DOI: 10.29326/2304-196X-2023-12-1-6-12

Modern approaches to production of safe and effective genetically modified rabies vaccines for animals

M. I. Doronin, A. Mazloum, D. V. Mikhalishin, M. N. Mitrofanova, A. Yu. Sukharkov, V. V. Kiseleva, A. V. Sprygin
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia

SUMMARY

Rabies is a dangerous zoonotic disease that affects the central nervous system, causes encephalomyelitis and paralyses and is almost invariably fatal. The disease causes significant economic losses associated with the death of animals, outbreak consequences, strict restrictions on domestic and international trade in livestock products, preventive and quarantine measures, laboratory tests. The World Organization for Animal Health recommends vaccination to control rabies. Taking into account that there is a lack of affordable high-quality vaccines to globally prevent and control the disease, stable, attenuated production strains of rabies virus with broad cross-activity against various variants of the pathogen shall be considered as ideal candidates to produce high-quality, safe and effective vaccines. Currently, some approaches are applied to reduce the virus virulence and improve safety of rabies vaccines. Reverse genetics is very popular now. It provides new approaches to study functions of a specific gene by analyzing phenotypic effects after direct manipulations with nucleotide sequences. The methods of reverse genetics have revolutionized molecular biology and have become a powerful tool to study genetics of RNA viruses. These methods are widely used to study rabies virus. The use of reverse genetics has made it possible to modify rabies virus production strains for manufacture of modern genetically modified rabies vaccines that induce a persistent and long-term immunity. The review briefly covers general approaches to development of viral vectors with the purpose to create genetically modified rabies vaccines.

Keywords: review, rabies virus, genes, genetically modified rabies vaccines, methods of reverse genetics

Acknowledgements: The work has been done as part of the research program "Creating protection tools for economically and socially significant animal diseases based on production strains of microorganisms selected by genomic sequencing", implemented within some measures of the Federal Program for Genetic Technologies Development for 2019–2027, taken under the Federal Project "Development of large-scale scientific and scientific-technological projects in priority research areas" of the national project "Science and Universities".

For citation: Doronin M. I., Mazloum A., Mikhalishin D. V., Mitrofanova M. N., Sukharkov A. Yu., Kiseleva V. V., Sprygin A. V. Modern approaches to production of safe and effective genetically modified rabies vaccines for animals. *Veterinary Science Today*. 2023; 12 (1): 6–12. DOI: 10.29326/2304-196X-2023-12-1-6-12.

Conflict of interest: The authors declare no conflict of interest.

For correspondence: Maksim I. Doronin, Candidate of Science (Biology), Head of Sector, Laboratory for FMD Prevention, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: doronin@arriah.ru*.

УДК 619:578.824.11:615.371:573.6

Современные подходы к созданию безопасных и эффективных генно-инженерных антирабических вакцин для животных

М. И. Доронин, А. Мазлум, Д. В. Михалишин, М. Н. Митрофанова, А. Ю. Сухарьков, В. В. Киселева, А. В. Спрыгин
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия

РЕЗЮМЕ

Бешенство является одним из опасных зоонозов, который вызывает поражение центральной нервной системы, приводит к энцефаломиелитам, параличам с неизбежным летальным исходом. Заболевание наносит значительный экономический ущерб, который связан с гибелью животных, ликвидацией последствий вспышек болезни, введением строгих ограничений, налагаемых на внутреннюю и международную торговлю продукцией животноводства, проведением профилактических и карантинных мероприятий, осуществлением лабораторных исследований. Для борьбы с бешенством Всемирная организация здравоохранения животных рекомендует вакцинопрофилактику. Для глобальной профилактики и борьбы с этим заболеванием производимого количества доступных высококачественных вакцин недостаточно. Стабильные аттенуированные производственные штаммы вируса бешенства с широкой перекрестной активностью против различных вариантов возбудителя являются идеальными кандидатами для создания надежных, безопасных и эффективных препаратов. На сегодняшний день применен ряд подходов для снижения вирулентности вируса и повышения безопасности антирабических вакцин. Большую популярность имеют методы обратной генетики, которые представляют собой новые подходы к исследованию функции конкретного гена путем анализа фенотипических эффектов за счет непосредственного манипулирования последовательностями нуклеотидов. Данная группа методов произвела революцию в молекулярной биологии, стала мощным инструментом для изучения генетики РНК-содержащих вирусов

и широко используется в исследованиях возбудителя бешенства. Применение методов обратной генетики позволило проводить модификации производственных штаммов вируса бешенства для использования при изготовлении современных генно-инженерных антирабических препаратов, вызывающих стойкий и длительный иммунитет. В представленном обзоре кратко изложены общие подходы к разработке вирусных векторов с целью создания генно-инженерных вакцин против бешенства.

Ключевые слова: обзор, вирус бешенства, гены, генно-инженерные антирабические вакцины, методы обратной генетики

Благодарности: Работа выполнена в рамках исследовательской программы «Создание комплекса средств защиты против экономически и социально значимых болезней животных на основе отобранных методами геномного секвенирования производственных штаммов микроорганизмов», осуществляемой при выполнении отдельных мероприятий Федеральной научно-технической программы развития генетических технологий на 2019–2027 годы, проводимых при реализации федерального проекта «Развитие масштабных научных и научно-технологических проектов по приоритетным исследовательским направлениям» национального проекта «Наука и университеты».

