virus to infect the cells. It was also observed that the infectivity of the recovered virus improved during subsequent passages (Figure 5). RNA extraction was performed from the virus produced in the BSR cell and sequencing of the virus glycoprotein gene confirmed the presence of a mutation in the recovered virus (Figure 6).

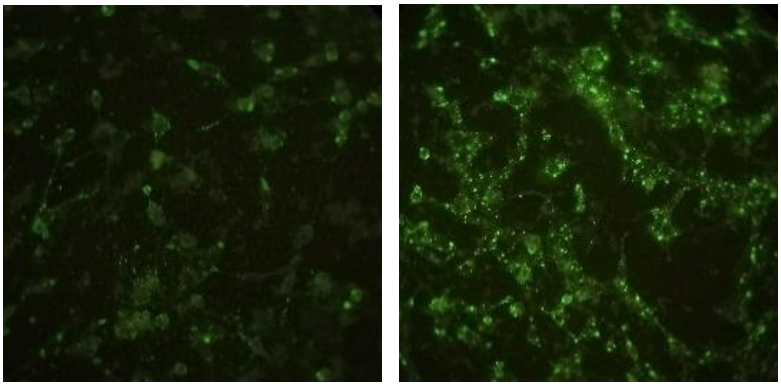

Figure 5. Increasing the virus's ability to infect during consecutive cultures: The left side of the virus-infected cells in the first passage of the virus, compared to the cells infected in the second passage of the virus (right).

Figure 6. Determination of glycoprotein sequence of non-mutated recovered virus (Rescued PV), control vaccine strain (PV) virus, mutated recovered virus (Rescued Modified PV). The location of the mutated nucleotide is shown in the figure with the arrow.

Discussion

In this study, using genetic engineering methods, molecular changes were created in the glycoprotein of rabies virus and the recombinant virus recovered. The virus has shown the ability to infect animal cell culture. In 2003, Nauto Ito et al. improved the recovery of rabies virus from cloned cDNA using reverse genetics without the vaccinia auxiliary virus **(18)**. In the present study, the recombinant rabies virus was recovered using a similar system, the desired change in glycoprotein gene was first altered and then transfected to T7-BHK cell with others viral proteins encoding genes. Rabies virus glycoprotein (RVGP) is the main antigen of the virus and is the only viral component of all new rabies vaccines available. Many new methods have been used since recombinant DNA technology became available for the expression of recombinant immunogenic viral glycoproteins (rRVGP). Recent studies on the development of rabies vaccines have focused on the expression of rRVGP in vivo by transferring the viral vectors into the body. This method is considered as the basis of biotechnology for the new generation of rabies vaccines **(27)**.

Conclusion

In this study, we tried to rescue the rabies virus by modifying the structure of the protein that is effective in the antigenicity and immunogenicity, so that it could be used in qualitative and quantitative tests required for rabies vaccine

Acknowledgment

This work was supported by the production and research complex of Pasteur Institute of Iran. Human rabies vaccine production department.

Conflict of Interest

Authors declared no conflict of interests.