Для цитирования: Доронин М. И., Мазлум А., Михалишин Д. В., Митрофанова М. Н., Сухарьков А. Ю., Киселева В. В., Спрыгин А. В. Современные подходы к созданию безопасных и эффективных генно-инженерных антирабических вакцин для животных. *Ветеринария сегодня*. 2023; 12 (1): 6–12. DOI: 10.29326/2304-196X-2023-12-1-6-12.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Доронин Максим Игоревич, кандидат биологических наук, заведующий сектором лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: doronin@arriah.ru.*

INTRODUCTION

Rabies is an acute viral disease affecting almost all mammalian species, including humans [1]. Once clinical symptoms appear, rabies is virtually 100% fatal. The World Health Organization estimates that the rabies virus transmitted via bites from infected animals causes more than 59,000 deaths worldwide.

To eradicate the disease successfully, it is necessary to vaccinate wild carnivorous animals (oral vaccination program); to vaccinate domestic animals; to ensure post-exposure and preventive immunization of people who seek medical help and to ensure preventive vaccination of people at risk, primarily, professional risk; to monitor ongoing anti-rabies measures, comprising a number of tasks and techniques. Taking into account that there is a lack of affordable, high-quality vaccines to globally prevent and control the disease; stable, attenuated production strains of rabies virus with broad cross-activity against various variants of the pathogen shall be considered as ideal candidates to make affordable, high quality, safe and effective vaccines [2], however, significant efforts shall be made.

Since the first rabies vaccine was developed at the end of the XIX century by Pasteur, the product has significantly improved, and vaccination is now used for both domestic and farm animals, as well as for reservoir species [3]. Brain tissues affected by the virus were used for a long time to make vaccines. Severe adverse reactions were observed when using rabies vaccines produced from nerve tissues or from tissues of developing avian embryos. The modern technologies of industrial cell cultivation and fermentation have significantly expanded the possibilities to produce high-quality vaccines with a specified amount of immunogenic components [2].

Inactivated and attenuated vaccines are widely used to control rabies, however, they have some disadvantages, most importantly, and some of them are related to safety and strict requirements for biosafety level in the laboratory. Modern rabies vaccines, which are chemically inactivated whole viral products combined with an adjuvant, or live vaccines (most often used in enzootic areas), have been very successful in reducing the number of outbreaks worldwide [4, 5]. However, when using live rabies vaccines, there is a risk that the used strain may easily revert to a virulent phenotype, which is extremely hazardous. In addition, there is an important problem related to commercial production. For this purpose the followings things are required: expensive production lines installed in BSL-3 facilities and a great amount of infectious agent for the vaccine production. An opportunity to bypass these restrictions has appeared, thanks to genetically modified vaccines, because their production is safe. The modified constructions are non-infectious and can be used to produce vaccines in BSL-2 laboratories. However, at the same time, it is necessary to prove that the virus obtained by reverse genetics is avirulent or it belongs to Pathogenicity Groups 3–4 [6–8].

In 1994, M. J. Schnell et al. cloned cDNA of each rabies virus gene and obtained a modified virus. Recently, due to a large amount of data on gene sequences, methods of reverse genetics have been used to determine their functions. The essence of this approach is to move "from genotype to phenotype", i.e. with the help of various genetic manipulations to change or to knock out a particular gene, and then analyze what these changes will result in [9]. At the same time, in contrast to classical approaches, reverse genetics allows to use nucleotide sequences to analyze a specific role of a gene in the phenotype.

Currently, there are four main methods: gene replacement/knockout based on homologous recombination; RNA interference/gene silencing; T-DNA insertion mutagenesis (T-DNA tagging); TILLING (Targeting Induced Local Lesions in Genomes). It should be noted that the reverse genetics greatly facilitates molecular and biological analysis of RNA-containing viruses due to a possibility to do tests directly with ribonucleic acid molecules [10].

Due to *in vitro* reversion, the viral RNA is converted into its corresponding cDNA. Further manipulations with the sequence of the resulting nucleic acid, such as site-specific mutation, deletion, insertion, replacement of a gene site, or the entire gene, can be performed at the cDNA level to study the structure and function of a particular gene [11]. Thus, M. J. Schnell et al. in 1994 used reverse genetics to study attenuated SAD-B19 strain of fixed rabies virus [9]. Later, many researchers began to use this powerful tool to study the molecular biology of rabies viruses belonging to genetic group RABV. Reverse genetics offer great opportunities for development of modern genetically engineered rabies vaccines, which are much safer than the conventional ones [10, 12, 13].

The review briefly covers general approaches to development of viral vectors with the purpose to create genetically engineered rabies vaccines. We have analyzed many publications on reverse genetics of negative-strand RNA viruses, as well as the history and development of rabies vaccines [2, 3, 5, 7, 14–16]. Methods of reverse genetics currently used to reduce virulence and improve safety of rabies vaccines are summarized in the paper. The paper also covers general points related to the use of these methods for negative-sense RNA viruses.

BRIEF DESCRIPTION OF RABIES VIRUS STRUCTURE

Rabies virus (RABV) is a neurotropic virus, a member of the *Lyssavirus* genus belonging to the *Rhabdoviridae* family. The virus of genetic group RABV causes most rabies cases [8].

The virion is a bullet-shaped particle, approximately 250 nm long and 70 nm in diameter. The rabies virus genome is a single-tranded RNA of negative polarity (11,000–12,000 bp in size), encoding the following five structural proteins in a conservative order of 3′→5′: nucleoprotein (N-protein), phosphoprotein (P-protein), matrix protein (M-protein), glycoprotein (G-protein) and RNA-dependent RNA polymerase (L-protein) [4].

Transcription starts from short-leader RNA synthesis, at the 3′ end of the genomic RNA. Viral nucleic acid serves as a matrix for transcription with an RNA-dependent RNA polymerase consisting of L- and P-proteins, which results in synthesis of mRNA for expression of N-, P-, M-, G- and L-proteins. Replication of negative-chain genomes leads to formation of antigenomes that serve as templates for synthesis of negative-strand genomes. Genomic and its complementary antigenomic RNA are tightly encapsulated by a nucleoprotein to form a helical ribonucleoprotein. Only ribonucleoprotein, but not a free RNA, is suitable as a template for replication and transcription. The viral capsid is surrounded by a membrane derived from the host cell, which interacts with the matrix protein and rabies virus glycoprotein [17–19].

Each link between the genes in the rabies virus genome contains a sequence that determines the end of the upstream gene, the intergenic region and the beginning for the downstream gene. These sequences function as a signal for polyadenylation, as well as for initiation, capping, and methylation of the downstream RNA. Intergenic regions of the rabies virus N-P, P-M, M-G and G-L (untranslated pseudogenic ψ-region) include 2, 5, 5 and 24 nucleotides, respectively [14, 20].

ANALYSIS OF RABIES VIRUS GENES FOR DEVELOPMENT OF GENETICALLY MODIFIED RABIES VACCINES

G-gene of rabies virus and its expression. Glycoprotein is a protein of rabies virus, located on the surface of the bullet-shaped virion. It is the main structural protein and the rabies virus antigen, which triggers a strong immune response. The G-protein has two major functions: it determines pathogenicity of the viral particle and induces humoral and cellular immunity against this pathogen [19, 21]. In addition, the glycoprotein ensures interaction between the virion and corresponding receptors on the cell surface resulting in its penetration, and determines the neurotropic nature of the infection [22]. It is important to note that, unlike field isolates, attenuated vaccine strains of rabies virus can synthesize higher levels of glycoprotein in infected neurons [23]. Rupprecht C. E. et al. focus on the fact that the attenuated strains of rabies virus cause mass apoptosis in neurons; however, in case of infection with pathogenic street isolates, these phenomena are much less frequent [8]. Artificially induced mutations of the G-gene make it possible to obtain materials for creation of a new-generation rabies vaccine based on genetically modified structures.

Rabies virus gene containing two and three copies of the G-gene. According to some researchers, modification of the rabies virus containing two copies of G-gene allowed to increase the expression of glycoprotein, which significantly improved the vaccine effectiveness due to an increase in their immunogenicity. At the same time, pathogenicity of the strains decreased sharply. The researchers also showed that the level of G-protein expression is inversely related to the rabies virus pathogenicity [21, 24, 25]. Increased glycoprotein synthesis is associated with enhanced apoptosis, which contributes to regulation of genes associated with host immune responses observed in neurons infected with attenuated virus strains [21].

Hosokawa-Muto J. et al. created R (NPMGGL) strain of recombinant rabies virus carrying double glycoprotein (G) genes. This structure was obtained with the help of reverse genetics using cloned cDNA of RC-HL strain. The biological properties of the created virus were compared with those of the recombinant RC-HL (rRC-HL) strain. The reproduction intensity of strain R(NPMGGL) in cell lines and virulence for adult mice were almost the same as those of strain rRC-HL. At the same time, the G-protein content in the purified virion of strain R(NPMGGL) and the level of glycoprotein expression in infected cells were 1.5 times higher than those in strain rRC-HL. Following serial passages of strain R(NPMGGL) in cell culture, G-protein expression level was the same and the virus infectious titer increased in the process of its adaptation to the cells. It was also demonstrated that strain R(NPMGGL) has higher immunogenicity than strain rRC-HL [6]. Thus, the modified rabies virus strain carrying a double G-gene will make it possible to develop new genetically modified rabies vaccines in future. It should also be noted that in this case we are talking about an inactivated vaccine, since the only modification is the duplication of G-gene, which significantly increases the concentration of immunogenic components and, as a consequence, the vaccine immunogenicity.

Tan Y. et al. conducted a research, where they used self-cleaving sequence of FMDV 2A gene to express

double or triple copies of rabies virus G gene from a one open reading frame obtained from human adenovirus type 5 (AdHu-5). Recombinant adenoviruses produce the virus in similar titers, which suggests that the insertion of double or triple copies of G-gene (rabies virus) associated with the 2A gene sequence (FMDV) does not affect the virus replication. The glycoprotein was effectively expressed by constructs containing 2A gene sequence and retained its antigenic properties. The 2A self-cleaving peptide mediated effective generation of individual rabies virus glycoprotein in the assessment of transient expression. Flow cytometry proved that G-gene expression levels were higher in recombinant adenovirus constructs carrying multiple copies of the rabies virus glycoprotein gene [26].

Thus, the increase in the G-gene expression level has a number of advantages for creation of genetically modified vaccines: 1) it significantly improves production capacity and biosafety; 2) reduces economic losses. These factors are crucial for modern production of safe, effective and affordable rabies vaccines. Therefore, the recombinant rabies virus expressing two or three copies of a glycoprotein is a candidate to develop new-generation genetically modified rabies vaccines.

Significant nucleotide substitutions in the rabies virus G-gene. According to M. Faber et al., the substitution of one amino acid at position 333 on the glycoprotein from a positively charged arginine (Arg) or lysine (Lys) residue for glutamine (Gln) or isoleucine (Ile) makes a virulent strain of rabies virus apathogenic for adult mice when administered intracerebrally [21]. At the same time, there is evidence that the glycoprotein amino acids at position 333 are not fully responsible for the virus pathogenicity, therefore, some rabies virus strains that have a substitution for Gln_{333} retain neuroinvasive abilities and pathogenicity [17].

Ito Y. et al. analyzed rabies virus strain RC-HL using reverse genetics and concluded that amino acids located between positions 164–303 in G-protein, especially amino acids at positions 242, 255 and 268, also play an important role in making the strain apathogenic [27]. M. Faber et al. found that only the amino acid substitution at position 194 of glycoprotein from asparagine (Asn) to lysine (Lys) is responsible for restoring pathogenicity in an apathogenic, attenuated rabies virus strain SPBNGA [21].

Taking into account the experience of many researchers, it can be concluded that when using recombinant strains of rabies virus that carry two or more G-genes encoding glycoprotein with mutations in positions 149, 194 and 333, the risk of returning to the pathogenic phenotype is significantly reduced.

Significant mutations in the M-protein of the rabies virus. The matrix protein of rabies virus is multifunctional, has a small molecular weight of about 20–25 kDa and a length of 202 amino acid residues. This phosphoprotein is represented by two isoforms M_1 and M_2, which differ from each other in phosphorylation degree. The matrix protein is structurally a bridge between the N- and G-protein. M-gene is much more conservative compared to P-protein. The matrix protein is believed to form a layer between the glycoprotein in the virion shell and the helical-shaped nucleocapsid nucleus consisting of RNA and N-, L-, P-proteins. M-protein is the main factor contributing to virion morphogenesis [28].

Finke S. et al. demonstrated that the M-protein establishes a balance between the virus transcription and replication [15, 16]. It interacts with the viral ribonucleoprotein, which condenses into a dense bullet-shaped form, and plays a key role in the assembly and budding of mature virions [29].

Wirblich C. et al. found that the matrix protein structure has an L-domain with four motifs, one of which is PPxY (PPEY) close to the amino terminus of M-protein. The authors created constructs with point mutations and revealed that PPEY is necessary for effective release of virion from the cell membrane. Amino acid deletions and substitutions in PPEY motif reduce the infection spread. Recombinant viruses constructed on this basis demonstrated reduced virulence for mice, while causing strong immune responses [30]. Thus, significant substitutions in the matrix protein of the rabies virus can create gene constructs for production of modern rabies vaccines.

Significant mutations in the P-gene of the rabies virus. Phosphoprotein is the most important structural protein of the rabies virus and it has molecular weight of 260 kDa and a length of about 330 amino acid residues. The expanded N-terminal region of the P-protein interacts with RNA polymerase. The P-gene encodes at least four proteins synthesized in an infected cell. The phosphoprotein has a chaperone function and binds to N that is not yet bound to viral RNA. The C-terminal domain of the P-protein binds to the "nucleoprotein–RNA" complex and attaches the polymerase complex [29].

Schnell M. J. et al. detected immunodominant P-protein site, which is located in the range of 191–206 amino acid residues and is an interferon antagonist [9, 31, 32]. Jacob Y. et al. determined that the light chain of cytoplasmic dynein involved in the intracellular transport of organelles interacts with the rabies virus phosphoprotein [33]. Deletion of LC8 binding domain in the P-protein significantly inhibited replication and transcription of the virus in neurons. It shall be noted that such a recombinant virus was characterized by a decreased level of gene expression in neuronal cell cultures, while the growth pattern on non-neuronal cells remained unchanged [17, 26]. Thus, amino acid mutations in the dynein-binding domain of the P-protein make it possible to reduce the rabies virus virulence, and the created recombinant viruses make it possible to develop genetically modified rabies vaccines.

SYNTHESIS OF INFECTIOUS RECOMBINANT VIRAL PARTICLES USING MODIFIED cDNA

In 1994 for the first time, M. J. Schnell et al. constructed rabies virus using cloned cDNA [9]. The schematic diagram of this process included at least simultaneous transfection into the cell of four plasmids encoding N-, P-, L-proteins and full-size cDNA of the virus. Rabies virus (-)RNA was reverse transcribed into positive-sense antigenomic cDNA (Fig.). Using amplification of a specific gene from full-length cDNA, three plasmids *p-N*, *p-P*, *p-L* expressing N-, P- and L-proteins of the rabies virus were constructed. In order to avoid mutations, the L-gene was assembled from gradually cloned fragments. Similarly, a plasmid carrying information about the entire genome of the rabies virus (*p-genome*) was constructed.

Viral cDNA was gradually assembled with the help of restrictases (as part of a plasmid), to create a full matrix

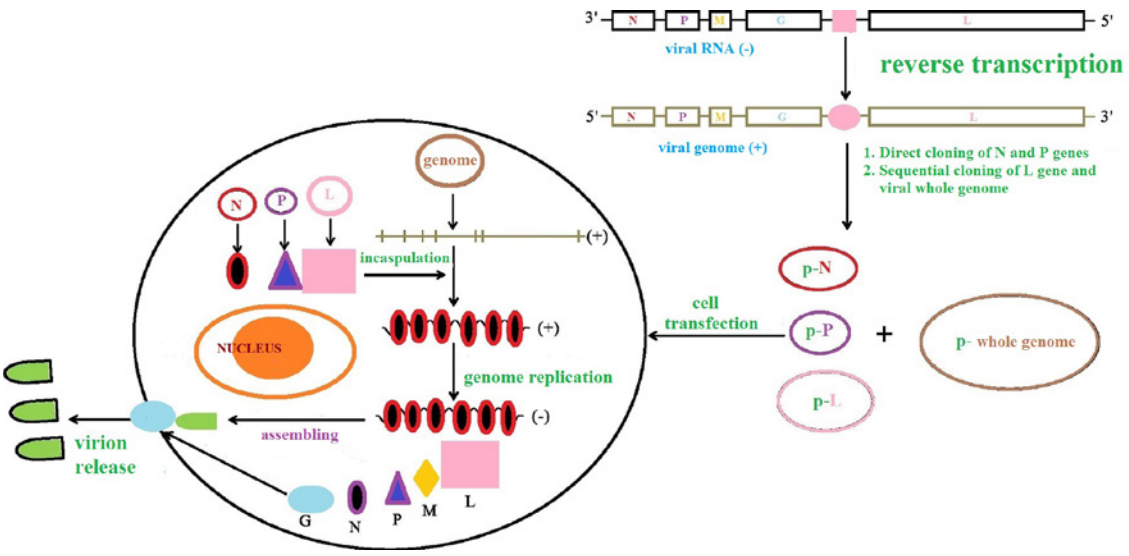

Fig. Synthesis of infectious recombinant rabies virus particles from cloned cDNA

for the rabies virus genetic material. For transcription of the constructed plasmids, T7-RNA polymerase or endogenous cellular RNA polymerases of type I or II were used. In order to obtain the exact 5′ and 3′ ends of the viral RNA, the cloned viral cDNA was flanked with autocatalytic ribozyme sequences. The N-, P- and L-proteins of the rabies virus expressed by three plasmids encapsulate the transcribed full-size antigenomic sense RNA to form a ribonucleoprotein, which functions as a matrix for further expression of structural viral proteins and amplification of rabies virus (-)RNA. The use of transcripts with antigenomic meaning is fundamental in this case, since only they can ensure the process of gene expression and avoid hybridization between genomic (-)RNA and plasmid (+)RNAs. The ribonucleoprotein shapes into a bullet using a matrix protein and separates from the cytoplasmic membrane, where glycosylated trimeric transmembrane compounds have accumulated. At the end of the process, infectious virions are formed, and the next cycle of infection begins [34–36].

Some researchers describe procedures that facilitate and increase effectiveness of creation of a viable rabies virus. Thus, to deliver T7 RNA polymerase, instead of infecting cells with recombinant smallpox vaccine virus, BSR-T7/5 cell line was created that expresses RNA polymerase [37]. Inoue K. et al. developed a segmented version of the virus and showed that additional nucleotides at the terminal end of the genome can affect the expression efficiency. Coding sequences of two ribozymes (HamRz and HdvRz) were "sewn" onto the 5′ and 3′ ends of the rabies virus genome to obtain full-sized virus transcripts with exact ends [35]. Such modifications significantly expand the possibilities of reverse genetics of the rabies virus nucleic acid for various cell lines and make it possible to quickly and efficiently generate a recombinant virus.

It should be noted that scientists also analyzed the ψ-region of the rabies pathogen genome. They found that the virus with a knocked out pseudogen was characterized by normal reproduction rates in biological test systems and did not differ from isolates or strains that have this region [9, 34]. Thus, the pseudogen ψ is an ideal target for insertions and makes it possible to carry out various genetic manipulations with the rabies virus genome.

CONCLUSION

Vaccination of animals is recommended in the Manual of the World Organization for Animal Health to control rabies. Currently, live, as well as cultural inactivated adsorbed and emulsion rabies vaccines are used for these purposes. At the same time, there is a number of disadvantages related to the vaccines, i.e. strict biosafety requirements for production laboratories and a fact that the attenuated strains may revert to virulence [3, 11, 14].

In order to reduce the virulence and increase the safety of rabies vaccines, methods of reverse genetics are currently used [38]. Some researchers demonstrate [9, 12, 31] that many properties of the rabies virus with non-segmented negative RNA are perfect to construct gene delivery vectors required for development of genetically modified rabies vaccines. The simple and conservative composition of the rabies virus genome makes it easy to use genetic engineering and to express modified genes.

The encapsidation of the rabies virus nucleic acid into a ribonucleoprotein has an advantage to significantly reduce the probability of recombination and thereby maintain genome stability [19, 38–41]. High frequency of reversion shall be taken into account during reproduction of the virus in an infected cell. It is associated with low accuracy of transcription with RNA polymerase, which indicates the need for simultaneous introduction of several modifications.

Based on the extensive research into modification of rabies virus genome using reverse genetics, it became possible to construct attenuated live strains which will help to develop modern genetically modified rabies vaccines, safe and effective. Currently, researchers focus on significant mutations introduced into the G-, M-, P-genes of rabies virus, leading to a loss of pathogenicity. Special attention is paid to gene constructs that include two or even three copies of the G-gene, which makes it possible to get suspensions with a high concentration of glycoprotein and induce a strong immune response when administered to animals. The application of reverse genetics for synthesis of infectious recombinant viral particles using modified rabies virus cDNA is also of great interest.

REFERENCES

1. Hidaka Y., Lim C. K., Takayama-Ito M., Park C. H., Kimitsuki K., Shiwa N., et al. Segmentation of the rabies virus genome. *Virus. Res*. 2018; 252: 68–75. DOI: 10.1016/j.virusres.2018.05.017.

2. Wu X., Smith T. G., Rupprecht C. E. From brain passage to cell adaptation: the road of human rabies vaccine development. *Expert. Rev. Vaccines*. 2011; 10 (11): 1597–1608. DOI: 10.1586/erv.11.140.

3. Briggs D. J., Nagarajan T., Rupprecht C. E. Rabies vaccines. *In: Rabies: Scientific Basis of the Disease and Its Management. Ed. by A. C. Jackson. 3rd ed*. Academic Press; 2013; Chapter 13: 497–526. DOI: 10.1016/B978-0-12-396547-9.00013-4.

4. Evans J. S., Horton D. L., Easton A. J., Fooks A. R., Banyard A. C. Rabies virus vaccines: is there a need for a pan-lyssavirus vaccine? *Vaccine*. 2012; 30 (52): 7447–7454. DOI: 10.1016/j.vaccine.2012.10.015.

5. Hicks D. J., Fooks A. R., Johnson N. Developments in rabies vaccines. *Clin. Exp. Immunol.* 2012; 169 (3): 199–204. DOI: 10.1111/j.1365-2249.2012.04592.x.

6. Hosokawa-Muto J., Ito N., Yamada K., Shimizu K., Sugiyama M., Minamoto N. Characterization of recombinant rabies virus carrying double glycoprotein genes. *Microbiol. Immunol*. 2006; 50 (3): 187–196. DOI: 10.1111/j.1348-0421.2006.tb03785.x.

7. Nel L. H. Vaccines for lyssaviruses other than rabies. *Expert. Rev. Vaccines*. 2005; 4 (4): 533–540. DOI: 10.1586/14760584.4.4.533.

8. Rupprecht C. E., Plotkin S. A. Rabies vaccines. *In: Vaccines. Ed. by S. A. Plotkin, W. A. Orenstein, P. A. Offit. 6th ed*. Elsevier Saunders; 2013: 646–668. DOI: 10.1016/B978-1-4557-0090-5.00036-7.

9. Schnell M. J., Mebatsion T., Conzelmann K. K. Infectious rabies viruses from cloned cDNA. *EMBO J*. 1994; 13 (18): 4195–4203. DOI: 10.1002/j.1460-2075.1994.tb06739.x.

10. Yin J., Wang X., Mao R., Zhang Z., Gao X., Luo Y., et al. Research advances on the interactions between rabies virus structural proteins and host target cells: accrued knowledge from the application of reverse genetics systems. *Viruses*. 2021; 13 (11):2288. DOI: 10.3390/v13112288.

11. Conzelmann K. K. Reverse Genetics of *Mononegavirales*: The Rabies Virus Paradigm. *In: Sendai Virus Vector. Ed. by Y. Nagai*. Tokyo: Springer; 2013: 1–20. DOI: 10.1007/978-4-431-54556-9_1.

12. Huang Y., Tang Q., Nadin-Davis S. A., Zhang S., Hooper C. D., Ming P., et al. Development of a reverse genetics system for a human rabies virus vaccine strain employed in China. *Virus. Res.* 2010; 149 (1): 28–35. DOI: 10.1016/j.virusres.2009.12.009.

13. Larsen D. D., Wickersham I. R., Callaway E. M. Retrograde tracing with recombinant rabies virus reveals correlations between projection targets and dendritic architecture in layer 5 of mouse barrel cortex. *Front. Neural Circuits*. 2008; 1:5. DOI: 10.3389/neuro.04.005.2007.

14. Conzelmann K. K., Cox J. H., Schneider L. G., Thiel H. J. Molecular cloning and complete nucleotide sequence of the attenuated rabies virus SAD B19. *Virology*. 1990; 175 (2): 485–499. DOI: 10.1016/0042-6822(90)90433-r.

15. Finke S., Conzelmann K. K. Recombinant rhabdoviruses: vectors for vaccine development and gene therapy. *Curr. Top. Microbiol. Immunol*. 2005; 292: 165–200. DOI: 10.1007/3-540-27485-5_8.

16. Finke S., Mueller-Waldeck R., Conzelmann K. K. Rabies virus matrix protein regulates the balance of virus transcription and replication. *J. Gen. Virol*. 2003; 84: 1613–1621. DOI: 10.1099/vir.0.19128-0.

17. Mebatsion T. Extensive attenuation of rabies virus by simultaneously modifying the dynein light chain binding site in the P protein and replacing Arg333 in the G protein. *J. Virol*. 2001; 75 (23): 11496–11502. DOI: 10.1128/JVI.75.23.11496-11502.2001.

18. Morimoto K., Shoji Y., Inoue S. Characterization of P gene-deficient rabies virus: propagation, pathogenicity and antigenicity. *Virus. Res*. 2005; 111 (1): 61–67. DOI: 10.1016/j.virusres.2005.03.011.

19. Wang Z. W., Sarmento L., Wang Y., Li X. Q., Dhingra V., Tseggai T., et al. Attenuated rabies virus activates, while pathogenic rabies virus evades, the host innate immune responses in the central nervous system. *J. Virol*. 2005; 79 (19): 12554–12565. DOI: 10.1128/JVI.79.19.12554-12565.2005.

20. Walker P. J., Dietzgen R. G., Joubert D. A., Blasdell K. R. Rhabdovirus accessory genes. *Virus. Res*. 2011; 162 (1–2): 110–125. DOI: 10.1016/j.virusres.2011.09.004.

21. Faber M., Faber M. L., Papaneri A., Bette M., Weihe E., Dietzschold B., Schnell M. J. A single amino acid change in rabies virus glycoprotein increases virus spread and enhances virus pathogenicity. *J. Virol.* 2005; 79 (22): 14141–14148. DOI: 10.1128/JVI.79.22.14141-14148.2005.

22. Etessami R., Conzelmann K. K., Fadai-Ghotbi B., Natelson B., Tsiang H., Ceccaldi P. E. Spread and pathogenic characteristics of a G-deficient rabies virus recombinant: an *in vitro* and *in vivo* study. *J. Gen. Virol.* 2000; 81: 2147–2153. DOI: 10.1099/0022-1317-81-9-2147.

23. Yan X., Prosniak M., Curtis M. T., Weiss M. L., Faber M., Dietzschold B., Fu Z. F. Silver-haired bat rabies virus variant does not induce apoptosis in the brain of experimentally infected mice. *J. Neurovirol*. 2001; 7 (6): 518–527. DOI: 10.1080/135502801753248105.

24. Zhang G., Wang H., Mahmood F., Fu Z. F. Rabies virus glycoprotein is an important determinant for the induction of innate immune responses and the pathogenic mechanisms. *Vet. Microbiol*. 2013; 162 (2–4): 601–613. DOI: 10.1016/j.vetmic.2012.11.031.

25. Tao L., Ge J., Wang X., Wen Z., Zhai H., Hua T., et al. Generation of a recombinant rabies Flury LEP virus carrying an additional G gene creates an improved seed virus for inactivated vaccine production. *Virol. J.* 2011; 8:454. DOI: 10.1186/1743-422X-8-454.

26. Tan Y., Liang H., Chen A., Guo X. Coexpression of double or triple copies of the rabies virus glycoprotein gene using a 'self-cleaving' 2A peptide-based replication-defective human adenovirus serotype 5 vector. *Biologicals*. 2010; 38 (5): 586–593. DOI: 10.1016/j.biologicals.2010.06.001.

27. Ito Y., Ito N., Saito S., Masatani T., Nakagawa K., Atoji Y., Sugiyama M. Amino acid substitutions at positions 242, 255 and 268 in rabies virus glycoprotein affect spread of viral infection. *Microbiol. Immunol*. 2010; 54 (2): 89–97. DOI: 10.1111/j.1348-0421.2009.00192.x.

28. Zandi F., Khalaj V., Goshadrou F., Meyfour A., Gholami A., Enayati S., et al. Rabies virus matrix protein targets host actin cytoskeleton: a protein-protein interaction analysis. *Pathog. Dis*. 2021; 79 (1):ftaa075. DOI: 10.1093/femspd/ftaa075.

29. Mebatsion T., Konig M., Conzelmann K. K. Budding of rabies virus particles in the absence of the spike

glycoprotein. *Cell.* 1996; 84 (6): 941–951. DOI: 10.1016/s0092-8674(00)81072-7.

30. Wirblich C., Tan G. S., Papaneri A., Godlewski P. J., Orenstein J. M., Harty R. N., Schnell M. J. PPEY motif within the rabies virus (RV) matrix protein is essential for efficient virion release and RV pathogenicity. *J. Virol*. 2008; 82 (19): 9730–9738. DOI: 10.1128/JVI.00889-08.

31. Schnell M. J., McGettigan J. P., Wirblich C., Papaneri A. The cell biology of rabies virus: using stealth to reach the brain. *Nat. Rev. Microbiol*. 2010; 8 (1): 51–61. DOI: 10.1038/nrmicro2260.

32. Schnell M. J., Tan G. S., Dietzschold B. The application of reverse genetics technology in the study of rabies virus (RV) pathogenesis and for the development of novel RV vaccines. *J. Neurovirol*. 2005; 11 (1): 76–81. DOI: 10.1080/13550280590900436.

33. Jacob Y., Badrane H., Ceccaldi P. E., Tordo N. Cytoplasmic dynein LC8 interacts with lyssavirus phosphoprotein. *J. Virol*. 2000; 74 (21): 10217–10222. DOI: 10.1128/jvi.74.21.10217-10222.2000.

34. Ceccaldi P. E., Fayet J., Conzelmann K. K., Tsiang H. Infection characteristics of rabies virus variants with deletion or insertion in the pseudogene sequence. *J. Neurovirol.* 1998; 4 (1): 115–119. DOI: 10.3109/13550289809113489.

35. Inoue K., Shoji Y., Kurane I., Iijima T., Sakai T., Morimoto K. An improved method for recovering rabies virus from cloned cDNA. *J. Virol. Methods*. 2003; 107 (2): 229–236. DOI: 10.1016/s0166-0934(02)00249-5.

36. Le Mercier P., Jacob Y., Tanner K., Tordo N. A novel expression cassette of lyssavirus shows that the distantly related Mokola virus can rescue a defective rabies virus genome. *J. Virol*. 2002; 76 (4): 2024–2027. DOI: 10.1128/jvi.76.4.2024-2027.2002.

37. Buchholz U. J., Finke S., Conzelmann K. K. Generation of bovine respiratory syncytial virus (BRSV) from cDNA: BRSV NS2 is not essential for virus replication in tissue culture, and the human RSV leader region acts as a functional BRSV genome promoter. *J. Virol*. 1999; 73 (1): 251–259. DOI: 10.1128/JVI.73.1.251-259.1999.

38. Wenqiang J., Yin X., Lan X., Li X., Liu J. Development of a reverse genetics system for the aG strain of rabies virus in China. *Arch. Virol*. 2014; 159 (5): 1033–1038. DOI: 10.1007/s00705-013-1919-9.

39. Wall N. R., Wickersham I. R., Cetin A., De La Parra M., Callaway E. M. Monosynaptic circuit tracing *in vivo* through Cre-dependent targeting and complementation of modified rabies virus. *Proc. Natl. Acad. Sci. USA*. 2010; 107 (50): 21848–21853. DOI: 10.1073/pnas.1011756107.

40. Wickersham I. R., Finke S., Conzelmann K. K., Callaway E. M. Retrograde neuronal tracing with a deletion-mutant rabies virus. *Nat. Methods*. 2007; 4 (1): 47–49. DOI: 10.1038/nmeth999.

41. Wickersham I. R., Sullivan H. A., Seung H. S. Production of glycoprotein-deleted rabies viruses for monosynaptic tracing and high-level gene expression in neurons. *Nat. Protoc*. 2010; 5 (3): 595–606. DOI: 10.1038/nprot.2009.248.

Received 03.11.2022
Revised 15.11.2022
Accepted 09.12.2022

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Maksim I. Doronin, Candidate of Science (Biology), Head of Sector, Laboratory for FMD Prevention, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0002-4682-6559, e-mail: doronin@arriah.ru.*

Ali Mazloum, Candidate of Science (Biology), Senior Researcher, Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0002-5982-8393, e-mail: mazlum@arriah.ru.*

Dmitry V. Mikhalishin, Doctor of Science (Veterinary Medicine), Head of Laboratory for FMD Prevention, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0003-1718-1955, e-mail: mihalishin_dv@arriah.ru.*

Maria N. Mitrofanova, Candidate of Science (Veterinary Medicine), Junior Researcher, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0003-0126-9653, e-mail: mitrofanova@arriah.ru.*

Andrey Yu. Sukharkov, Candidate of Science (Biology), Head of Reference Laboratory for Rabies and BSE, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0001-5326-2440, e-mail: suharkov@arriah.ru.*

Valeriya V. Kiseleva, Leading Biologist, Reference Laboratory for Rabies and BSE, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0001-7114-6267, e-mail: kiseleva_vv@arriah.ru.*

Alexander V. Sprygin, Doctor of Science (Biology), Senior Researcher, Reference Laboratory for Bovine Diseases, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0001-5982-3675, e-mail: sprygin@arriah.ru.*

Доронин Максим Игоревич, кандидат биологических наук, заведующий сектором лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0002-4682-6559, e-mail: doronin@arriah.ru.*

Мазлум Али, кандидат биологических наук, старший научный сотрудник референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0002-5982-8393, e-mail: mazlum@arriah.ru.*

Михалишин Дмитрий Валерьевич, доктор ветеринарных наук, заведующий лабораторией профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0003-1718-1955, e-mail: mihalishin_dv@arriah.ru.*

Митрофанова Мария Николаевна, кандидат ветеринарных наук, младший научный сотрудник информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0003-0126-9653, e-mail: mitrofanova@arriah.ru.*

Сухарьков Андрей Юрьевич, кандидат биологических наук, заведующий референтной лабораторией по бешенству и BSE ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0001-5326-2440, e-mail: suharkov@arriah.ru.*

Киселева Валерия Владимировна, ведущий биолог лаборатории по бешенству и BSE ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0001-7114-6267, e-mail: kiseleva_vv@arriah.ru.*

Спрыгин Александр Владимирович, доктор биологических наук, старший научный сотрудник референтной лаборатории болезней крупного рогатого скота ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0001-5982-3675, e-mail: sprygin@arriah.ru.*